Venom allergies may be immune response gone awry, study says

By Molly Sharlach

For most people, a bee sting causes temporary pain and discomfort, but for those with a venom allergy, the consequences can be devastating: They experience anaphylactic shock, including a drop in blood pressure, itchy hives and breathing problems, and may die if not promptly treated.

New findings by School of Medicine scientists may provide an evolutionary explanation for severe allergic reactions. In a paper published online, scientists have found that severe allergic reactions to bee stings may have an evolutionary explanation.

Scientists have found that severe allergic reactions to bee stings may have an evolutionary explanation.

Scholar leads effort to allow comments on articles in PubMed

By Rosanne Spector

What would you do if you saw an error in a medical journal article? If you’re biostatistician Rob Tibshirani, PhD, you’d try to correct it. And then, because there’s no easy way to do that, you’d get frustrated and think, I wish I could help those less fortunate than myself.

Rob Tibshirani helped to establish an online forum for commenting on articles indexed in PubMed.

Leonard Herzenberg, who developed key cell-sorting technology, dies at 81

By Krista Conger

Leonard Herzenberg, PhD, professor emeritus of genetics at the School of Medicine, died Oct. 22 at Stanford Hospital. His wife and longtime collaborator, Leonore (Lee) Herzenberg, and their dog, Gigi, were by his side.

Leonard Herzenberg, PhD, professor emeritus of genetics at the School of Medicine, died Oct. 22 at Stanford Hospital.

Technique converts fat cells to liver cells

By Bruce Goldman

In a feat of modern-day alchemy with huge potential for regenerative medicine, School of Medicine scientists have developed a fast, efficient way to turn cells extracted from routine liposuction into liver cells.

Leonard Herzenberg

In a feat of modern-day alchemy with huge potential for regenerative medicine, School of Medicine scientists have developed a fast, efficient way to turn cells extracted from routine liposuction into liver cells.

Rob Tibshirani

Rob Tibshirani helped to establish an online forum for commenting on articles indexed in PubMed.

Leonard Herzenberg

Leonard Herzenberg helped to establish an online forum for commenting on articles indexed in PubMed.
By Krista Conger

In yet another coup for a research concept known as "big data," researchers at the School of Medicine have developed a computerized algorithm to understand the complex and rapid choreography of hundreds of proteins that interact in mindboggling combinations to govern how genes are flipped on and off within a cell. To do so, they coupled findings from 238 DNA protein-binding experiments performed by the ENCODE project — a massive, multiyear international effort to identify the functional elements of the human genome — with a data-mining-based technique to identify binding patterns among the proteins themselves.

The analysis is sensitive enough to have identified many previously unsuspected, multipartner trysts. It can also be performed quickly and repeatedly to track how a cell responds to environmental changes or crucial developmental signals.

"At a very basic level, we are learning who likes to work with whom to regulate around 20,000 human genes," said Michael Snyder, PhD, professor and chair of genetics. "If you had to look through all possible interactions pair-wise, it would be ridiculously impossible. Here we can look at thousands of combinations in an unbiased manner and pull our important and powerful information. It's a completely unprecedented level of understanding."

Snyder is the senior author of a paper, published Oct., in Cell, describing the research. The lead authors are postdoctoral scholars Dan Xie, PhD, Alan Boyle, PhD, and Linfeng Wu, PhD.

Proteins control gene expression by either binding to specific regions of DNA, or by interacting with other factors to control DNA-bound proteins to modulate their function. Previously, researchers could only analyze two to three proteins in combinations at a time, and were unable to see the true complexities of the interactions among proteins and DNA that occur in living cells.

The challenge resembled trying to figure out interactions in a crowded mosh pit by studying a few Waltz couples in an otherwise empty ballroom, and it is severely limited what could be learned about the dynamics of gene expression.

The ENCODE, for the Encyclopedia of DNA Elements, project was a five-year collaboration of more than 640 scientists in 52 labs around the world to reveal the complex interplay across entire genomes, regions and RNA molecules that govern when and how genes are expressed. The project has been generating a treasure trove of data for researchers to analyze for the last eight years.

In this study, the researchers combined data from genomics (a field devoted to the study of genes and their products (which focuses on proteins and their interactions). They studied 128 proteins, called trans-acting factors, which are known to regulate gene expression by binding to regulatory regions within the genome. Some of the regions control the expression of nearby genes; others affect the expression of genes far across a chromosome.

The researchers used 238 data sets generated by the ENCODE project to study the specific DNA sequences bound by each of the 128 trans-acting factors. But these factors are not monogamous; they bind many different sequences in a variety of protein-DNA combinations.

Xie, Boyle and Snyder designed a machine-learning algorithm to analyze all the data and identify which trans-acting factors are more likely to work with which DNA sequences they prefer.

Wu then performed immunoprecipitation experiments, which use antibodies to identify protein interactions within the cell nucleus. In this way, they were able to tell which proteins interacted directly with one another, and which were seen together because their preferred DNA binding sites were adjoining.

Before our work, only the combination of two or three regulatory proteins were studied, which oversimplified how gene regulators collaborate to find their targets.

"With our method, we are able to study the combination of more than 100 regulators and see a much more complex structure of collaboration. For example, it had been believed that a key regulator of cell proliferation called FOXS typically only works with JUN protein family members. We showed, in addition to JUN, FOXS has different partners under different circumstances. In fact, we found almost all the canonical combinations of two or three trans-acting factors have many more partners than we previously thought."

To broaden their analysis, the researchers included data from other sources that exploited protein-binding patterns in five cell types. They found that patterns of co-localization among proteins, in which several proteins are found clustered closely on the DNA to govern gene expression, vary according to cell type and the conditions under which the cells are grown.

They also found that many of these clusters can be explained through interactions among proteins, and that not every protein bound to DNA directly.

"We'd like to understand how these interactions work together to make different cell types and how they gain their unique identities in development," Snyder said. "Furthermore, diseased cells will have a very different type of wiring diagram. We hope to understand how these cells go astray."

Other Stanford co-authors include life science research assistant Je Zhai and life science research associate Triupi Kawi, PhD.

The study was supported by the National Human Genome Research Institute. The Department of Genetics also supported the work.

Male birth defect weakly linked to pesticide exposure, study finds

By Louis Berger

A study of several hundred chemicals used in agriculture to produce pesticides has found only weak evidence that any of them are associated with an increased risk of a common birth defect in male infants.

The study, led by epidemiologists at the School of Medicine, analyzed thousands of birth records and commercial pesticide applications from 57 counties of the San Joaquin and Sacramento valleys, which are among the most highly pesticide-used areas in California. The researchers aimed to determine whether children were at increased risk of hypospadias if their mothers had used pesticides while pregnant.

"We didn't see many chemicals that suggested an increased risk, and of those that did, most of them were not consistently found in our data," said Susan Carmichael, PhD, associate professor of pediatrics and lead author of the study published Oct. 28 in Pediatrics. "It is good news that such exposures are rare, but at the same time, when exposures are rare, it makes studies harder to do."

Approximately five of every 1,000 male infants are born with hypospadias, but the cause is usually unknown.

Most previous studies of pesticides and hypospadias focused on risks associated with occupations that involve the use of pesticides. Some studies have suggested slightly increased risks for infants whose mothers work around pesticides, but many studies suggest no association.

The researchers worked with data on births in the counties of Fresno, Kern, Merced, Madera, San Benito, San Joaquin, Stanislaus and Tulare. The Central Valley, composed of the San Joaquin and Sacramento valleys, has one of the highest rates of pesticide usage in the nation.

The study population included all male infants born from 1991 to 2004 to mothers residing in any of the eight counties at the time of birth. The study sample comprised 609 cases of hypospadias, as well as 2,195 controls randomly selected for comparison.

The researchers considered pesticides used within 500 meters of the mother's residence during weeks one to 14 of each pregnancy. Urethral development typically occurs between four and eight weeks after conception.

"Hypospadias has a significant impact on public health, as it often requires surgical correction. Approximately 600,000 to 900,000 American males alive today were born with some degree of hypospadias. Even after correction, individuals may face impaired sexual function and emotional and social difficulties stemming from the condition. More concerning to parents, and a defect in the genital structure often causes special concern," said William Kennedy, MD, associate professor of urology at Stanford and associate chief of pediatric urology at Lucile Packard Children's Hospital.

"Parents are reluctant to talk to anyone—even medical professionals—about the baby's condition," Kennedy added. "This can be very stressful for parents, who worry that their child may have underlying health problems resulting in poor outcomes."

In addition to exposures to individual chemicals, the researchers looked at exposure to multiple chemicals, but found no evidence to suggest that mothers' exposures to multiple pesticides were related.
Joseph Woo tapped to head cardiothoracic surgery

Joseph Woo, MD, a nationally recognized heart surgeon and leading researcher in new approaches to cardiovascular care, has been appointed chair of the Department of Cardiothoracic Surgery at the School of Medicine.

He will start Jan. 1. “Joseph is a distinguished research physician, and educator, who will lead our distinguished Department of Cardiothoracic Surgery to new levels of excellence,” said Lloyd Minor, MD, dean of the School of Medicine. “Stanford Medicine is fortunate to have been able to recruit someone with his talents and vision.”

Philip Oyer, MD, the Roy B. Cohn-Theodore A. Falasco Professor of Cardiothoracic Surgery, has been acting as interim department chair since the former chair, Robert Robbins, MD, left Stanford a year ago to head up the Texas Medical Center in Houston.

Amir Dan Rubin, president and CEO of Stanford Hospital & Clinics, said, “We are so thrilled to welcome Dr. Woo to Stanford, as he has been an innovator in advancing the leading edge of cardiac care while delivering highly coordinated, patient-centered care. Dr. Woo not only has built world-class programs, but has been a role model for treating patients with caring, compassion and consideration — what we at Stanford call C-I-CARE.”

Woo, 46, is currently a professor of surgery at the University of Minnesota, Minneapolis, where he has been on the faculty since 2008 and directs the Minimally Invasive and Robotic Cardiac Surgery Program and the Cardiac Transplantation and Mechanical Circulatory Support Program.

He has a successful career in the operating room, classroom and laboratory. As a surgeon who performs 500 to 400 heart surgeries a year, he has built a thriving research program. His research encompasses basic, translational and clinical projects. His laboratory, funded by the National Institutes of Health, investigates new pathways to myocardial repair through angiogenesis — the process through which new blood vessels form from pre-existing vessels — stem cells and tissue engineering. As an educator, he has mentored many surgeons.

“What we all know is that the complexity of cardiovascular disease is increasing, and we need to improve care. It is a field ripe for innovation. This means we need to ask new questions, rethink the way we do things and find new ways of measuring disease,” said Woo.

“Dr. Woo is an exceptional researcher, clinician and educator, he is passionate in training the next generation of thought leaders in CT surgery. I think he epitomizes the rare breed of cardiothoracic surgeon who’s a triple threat,” said Woo’s predecessor, Joseph Mussallem, MD, PhD, who is now the director of the Minimally Invasive and Robotic Cardiovascular Surgery Program. He has advanced the field of complex valve repair and serves as principal investigator for several clinical device trials and translational scientific clinical trials, such as delivering stem cells during coronary artery bypass grafting and mechanical heart pump implantation.

“I am thrilled to be working closely with him to take this distinct program to even greater heights.”

Woo is married and has two teenage children.

$11.4 million grant launches Center for Collective Cell Decisions

By Molly Sharlach

A new center at Stanford will bring together a diverse group of faculty with a common goal: to understand the complex behaviors within the context of a collective multicellular system. The Center for Collective Cell Decisions has been established with a five-year, $11.4 million grant from the National Centers for Systems Biology, part of the National Institutes of General Medical Sciences.

“Cells do everything in the context of what other cells around them are doing,” said center co-director James Ferrell, MD, PhD, professor of chemical and systems biology, said the new center will “help students and postdocs learn how to do systems biology and make new connections with colleagues who do similar work.”

The center will focus on deciphering the regulation of three fundamental yet complex cellular processes: vision, migration and differentiation, with applications to cancer biology, cell biology and developmental biology, respectively. Cell migration, for example, is vital to the process of healing a wound, as cells must move in a coordinated fashion to properly fill the gap with new tissue.

To create these synchronized activities, groups of cells may employ feedback loops and signaling pathways similar to individual cells. Alternately, cells may draw on a different set of ‘social skills’ to tune their behaviors within the context of a collective multicellular tissue,” Ferrell said.

Established in 2003, the National Centers for Systems Biology program promotes research that draws on mathematics and of biochemistry. “We want to put forward the basic rules and logic of these collective decisions,” said Ferrell.

Systems biology emerged more than a decade ago as biologists realized that studying a single cell, gene or protein in isolation was inadequate to describe the complexity of a biological system. In addition to biology and chemistry, the field draws on mathematics and computer science to understand the circuitry of interacting cells and molecules.

The new center aims to build stronger connections among Stanford researchers and students interested in the field through journal clubs, seminars and annual retreats. Human Diseases, the other co-director and a professor of chemical and systems biology, said the new center will “help students and postdocs learn how to do systems biology by bringing faculty together connecting with colleagues who do similar work.”

“Cells do everything in the context of what other cells around them are doing,” said center co-director James Ferrell, MD, PhD, professor of chemical and systems biology, said the new center will “help students and postdocs learn how to do systems biology and make new connections with colleagues who do similar work.”

The center will focus on deciphering the regulation of three fundamental yet complex cellular processes: vision, migration and differentiation, with applications to cancer biology, cell biology and developmental biology.

Put their babies at an increased risk of hypoxic-ischemic encephalopathy

These results extend what we know, but at the end of the day they need to be part of a bigger picture. We can really be sure whether there is, or is not, a real risk associated with these chemicals,” said Gary Shaw, PhD, professor of pediatrics and of environmental health sciences. Shaw and his colleagues have been involved in the study of how ambient pollutants influence human health for more than a decade. They have been investigating whether the environmental pollutants of concern are embroiled in the pathogenesis of hypoxia-ischemia in the newborn. Hypoxia-ischemia is the injury to the brain that occurs when there is too little oxygen reaching the brain tissues in the blood supply. It results in poor neurological outcomes and is the leading cause of non-traumatic death in children. Prevention of hypoxic-ischemic encephalopathy is possible through better understanding of the mechanisms that underlie the development of brain injury.

Put their babies at an increased risk of hypoxic-ischemic encephalopthy

“Cells do everything in the context of what other cells around them are doing,” said center co-director James Ferrell, MD, PhD, professor of chemical and systems biology, said the new center will “help students and postdocs learn how to do systems biology and make new connections with colleagues who do similar work.”

The center will focus on deciphering the regulation of three fundamental yet complex cellular processes: vision, migration and differentiation, with applications to cancer biology, cell biology and developmental biology.

Put their babies at an increased risk of hypoxic-ischemic encephalopothy

“Cells do everything in the context of what other cells around them are doing,” said center co-director James Ferrell, MD, PhD, professor of chemical and systems biology, said the new center will “help students and postdocs learn how to do systems biology and make new connections with colleagues who do similar work.”

The center will focus on deciphering the regulation of three fundamental yet complex cellular processes: vision, migration and differentiation, with applications to cancer biology, cell biology and developmental biology.

Pesticide continued from page 2

put their babies at an increased risk of hypoxic-ischemic encephalopathy

“Cells do everything in the context of what other cells around them are doing,” said center co-director James Ferrell, MD, PhD, professor of chemical and systems biology, said the new center will “help students and postdocs learn how to do systems biology and make new connections with colleagues who do similar work.”

The center will focus on deciphering the regulation of three fundamental yet complex cellular processes: vision, migration and differentiation, with applications to cancer biology, cell biology and developmental biology.
Program celebrates seven years of funding med-tech projects

By Kris Newby

Members of Stanford’s medical technology community gathered recently to celebrate the 100th birthday of the late inventor Wallace Coulter and his foundation’s support of biomedical innovation at the university.

During the first seven years of the Wallace H. Coulter Translational Research Grant Program at Stanford, its 47 sponsored projects have resulted in 21 patents and 10 venture-funded start-up companies.

“This program has succeeded in giving young entrepreneurs the confidence to keep going,” said Stanford President John Hennessy. He was joined onstage Sept. 20 at the Hass Plattner Institute of Design by Lloyd Minor, MD, dean of the medical school, as well as by a group of Coulter-supported inventors and the CEOs working to bring medical technologies to market.

Each of the entrepreneurs spoke about their personal challenges in moving discoveries from the idea stage to patents. This process, called the “translational pipeline,” typically takes four years or more for medical devices because of the difficulty of clinical testing, obtaining regulatory approvals and acquiring early-stage venture funding.

Also attending was Elias Caro, vice president of technology development at the Coulter Foundation, who presented a plaque to Stanford commemorating the 100th birthday of the late inventor Wallace Coulter and his medical technology community.

An electrical engineer and a basement tinkerer with 85 patents, Coulter was no stranger to the translational pipeline. In the 1940s, he invented a method and a device for counting red blood cells and white blood cells in whole blood, a ubiquitous diagnostic test. The method also is now used for quality control in the pharmaceutical, biotechnology, food, beverage and consumer industries.

Coulter ultimately founded a diagnostics company, Coulter Corp., to market his many practical inventions. In 1998, his company was acquired by Beckman Instruments, and, after his death, the Coulter Foundation was established to carry on Coulter’s legacy of bringing useful biomedical inventions to market to improve patient care.

Today, the Coulter program at Stanford awards up to $100,000 to collaborative teams who use bio-engineering methods to tackle pressing medical problems. In addition to providing early-stage funding for these projects, the faculty in the Stanford Biodesign program, Bio-X initiative, Bioengineering Department and Center for Clinical and Translational Education and Research (Spectrum) provide these teams with the advice needed to navigate the difficult process of moving new inventions into everyday patient use.

For details on applying for Coulter awards, visit http://bioengineering.stanford.edu/coulter/grantsinfo.html. The application deadline for the next round of awards is Jan. 17. To qualify for funding, teams must include a physician and a biomedical engineer.

Kris Newby is the communications manager for Spectrum, the Stanford Center for Clinical and Translational Education and Research.

Venom continued from page 1

Oct. 24 in Immunity, the researchers showed that mice injected with a small dose of bee venom were later resistant to a potentially lethal dose of the same venom. The study is the first experimental evidence that the same immune response involved in allergies may have served to evolve a protective role against toxins.

The study builds on earlier work by the researchers characterizing the innate immune response to snake venom and honeybee venom. Innate immune responses occur in subjects exposed to a foreign substance, such as a pathogen or a toxic material like venom, for the first time. Immune cells called mast cells, which reside in most of the body’s tissues, are poised to unleash signals that turn on defense responses when a pathogen or toxin intrudes. In a previous study, the researchers found that mast cells produce enzymes that can detoxify components of snake venom, and that mast cells can also enhance innate resistance to honeybee venom.

Such innate immune responses do not require prior immunization or the development of specific antibodies. By contrast, during an adaptive immune response, the immune system generates antibodies that recognize the invading pathogen or toxin; this process makes it possible to vaccine against infectious diseases. Adaptive immunity is usually a faster, more specific and more effective form of defense than innate immunity.

In allergic reactions, a type of antibody called IgE binds to the surface of mast cells and prompts them to initiate an adaptive immune response when exposed to the antigen recognized by that IgE. “The functions of IgE and mast cells are mostly known in the context of allergies,” said Thomas Marichal, DVM, PhD, a postdoctoral scholar and co-lead author of the study.

“It was kind of a dogma that most IgE-related responses are detrimental,” said postdoctoral scholar Philipp Starkl, PhD, the other lead author. “We and others speculated that there should be some very positive evolutionary pressure to keep these cells and these anitbody responses because if they were just bad and deleterious, they would have been eliminated.”

The researchers hypothesized that IgE might be required for protection against a lethal sting, and that allergies are an extreme, and maladaptive, example of this type of defense. This idea, known as the toxin hypothesis of allergy, was first proposed by Magie Profet in 1991, but was largely ignored by immunologists until recently.

To find out whether adaptive immune responses could help mice resist bee venom, Marichal and Starkl first injected mice with a low dose of venom, equivalent to one or two stings. The mice developed more venom-specific immune cells, and higher levels of IgE antibodies against the venom, than control mice injected with a salt solution.

Three weeks later, they injected both groups of mice with a potentially lethal dose of venom, similar to five bee stings. The immunized mice had less hypothermia and were three times more likely to survive than the control mice. Moreover, they did not develop the anaphylactic reaction characteristic of severe allergies.

To determine whether IgE antibodies were required for this protection, the team injected mice with several types of mutations: mice without IgE, mice without functional IgE receptors on their mast cells, and mice without mast cells. The IgE-deficient mutant mice were previously developed by Hans Oertgen, MD, PhD, associate professor of pediatric immunology at Harvard Medical School and a co-author of the study.

In all three groups of mutant mice, pre-immunization with a low dose of bee venom did not confer protection against a lethal dose, suggesting that the protection depends on IgE signaling and mast cell activation. “That was pretty exciting for us,” said Marichal. “It was the first time we could see a beneficial function for these IgE antibodies.”

Pre-immunization with a low dose of venom from the Russell’s viper also protected mice from a higher dose of venom from this snake, which is one of the “big four” species responsible for most snakebite deaths in India. So the researchers believe the response could be generalized to different types of toxic venoms.

“Our findings support the hypothesis that this kind of venom-specific, IgE-associated, adaptive immune response developed, at least in evolutionary terms, to protect the host against potentially toxic amounts of venom, such as would happen if the animal encountered a whole nest of bees, or in the event of a snake bite,” said Stephen Galli, MD, professor and chair of pathology and the co-senior author of the study. “Anaphylaxis probably represents the extreme end of a spectrum of IgE-associated reactivity, which in some unfortunate individuals is either poorly regulated or excessively robust, so the reaction itself can become dangerous to them.”

Galli cautioned that it’s not yet known whether IgE responses also protect humans from the toxic effects of arthropod or reptile venom, but it would be unthinkable to test lethal doses of venom in humans. Reptile and arthropod venoms are complex chemical cocktails. Some venom components have evolved to mimic chemicals made by the human body, while others are called conotoxins, which cause blood vessels to constrict during bacte- rial infections. At the same time, mammals have evolved immune responses to venoms that have evolved to incapacitate into maladaptive allergic reactions.

“We experience allergies in a much cleaner world, where we don’t have the same threats of venomous creatures and potentially toxic food that existed for much of our evolutionary history,” said Galli. “And so we’re left with this residual type of reactivity that seems completely mysterious and pointless and harmful. This is the first evidence, that we know of, indicating that IgE-associated ‘allerg-ic-type’ immune responses can actually reduce the toxicity of naturally occurring venoms.”

Other Stanford co-authors were instructor Laurent Lionel Reber, PhD; Janet Kalesnikoff, PhD, associate director of the Cardiovascular Institute; senior research scientist Mindy Tsai, DMSc; and Martin Metz, MD, PhD, a former post-doctoral scholar at Stanford and co-senior author of the study, now a professor of dermatology and allergy at Charité-Universitätsmedizin Berlin.

The study was funded by the German 1 Research Foundation, the National Institutes of Health, a Marie Curie International Fellowship, the Max Kade Foundation, the Austrian Academy of Sciences and the Austrian Science Fund. The Department of Pathology also supported the work.

Molly Sharlock is a writing-intern in the medical school’s Office of Communicating Public Affairs.
First-year anesthesia resident Jason Johns, MD, was pleased with the way his patient, a man in his early 70s, responded to his virtual signs during a four-hour surgical procedure at Stanford Hospital. “We were monitoring his blood loss and his numbers looked OK,” he said, but when he saw him up, his blood pressure dropped and he went into cardiac arrest.

In the minutes that followed, Johns and his colleagues were surprised they knew should be done immediately. They turned the man on his back, began chest compressions and administered the standard medical treatments. Johns also did something else as soon as those basic procedures were under way. “I grabbed the emergency manual and started reading off things,” he said. “You make sure you’ve covered your bases and done everything.”

Johns had trained briefly with the manual in simulation settings. He’d also read the emails reminding him and other Stanford medical personnel of its presence in the operating rooms. When the moment came that an unexpected cardiac arrest threatened the life of his patient, he reached for it.

That manual — with its laminated pages and brightly-colored graphic design, hung by a sturdy metal chain within easy reach of any member of a surgical team — represents medicine’s next step in using cognitive aids to support practitioners in doing the best job possible. “In medicine, where stress is a constant and knowledge is ever-expanding,” Johns said, “there’s always a part of you that wonders, ‘Have I thought of everything?’”

The value of such cognitive aids is detailed in a paper published in the November issue of *Anesthesia & Analgesia*. Johns and David Gabai, MD, professor of anesthesia and associate dean for immersive simulation, described the work of multiple Stanford teams that developed and implemented the emergency manual through a combination of research, training and feedback based on practical use. The emergency manual is now in use at Stanford and other hospitals.

It covers protocols for 24 conditions and circumstances. Some, like how to deal with a patient’s bradycardia (a slow and unstable heartbeat), will be familiar only to medical professionals. Others, like how to handle a hospital-wide power failure, address what to do first, and thereafter, in such circumstances.

In an editorial accompanying the Stanford paper and another on the same topic, David Gaba, MD, professor of anesthesia and associate dean for immersive simulation and simulation-based training at the School of Medicine, makes the connection between the value of cognitive aids and the spreading adoption of relevant crisis checklists in medicine. His value, Gaba wrote, transcends the pejorative nicknames, like cheat sheets, and crib sheets. While in the past the use of these aids was viewed as a sign of weakness and stupidity, many of the literature, he noted, to strong trainees and ex-perienced clinicians that their use is actually a sign of strength and wisdom and that failing to use them is a sign of weakness and perhaps hubris.

Sara Goldhaber-Fiebert, MD, lead author of the study and clinical assistant professor of anesthesia at Stanford who helped develop and implement the manual, said cognitive aids help medical teams efficiently and effectively deliver optimal care to their patients during critical events. “The emergency manual helps teams fill the gap between what they have done and what their patient needs,” she said. “The goal is not to replace vital clinical actions, getting appropriate help or exercising good clinical judgment. Clinicians who have trained on why and how to use emergency manuals in teams can find helpful information quickly and adapt their actions to ensure the best care.”

For Johns and David Gabai, the development of the manual was an immersive training module in cognitive aids and emergency manuals. Their value, Gaba wrote, transcends the pejorative nicknames, like cheat sheets, and crib sheets. While in the past the use of these aids was viewed as a sign of weakness and stupidity, many of the literature, he noted, to strong trainees and experienced clinicians that their use is actually a sign of strength and wisdom and that failing to use them is a sign of weakness and perhaps hubris.

“Remembering nine out of 10 actions for 10 minutes is not enough to meet the life for the patient who has the one in prob-lem,” said the paper’s senior author, Steven Howard, MD, Stanford associate professor of anesthesia. “Our memory is imperfect, more so under stress and that’s the reason why emergency manuals, cognitive aids and checklists are critical.”

While some cognitive aids for a handful of medical situations are in common use, the emergency manual developed by the Stanford Anesthesia Cognitive Aid Group — whose core members, in addition to Goldhaber-Fiebert, Gabai and Howard, are Larry Chu, MD, associate professor of anesthesia, and Kyle Harrison, MD, clinical assistant professor of anesthesia — provides a comprehensive pass at building a reference of responses to periprocedural critical events.

The development group examined every aspect of the emergency manual, down to details whose importance might not seem immediately obvious, including the colors, typefaces, boldfacing of words, size of pages, binding and physical placement within a working space. Over and over, the implementation team tested the manual in simulation with a full medical team, refining elements based on feedback from its users. Both its wording and design went through many versions to reach the goal of allowing time-pressed teams to deliver optimal care efficiently. It will continue to evolve as feedback from clinical use is gathered, Goldhaber-Fiebert said.

“We learned from simulation testing that it is not enough just to put the manual in the OR,” Goldhaber-Fiebert said. “We need to train people to use it. The ‘what’ is not enough. By training with these manuals, people reinforce their knowledge of both content and format. They learn how to efficiently access potentially lifesaving information and why using a manual like this is a good idea.”

The implementation team — which included anesthesiologists, nurses, surgeons, anesthesia technicians, hospital leadership and others — led accessibility and feedback sessions. Those simulations helped the team decide to hang the manual from a 6-foot beaded chain on a hook on the side of the anesthesia cart. Soon after, OR nurses requested a second copy be hung near the nursing phone. Computer and anesthesia technicians wanted copies where they work to help them anticipate the equipment that might be needed during specific emergencies.

Beyond the knowledge that is reinforced, “just the act of training to use the manual promotes team building and communication, which is critical not just in emergencies, but all the time,” said Bryan Bohman, MD, the former chief of staff at Stanford Hospital and an anesthesiologist who contributed to the implementation of the Stanford manual.

The Anesthesia & Analgesia paper also addresses other key elements in the best use of emergency manuals in combination with teamwork concepts such as leadership, roles and responsibilities, communication within the team, and planning. Such issues are not new: Stanford has had a simulation-based course in anesthesia-crisis resources for more than 20 years that included cognitive aids or emergency manuals. In recent years, the Anesthesia Department has incorporated an immersive training module in cognitive aids and emergency manuals. This type of training is gradually being expanded to include all personnel in Stanford’s operating rooms. A 2006 study led by Harrison found teams that consulted a cognitive aid performed better in the critical actions and did so more quickly than teams working only from memory.

Stanford is not alone in its efforts to develop emergency manuals. In 2012, members of the Stanford Anesthesia Cognitive Aid Group joined with other clinician groups to form the Emergency Manual Implementation Collaborative at a national meeting of the American Society of Anesthesiologists. Goldhaber-Fiebert is a member of that collaborative, as are Howard and Gabai, who was a key contributor in crafting and testing the manual. The collaborative is dedicated specifically to the adoption of crisis checklists in many acute care areas, focusing first on the operating room and related sites.

Cognitive aids and references are on the collaborative’s website at http://www.emergencymanuals.org, and the Stanford emergency manual can be downloaded for free at http://emergencymanualstanford.edu. Since becoming available last spring, more than 3,500 people have downloaded a copy, including many hospital leaders and clinicians.

Johns, now a second-year resident, has grown in confidence, but still sees the manual as a key resource. “Having the manual is reassuring, especially when you’re going through these catastrophic events,” he said. “There’s always a certain part of you that wonders, ‘Have I thought of everything?’”

Sara Wykes is a writer for the Stanford Hospital & Clinic communications office.

See story on page 20...
The catch, though, said Tibshirani, is that journals sometimes decline to publish corrections, or when they publish them they limit the length. Explaining a problem in a few hundred words can be very difficult, he said.

A further hitch is the lack of a centralized location for comments. With more than 20,000 biomedical journals in existence, it’s not realistic to expect many people to notice when a correction is made.

In fact, once a correction is made, it’s often ignored. Research by John Ioannidis, MD, PhD, professor of medicine at Stanford, has documented research papers that have been discredited, yet were cited for years afterward. “If the research was published in a high-ranking journal, it’s considered the truth. The culture is that it’s set in stone,” Tibshirani said.

In some ways, it’s surprising that the leaders of the National Institutes of Health have allowed comments to go forward, Tibshirani said. He knew there was considerable concern that negative comments could harm the reputation of NIH-funded research or be used to attack competitors, and that comments both positive or negative could have financial ramifications.

To keep commenters responsible and to make any potential conflicts of interest transparent, no anonymous comments are allowed, and commenting is restricted to the scientific community, at least during the pilot period, Tibshirani said.

“Getting the go-ahead for even this much openness was not simple.”

When Tibshirani first brought up the idea with Brown, they went into Brown’s Stanford office and skyped David Lipman, MD, director of the National Center for Biotechnology Information, to see if he thought it was feasible. In fact, Lipman had been thinking about doing something like this for nearly a decade: “But they asked how we could manage it, and until recently I didn’t think it would get the support of NIH leadership.”

But he told them he thought it was worth a try: “It helped that the request was coming from out of the community, not within. It’s a long shot.”

It also helped that Brown is an older friend of the director of the National Cancer Institute, Harold Varmus, MD. Brown presented the idea to Varmus, who also liked it.

So in September of 2012, Lipman proposed the site to the NIH steering committee, consisting of NIH director Francis Collins, MD, PhD, and 10 other directors from various institutes, and got the go-ahead to develop the idea.

“At that point, I realized I’m not going to develop the idea,” Lipman said. “Rob and Pat should organize themselves and get others involved. Much of the discussion should take the lead. I wanted to see if there’s an active group of people who care enough about it.”

Tibshirani became the community organizer and worked with Brown to involve about 500 people to discuss the ground rules and begin using an early version of the site. “We hashed over the basic ideas such as the level of inclusiveness—Do you allow anonymous comments? And we began commenting,” Tibshirani said. They had posted just over 100 comments to the site by early October.

Anonymity was the most contentious issue, Tibshirani said. Anonymity’s big draw is that it would allow scientific critics to engage without jeopardizing their own careers. Ivan Oransky, MD, president of MedPage Today and co-founder of RetractionWatch, argued that anonymity was not enough. “There is no anonymity without accountability,” he said. “If you publish anonymous comments, you would not have the quality of the interchange be high.”

So far, we have, for now, to identify ourselves.

Lipman said, “In the beginning, it’s balancing inclusiveness and relevance.”

You want to have as many people as possible contribute but want to say to be in there. That obviously includes more people than who have had papers published in PubMed. But who do you draw the line? The thinking for now is if I want to be able to comment on your paper, you should be able to comment on my paper,” he said.

Ultimately, the organizers hope to allow the public general to view comments, but not PubMed Commons and register to post them, as well.

Herzenberg continued from page 1

planning the next set of experiments.

“Wealth the Herzenbergs, tens of thousands of people for about $14,000. They published their Science in 1969. They brought back to the University of California, San Francisco, where they developed a machine to sort cell-sized particles by volume, and it was a success. Not only did he change forever how cell biology is done, he was an inspiring teacher, a deep thinker and a man of high moral conduct,” said Hiroshi Lipman. 

Leonard Herzenberg was best known in the scientific arena for developing, in collaboration with his wife, the fluorescence-activated cell-sorter. Like a coin sorter that separates a jumble of change into neat stacks of quarters, nickels, dimes and pennies, the FACS sorts cells according to fluorescent tags attached to their surfaces and 1,000 times faster than the human eye can process.

Researchers can couple the fluorescent tags to antibodies that home in on and attach to molecules produced only by cells of certain cell types. Because we now have millions of rare immune stem cells for further study, or identify stem cells and other populations of cells that are waxing and waning in conditions such as HIV. The possibilities of the technology, also known as flow cytometry, are limited only by the creativity of the users.

"Len was an amazing person in many respects," said Michael Snyder, PhD, a professor and chair of genomics at Stanford. "Scientifically, he was a giant. The FACS technology was transformative to many fields, and it is still largely used today 40 years after its development and dissemination.

But beyond that, and he and Lee have been just fantastically warm and outgoing people who helped make our department feel a family. I feel a lot of Otronino gratitude to this."

The scientific problem in the 1960s that initially set Leonard Herzenberg on his path toward the cell sorter was pretty mundane: His eyes hurt. “I was sitting in the lab one day counting immunofluorescent cells under the microscope, and I said, ‘There’s got to be some kind of machine that can do this.’” He discovered that scientists at Los Alamos National Laboratory, in New Mexico, had developed a machine that could count cells volume in order to analyze the lung contents of mice and rats exposed to fallout from atomic bomb testing.

With characteristic zeal, he headed to New Mexico. With Francis Collins, MD, PhD, and 10 other members he convinced Russ Hulett and William Bonner, two engineers in the laboratory of Genetics Department chair J edward Lipman, MD, Ph.D, to help him modify the Los Alamos plans. Together, they and their colleagues cobbled together the predecessor of the first FACS, dubbed “The Whizzer,” in the basement of the medical school for about $14,000. They published their success in Science in 1969.

By the early 1970s, the group had incorporated a laser to make the machine brighter, and added members of the team invited the inventor of the ink-jet printer, Dick Swett, to hone the machine’s droplet-sorting technique. By 1973’s first FACS and then with the Whizzer backed up to Stanford.

During Tibshirani’s discussions with researchers as the site was being developed, he learned that many were worried about its repercussions, or just didn’t see the point. “They say you could trash someone’s reputation,” Tibshirani said.

But is that so bad? “My thought is if someone publishes something really wrong, maybe their reputation shouldn’t be trashed but it should be at least tarnished. Should the facts not come up? I think yes, they should. It’s in everyone’s interest.”

From Lipman’s perspective, positive comments are of even more interest than corrections or comments. “In the case of articles that interest me in all the articles that are being published. One way is to find out how interesting you thought the article was good,” he said. “If I could have a growing number people to follow, and they recommend even just one paper a month, I think that would be fantastic.”

During Tibshirani’s discussions with researchers as the site was being developed, he learned that many were worried about its repercussions, or just didn’t see the point. “They say you could trash someone’s reputation,” Tibshirani said.

But is that so bad? “My thought is if someone publishes something really wrong, maybe their reputation shouldn’t be trashed but it should be at least tarnished. Should the facts not come up? I think yes, they should. It’s in everyone’s interest.”

Len and Lee were just the personal friends of the director of the National Cancer Institute, Harold Varmus, MD. and is still regularly used today 30 years after its development. The FACS technology was transformative to many fields, and is still largely used today 40 years after its development and dissemination.

But beyond that, and he and Lee have been just fantastically warm and outgoing people who helped make our department feel a family. I feel a lot of Otronino gratitude to this."

The scientific problem in the 1960s that initially set Leonard Herzenberg on his path toward the cell sorter was pretty mundane: His eyes hurt. “I was sitting in the lab one day counting immunofluorescent cells under the microscope, and I said, ‘There’s got to be some kind of machine that can do this.’” He discovered that scientists at Los Alamos National Laboratory, in New Mexico, had developed a machine to sort cell-sized particles by volume in order to analyze the lung contents of mice and rats exposed to fallout from atomic bomb testing.

With characteristic zeal, he headed to New Mexico. With Francis Collins, MD, PhD, and 10 other members he convinced Russ Hulett and William Bonner, two engineers in the laboratory of Genetics Department chair J edward Lipman, MD, Ph.D, to help him modify the Los Alamos plans. Together, they and their colleagues cobbled together the predecessor of the first FACS, dubbed “The Whizzer,” in the basement of the medical school for about $14,000. They published their success in Science in 1969.

By the early 1970s, the group had incorporated a laser to make the machine brighter, and added members of the team invited the inventor of the ink-jet printer, Dick Swett, to hone the machine’s droplet-sorting technique. By 1973’s first FACS and then with the Whizzer backed up to Stanford.
covery,” said Stanley Cohen, PhD, professor of genetics and the Kwoh-Ting Li Professor. “It was a time of women’s equality — he and his associates were able to achieve the conversion within nine days with an efficiency of 37 percent, as opposed to the vastly lower yield obtained with the prior method (12 percent) or using iPS cells. (Peltz said improvements since the study’s publication now enable yields exceeding 50 percent within seventy days.)

Dan Xu, PhD, a postdoctoral scholar and the study’s lead author, adapted the spherical culture methodology from early embryonic-stem-cell literature. Instead of using a biocompatible and bioactive dish, the human induced-pluripotent stem cells were cultured in a liquid suspension in which they form spheroids. “This seems to make the appropriate microenvironment for i-Heps.”

When they had enough cells, the investigators tested them by injecting them into immune-deficient laboratory mice that accept human grafts. These mice were bioengineered in 2007, in a collaboration between Pelz’s lab and study co-author Toshihiko Nishimura, MD, PhD, and other scientists at the Tokyo-based Cen-

tury. “In the 1970s, the message to women scientists was that you couldn’t expect to have a family and be a scientist.”

Len when they were both graduate students at Caltech. “Leonard Herzenberg was born Nov. 5, 1931, in Brooklyn, N.Y. He attended Brooklyn College, where he met Leonore, and earned a bachelor’s degree there in 1952 in biology and chemistry. He then attended graduate school at the California Institute of Technol-

“...choosing,” he remarked, to “carry a pipette rather than a gun for my country.” There, he learned how to grow mammalian cells in the laboratory of eminent pa-

thologist and mammalian cell biologist Harry Eagle. Never had the School of Medicine been so vibrant or fruitful. “Len and the Herzenbergs have trained hundreds of scientists.”

The Herzenbergs’ years at Stanford were marked by extraordinary cross-disciplinary collaborations. In the late 1970s, the laboratory of David Baltimore and the Herzenbergs began working together on the production of monoclonal chimeric antibodies, each a blend of mouse biochemist Paul Berg, PhD. Their goal was to create a antibody that would be more readily accepted by the human immune system when used to treat rheumatoid disease and many other conditions, and the patent stands as the most profitable ever

At the time of Herzenberg’s graduate studies, the small genetics department at CalTech included seven future Nobel laureates: George Beadle, Max Delbruck, Ed LENI, Robert Hubel, Roger Sperry, James Wat-

son and Barbara McClintock. Two-time Nobel prize recipient Linus Pauling had a lab nearby. “Creative postdoctoral scholars, Vernon Oi, began collaborating with Leonore, and earned a bachelor’s degree there in 1952 in biology and chemistry. He then attended graduate school at the California Institute of Technol-

ogy, where he earned a PhD in 1955 in biochemistry and molecular biology.

At the time of Herzenberg’s graduate studies, the small genetics department at CalTech included seven future Nobel laureates: George Beadle, Max Delbruck, Ed LENI, Robert Hubel, Roger Sperry, James Wat-

The Herzenbergs also became politically active at the time, working with Pauling and others to start a lo-

The Stanford team knew it was possible, though. Already, two months after injection of i-Heps, the transplanted cells had integrated into the liver, expressed human bile duct formation. Other tests indicated that the spherically cultured i-Heps resembled natural human liver. “Blood tests also revealed that the mice’s new liver tis-

ue was discharging its waste-filtration responsibility. Also, the mice produced human bile, and transplanted cells had integrated into the liver, expressed human bile duct formation. Other tests indicated that the spherically cultured i-Heps resembled natural human liver.”

Importantly, two months after injection of i-Heps produced by spherical culture, there was no evidence of tumor formation. But mice in which IPS-cell-originated i-Heps were introduced developed multiple tumors, which could be felt through the body surface within three weeks. At 1,500 grams, a healthy human liver is more than 800 times the size of a mouse’s and contains about 200 billion cells. “To be successful, we must regenerate about half of the human liver,” said Pelz. “With spherical culture, he said, close to a bil-

ion injectable i-Heps can be produced from 1 liter of medium, and yields have been greatly improved with pro-

duction procedures. The cell replication that takes place after injection expands that number further, to over 100 billion cells.

That could be enough to substitute for a human liver transplant, Pelz said. Stanford’s Office of Technology Licensing has filed a patent on the use of spherical cul-

tures for hepatocyte induction. Pelz’s group is optimizing the culture and injection techniques, talking to the U.S. Food and Drug Administration, and gearing up for tests in living mice. The new method could be ready for clinical trials within two to three years, he estimated.

International Stanford co-authors were Jeffrey Glenn, MD, PhD, associate professor of medicine; Sara Michie, MD, professor of pathology; Gordon Lee, MD, professor of medicine and pediatrics; and Neesa Cash and his colleagues conducted the first clinical trials of oral rehydration therapy in adult cancer patients with diarrhea caused by infectious diseases. His other interests include research ethics and the scale-up of health pro-

grams in low-income countries.

Cash will be in conversation with Paul Costello, chief communications officer at the medical school, Stephen Luby, MD, professor of medicine and pediatrics, and the Stanford Center for Innovation in Global Health, will in-

roduce Cash. The event, which is free and open to the pub-

lic, is part of the Conversations in Global Health series organized by Center for Innovation in Global Health.

“Len’s influence will continue to be felt for decades in our department and around the world, extending even to people who never met him,” said Snyder, chair of the Genetics Department, who noted that the Herzen-

bergs have trained hundreds of scientists. One of them, Paula Kavathas, PhD, is a professor of laboratory medicine and immunology at Yale Uni-

versity. “In the 1970s, the message to women scientists was that you couldn’t expect to have a family and be a scientist. Len, however, encouraged me at a high level.”

“Len made you feel good — made you want to engage with him and talk with him. He and Lee were remarkable team and a very, very special couple.”

Leonard Herzenberg is survived by four children — Beri, Jana, Michael and Eric (Rick) — and four grandchildren.

Plans for a memorial symposium will be announced at a later date. The family requests that, in lieu of flowers, donations in Len’s memory be made to the Len and Leonore Herzenberg Fund at the Department of Medicine. Gifts may be sent to Stanford Medical Center Development, #3172 Porter Drive, Suite 210, Palo Alto, CA 94304, or made online at http://medi-calging.stanford.edu.

The event, which is free and open to the pub-
lic, is part of the Conversations in Global Health series organized by Center for Innovation in Global Health.
Cameron Bozdog, of Atherton, was happy and active. The 13-year-old enjoyed running, swimming and playing soccer. Then, her left leg started hurting. It wasn’t just any old hurt, but a hurt that eventually became so bad that she couldn’t even put a sock on.

It turns out Cameron was entering the mysterious world of complex regional pain syndrome, or CRPS — an often-sudden condition in which the brain registers severe, unmitigating pain from a limb, even when no injury or trauma is apparent.

CRPS is difficult to diagnose and tough to treat, especially in children. “We don’t know exactly what causes it,” said Dr. Karen Kane, MD, director of the Pediatric Pain Management Program at Packard Children’s and professor of anesthesiology at the School of Medicine. “However, it’s likely that the inflammation of the spinal cord and brain that are misinterpreted as pain in what should be a normal limb.”

That’s why Cameron came to Packard Children’s, home to the largest and most successful pediatric pain management program in California. In collaboration with the hospital’s Pediatric Pain Rehabilitation Center, Cameron was successfully treated with a get-your-life-back protocol that has worked for hundreds of kids visiting the program from throughout the United States.

At first, her doctors thought the pain could stem from inflammation of the tendons in the heel. But walking boots and braces were not fixing the problem. So she was referred to the specialist at Packard Children’s. “By the time we saw Cameron in April, she was definitely starting to show signs of complex regional pain syndrome,” said Brenda Golianu, MD, associate professor of medicine and the Department of Pediatrics, and is medical director of the Pediatric Pain Rehabilitation Center. “Cameron was treated five days a week, eight hours a day. Intense! Yes. “Each day included several hours of physical, occupational and aquatic therapy,” said Sarah Jomá, the rehabilitation therapist, who noted that at first Cameron could not put her foot in the water because of pain from the sensation. Additionally, there were psychological counseling and family therapy sessions, plus lots of work on distraction techniques.

The team’s goal was to desensitize the limb experiencing CRPS, which would rewire the misfired nerve signals being sent to the brain. “We make this happen by retraining the brain and working through the things that are causing pain,” Niswonger said. For Cameron, this included gradually walking on grass, standing on the foot and other activities that would encourage the process of recalibrating the nerves. Then, a big day. On Aug. 14, Cameron and Niswonger took a four-block walk to a local market, a trek that a few weeks earlier seemed impossible. “That was huge,” said Roxanne, the girl’s mother.

Despite several months of incredible pain, Cameron stayed focused on recovery. “I was kind of scared,” she said, “but I did not want to be like this the rest of my life. I tried to be confident every day and persevere.”

“Their lives are in the hands of the medical professionals, but it turns out a lot of their healing comes from their own determination,” Golianu said.

At the painful journey, mom saluted her daughter and an experienced care team that made family life normal again.

“They said Cameron would eventually walk out of there wearing shoes and socks again,” recalled Roxanne, who brought Cameron some new Nikes for the occasion. “They were right.”

Robert Dicks is the senior media relations director for Lucile Packard Children’s Hospital.