

SCIENTISTS WORK FURIOUSLY TO TACKLE COVID-19

BY RUTHANN RICHTER

When the SARS-CoV-2 coronavirus spread throughout the world last year, triggering a global pandemic and a university-wide shutdown, scientists across the Stanford University campus had to curtail their lab operations—and think creatively about how to carry on their research. Those at the Beckman Center for Molecular and Genetic Medicine proved nimble at adapting, with many quickly turning their attention to the new viral threat.

Indeed, over the last several months, despite the many limitations imposed by the pandemic, research on SARS-CoV-2 flourished at the Beckman Center and its affiliated labs. Many scientists pivoted their research efforts to exploring how the virus acts in the body to cause the disease known as COVID-19, and to developing innovative potential solutions.



LUCY SHAPIRO, PH.D.

Virginia and D.K. Ludwig Professor of Cancer Research
Director, Beckman Center for Molecular and Genetic Medicine

“The response of the Stanford community has been one of great responsibility, as well as dedication, in very challenging times, where our labs have been downsized and people have been forced to work difficult schedules, essentially doing shift work,” said Lucy Shapiro, Ph.D., the Virginia and D.K. Ludwig Professor of Cancer Research and director of the Beckman Center.

“Even in the face of very difficult working conditions, the desire to help and to take projects to fruition has been incredibly impressive.”

The Beckman Center’s mission—for more than thirty years—has been to focus on basic science, with an eye to developing solutions for the betterment of humanity. In the COVID-19 pandemic, that mission became an urgent one, with the pressure of finding immediate solutions to save as many lives as possible.

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The results of these concentrated SARS-CoV-2 research efforts have been exceptional. Over the last few months, Stanford scientists developed new vaccine candidates in record time, created a new model for testing vaccines, and devised a unique approach to preventing infection. They also conducted intensive studies on the impact of SARS-CoV-2 on the immune system and on the lungs, which are a major target of infection. Many of these research programs built on years of earlier basic research.

Throughout this time, the scientists have been able to take advantage of Beckman’s technology service centers, which continued to operate during the pandemic under strict safety protocols.

“The fact that we were able to continue and provide these services, again through very difficult times, is a testament to the people who direct the service centers,” Dr. Shapiro said. “They have consistently responded to every request. It’s also important to acknowledge all the people who work at the lab benches, at the lab centers, and at the service centers, as well as the principal investigators who had the vision and stamina to see where we must go, not only as a scientific community, but as an economy to help protect global health.”

THE HUNT FOR AN IDEAL VACCINE

For decades, Peter S. Kim, Ph.D., the Virginia and D.K. Ludwig Professor of Biochemistry, has focused on creating vaccines to fight some of the world’s major scourges—HIV, Ebola, and pandemic flu. When the coronavirus struck, he paused that work and his lab immediately shifted its attention to the new foe. Dr. Kim’s experience in vaccine development suggested

this could be a huge challenge: Scientists can spend up to a decade developing a vaccine and sometimes, as with HIV, they may not succeed at all.

In a matter of months, however, Dr. Kim’s team was able to produce a vaccine prototype for SARS-CoV-2



PETER S. KIM, PH.D.

Virginia and D.K. Ludwig Professor of Biochemistry

that is effective in mice and has several advantages over the vaccines currently in use. He said he hopes to build a vaccine for global distribution—a simplified version that can be delivered in one dose, rather than two, that doesn’t require refrigeration, and is inexpensive to make.

“As with the Ebola vaccine, our goal is to develop a vaccine that will be a single shot, because in many places around the world, recalling somebody a month later is fraught with logistical issues,” Dr. Kim said. “We also want to create a vaccine that is stable at room temperature. Even in this country, having to deal with frozen vaccine is creating all sorts of issues. Just imagine trying to do that in low- or middle-income countries. If we want to solve this pandemic, we have to solve it on a global scale.”

Dr. Kim's prototype takes advantage of the unique crown-like spikes on the surface of the SARS-CoV-2 coronavirus (corona is Latin for "crown"). These spike proteins are crucial to the virus, as it uses them to attach to a cell and gain entry, so it can use the cell's machinery to reproduce. The spike proteins, however, can also be used as antigens—molecules that trigger an immune response in the body.

To deliver the spike proteins, the researchers relied on a ferritin-based system originally developed at the National Institutes of Health and known to be safe in humans, as it was tested in two clinical trials for a flu vaccine, Dr. Kim said. Ferritin, an iron-carrying protein, naturally self-assembles to create a nanoparticle, similar to a tiny ball, to which the researchers attached the spike protein.

The researchers then immunized mice, using the nanoparticle together with the full spike protein, as well as the nanoparticle with a truncated version that deleted an end piece of the spike. They also tested a version that contained the full and shortened versions of the protein without the nanoparticle.

In those vaccine candidates containing the nanoparticle, "We found that after a single injection, the mice produced antibodies, and those antibodies were capable of neutralizing the virus," Dr. Kim said.

Furthermore, the antibodies produced were more than twice the number of those found in the plasma of patients who've recovered from COVID-19, the researchers found. And surprisingly, the vaccine variant containing the shortened spike protein elicited the best antibody response. The scientists described their work in a January 5, 2021, paper in *ACS Central Science*.

The next step is to test the vaccine in primates to see if it generates the same level of neutralizing

antibodies found in mice, Dr. Kim said. The researchers would also have to conduct safety studies in animals before they could apply to the federal Food and Drug Administration (FDA) to begin testing in humans.

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— Peter S. Kim, Ph.D.

"We are doing the planning for all of that, but we need the funding," said Dr. Kim, whose decade as president of Merck Research Laboratories has given him the perspective and experience needed to navigate the process.

While the research showed the vaccine was effective in a single dose, the team is still working to prove that it can survive without refrigeration. Dr. Kim said he also hopes it can be freeze-dried to make distribution even easier. In addition, this vaccine could be much cheaper to produce than the mRNA vaccines made by Moderna and Pfizer that are currently authorized by the FDA for emergency use. And it's not likely to have the same potential for side effects as vaccines based on viral delivery systems, he said.

In developing the prototype, Dr. Kim said he relied on collaborations with many Stanford colleagues, including those at the Stanford Linear Accelerator Center (SLAC) who used electron cryo-microscopy

to confirm the 3D structure of the spike ferritin nanoparticles, as well as colleagues at the Stanford Blood Center, who provided the plasma from recovered patients.

“It’s very exciting to be at Stanford where we have the hospital, the medical school, SLAC, all in one place; they all came to bear,” he said. “It’s the sort of collaborative interdisciplinary work that people at the Beckman Center and Chem-H (the Stanford Chemistry, Engineering & Medicine for Human Health institute) have fostered.”

Dr. Kim said it’s astounding that scientists throughout the United States have been able to respond so quickly to the epidemic with effective vaccines.

“It really has been remarkable,” he said. “There’s never been a situation where we’ve developed a vaccine against an infectious disease in less than a year. It builds upon years of work, both with mRNA vaccines as well as the viral vector vaccines. People should not underestimate what a tremendous accomplishment this was—how it builds on the investment in basic science over the decades.”

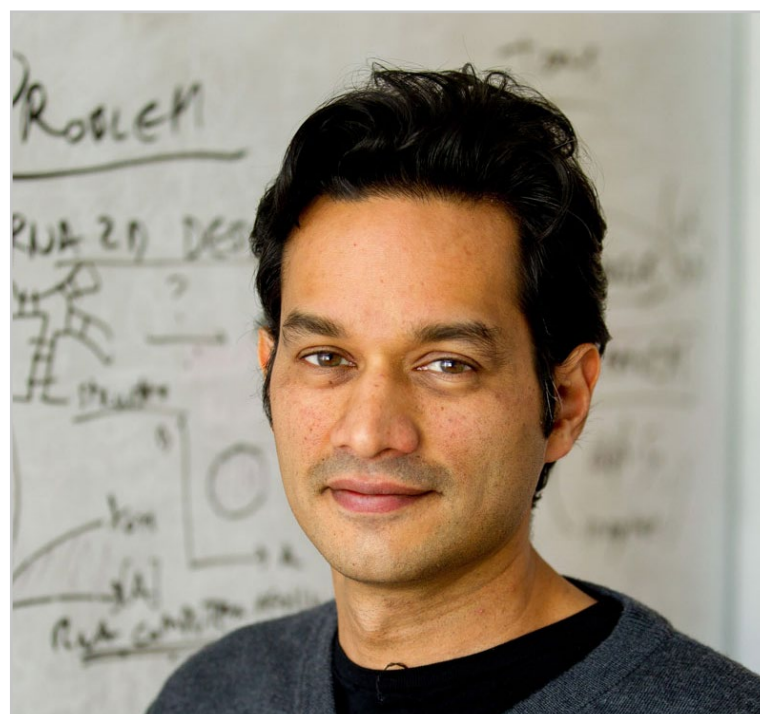
AN INVENTIVE WAY TO BUILD A VACCINE

Like Dr. Kim, Rhiju Das, Ph.D., an associate professor of biochemistry, is working to build a COVID-19 vaccine with global potential—one that can reach billions of people quickly. That means the vaccine would have to be easy to distribute and be able to retain its potency over time at room temperature.

To accomplish that, Dr. Das is using the power of multiple minds, enlisting the help of imaginative citizen-scientists from around the world to solve what he calls a unique “molecular puzzle.”

In March 2020, Dr. Das issued an OpenVaccine challenge, a new project hosted on his decade-old gaming platform Eterna (<https://eternagame.org>), which in the past has been used to develop an inexpensive tuberculosis (TB) diagnostic and other useful medical tools. The OpenVaccine challenge called on thousands of gamers to help create a vaccine based on an RNA structure that would remain stable at various temperatures, long enough to survive a trip across the globe for shipping and distribution.

“In February, we knew RNA vaccines would be the first to be authorized, as mRNA technology was heating up,” Dr. Das said. “So the primary goal was this problem of trying to stabilize the vaccines. We’ve had some really remarkable results.”



RHIJU DAS, PH.D.

Associate Professor of Biochemistry

Dr. Das and his colleague Maria Barna, Ph.D., an associate professor of genetics, have discovered mRNA design rules and invented experimental technologies that can produce mRNA molecules with dramatically increased stability compared to the existing ones produced by Moderna and Pfizer. They are now testing

whether the formulated vaccine has to be frozen to remain viable, as the current mRNA vaccines do, and whether it could potentially be packaged in syringes and shipped just as the flu vaccine is now.

“It’s really exciting. Our work has the potential to affect billions of people on a rapid time scale, as well as to lower the costs and barriers associated with future mRNA medicines.”

— Rhiju Das, Ph.D.

In addition, the researchers have figured out how to rapidly redesign the RNA structure so the vaccine will be effective against some of the newer, more dangerous strains of SARS-CoV-2 that have emerged, Dr. Das said. They are now working with an

industry partner and hope to begin clinical trials soon.

“It’s really exciting for us,” said Dr. Das. “Our work has the potential to affect billions of people on a rapid time scale, as well as to lower the costs and barriers associated with future mRNA medicines.”

In the OpenVaccine challenge, participants started by tackling weekly RNA puzzles. RNA, which encodes the genetic information for proteins, folds into complex, three-dimensional shapes, which enable it to function properly. But RNA molecules tend to be “floppy,” contorting into folds that lead to the RNA cutting itself, Dr. Das said. The goal was to find designs that would remain stable.

Dr. Das said thousands of people participated in the challenge, with about 100 making fundamental contributions to the design process. He said the project also benefited from some recent computational advances he and his colleagues had

made in managing large amounts of data on RNA folding patterns on Eterna.

“Although we weren’t working on vaccines then, those large datasets ended up having a huge impact on how we tackled RNA vaccines,” he said.

The process yielded hundreds of promising designs that the researchers tested in the lab to see if they were translatable in human cells and if they maintained their stability over time. Dr. Barna developed a new technology for this purpose, using her expertise in ribosomes. There are millions of ribosomes inside each cell that are essential to the translation process, as they take encoded information from RNA to produce proteins.

Dr. Barna and her colleagues were able to determine which RNA structures would translate the best by measuring the number of ribosomes associated with them—the more ribosomes, the better the translation. They also used the technology to measure the ability of the RNAs to survive over time.

“We can take measurements by putting the RNA in the cells, then taking measurements 6, 8, 10, and 12 hours later to see how long the RNA is hanging around, as a measure of how stable it is,” Dr. Barna explained.

She said the project broke new ground by showing that putting more structure in RNA would not hamper its translational ability.

“It was a huge conceptual advance,” she said. “In the past, people thought it would never work. But we found the RNA can be more structured and still be well-translated in the cell.”

Dr. Das used crowdfunding to help support the research and has posted the resulting sequences in the public domain so any company or academic institution

can make use of them. “That’s really important and something I’m really proud of,” he said.

In addition to that research, Dr. Das and colleagues in the lab of Jeffrey S. Glenn, M.D., Ph.D., a professor of medicine (gastroenterology and hepatology) and of microbiology and immunology, developed an anti-viral that takes a different approach to combatting the



MARIA BARNA, PH.D.

Associate Professor of Genetics

coronavirus. Most anti-virals target the spike protein, the characteristic crown-like spike on the surface of the virus. This molecule targets the viral genome itself.

“We realized that there are likely really important parts of the RNA genome where, if you could design molecules that bind up those regions, you may be able to block infection and viral replication,” Dr. Das said.

“What’s cool about that idea is it will allow for any future pandemics, for immediate design of anti-virals on demand for any new pathogen.”

The researchers collaborated with Wah Chiu, Ph.D., the Wallenberg-Bienenstock Professor, who discovered a way to visualize the elaborate, three-

dimensional structures of pieces of RNA using cryo-electron microscopy. The scientists used that technology to image pieces of the coronavirus RNA, and then looked for molecules to disrupt those structures, he said.

Dr. Das and his colleagues have had promising results with the anti-virals in cell cultures and are advancing to animal testing.

“A lot of the work has been done on the basis of experiments, technologies, and datasets uniquely available at Stanford,” Dr. Das added. “At Beckman, I feel the environment and the labs we have, and the programs to defray the cost of the equipment, have been really important for setting the foundation for what we have been able to do.”

Dr. Barna, who is a basic scientist, said this was her first experience in seeing her findings move into a potential clinical application.

“It’s really amazing,” she said. “We’ve worked nonstop. Even though it’s been tiring, keeping an eye on the impact to humanity has pushed us to really keep going.”

A NEW TESTBED FOR VACCINES

Scientists typically test vaccine candidates in mice as a first step to introducing them into humans. But mice are notoriously unreliable test subjects, noted Mark M. Davis, Ph.D., the Burt and Marion Avery Family Professor, director of the Stanford Institute for Immunity, Transplantation and Infection, and a Howard Hughes Medical Institute investigator.

“Mice respond to every vaccine,” said Dr. Davis.

“They are extremely vaccine-friendly. There are many vaccines that have worked spectacularly in mice, but not in humans. No one really knows why.”

He said the recent history of vaccinology is “mostly failure,” citing TB, HIV, dengue, and malaria vaccines as examples. That’s due in part to misleading mouse models.

Now Dr. Davis has found a surprising human model for vaccine testing—one that could make vaccine development cheaper, faster, and more predictable. It’s the tonsil, an olive-sized organ in the throat that is a key part of the immune system. Dr. Davis likens the tonsil to a giant lymph node.

Each pair of tonsils contains about two billion cells—mostly B cells and T cells that comprise the body’s first line of defense against invading pathogens. Tonsils are readily available in the United States, as about half a million are discarded each year after surgery, Dr. Davis said.

In a recent study, Dr. Davis and his colleagues obtained tonsils from local patients who’d had them removed because the organs were obstructing their breathing during sleep. The researchers separated out the various cells in the tonsils and placed them in a laboratory culture. The cells then naturally reassembled into clusters that resemble the germinal centers in lymph nodes, where antibodies are produced.

Postdoctoral fellow Lisa Wagar, Ph.D., now an assistant professor at the UC Irvine School of Medicine, then introduced a flu vaccine into the mix.

“She was able to show that if you gave the tonsil a flu vaccine, the tonsil actually responded and made antibodies after a week,” Dr. Davis said. “With that, we really knew this was the way to go.” In other words, the system reacted exactly as lymph nodes do when people get a vaccine: It pumped out antibodies primed to fight off the offending pathogen.



MARK M. DAVIS, PH.D.

Burt and Marion Avery Family Professor
Director, Stanford Institute for Immunity, Transplantation and Infection
Howard Hughes Medical Institute Investigator

Dr. Davis said he had no luck in getting their study published until COVID-19 hit, when suddenly there was intense interest in vaccines. He also began working with Vaxart, Inc., a vaccine developer based in South San Francisco, which had a COVID-19 vaccine candidate in the works.

“Sean Tucker, the chief scientific officer, called me and said, ‘Would you have a use for this COVID-19 vaccine we’re developing?’” Dr. Davis recalled.

“I said, ‘Yeah, let’s put it in the tonsils.’ We got a response—a few of the subjects’ tonsils had

“The tonsil model is ideal for studying a wide range of human immunological interactions, something that’s not been available until now.”

— Mark M. Davis, Ph.D.

noticeable antibodies to the vaccine. It wasn't super robust. It wasn't as robust as the flu vaccine response, but it was detectable. It helped validate the system and showed it was something that could be broadly applied." The researchers reported their results in January 2021 in *Nature Medicine*.

Dr. Davis said the tonsil system is ideal for testing vaccines as it produces quick results, saving both time and money. Scientists could try out hundreds of candidates in a relatively short period to see which ones produce the best response.

"Say you want to make the ultimate COVID-19 vaccine," he said. "What you'd want to do is make a thousand different flavors. But if all you could do is test it in mice, it wouldn't help you very much. If you test it in tonsils, however, you'd be much more likely to find the version that works best in people."

There is a lot of variation in how people respond to vaccines. In the flu vaccine study, for instance, the tonsils from 13 children produced antibodies, but those from two others did not.

"People are different, and they respond differently," said Dr. Davis. "The goal is to optimize it to make a more effective response. This is a way to optimize, as you could test 100 or 1,000 variations and pick the one that gets the best results in the greatest variety of tonsils."

Dr. Davis said the tonsil model is ideal for studying a wide range of human immunological interactions, something that's not been available until now.

"We have a very difficult time in human work doing mechanistic experiments, as you can't slice and dice people," he said. "The better thing is to show some important function in a dish. It means you can access every cell and manipulate it to test hypotheses *in vitro*. We've not really been able to do that before."

Dr. Davis is now looking at the phenomenon of why very young children are susceptible to infection but then suddenly become more resistant after they reach age five. "No one has the slightest idea why that is. Since we can get tonsils in those age groups and in adults, it would be interesting to define what is changing, how the immune system is getting better."

He's also interested in the immune response to cancer. "Tumors appear all the time and are normally eliminated by the immune system. It's probably the rare ones that survive and end up growing and doing harm," he said. In a tonsil system, scientists could introduce cancer cells and then see what kind of response the cells engender.

The model also could open a new window on how the body is able to marshal its forces against infection, rather than be overwhelmed by harmful invaders.

"We can put live flu in the tonsils, and the virus doesn't take over," Dr. Davis said. "In fact, there is an immune response. It must be that there is enough happening to trigger the response so the infection doesn't go anywhere. It's eliminated. The ability to see that in a dish will be transformational in terms of understanding just what happens with an infection."

HOW SARS-COV-2 ALTERS THE IMMUNE SYSTEM

Catherine Blish, M.D., Ph.D., a professor of medicine (infectious diseases), has found that the SARS-CoV-2 virus can wreak havoc on the immune system in unique and surprising ways. An infectious disease specialist, Dr. Blish has spent years stalking some major killers—HIV, Zika virus, dengue, flu, and most recently, tuberculosis. But when the 2020 lockdown forced her to abandon her TB research, she began to focus exclusively on SARS-CoV-2.

Dr. Blish's studies show that virtually all immune cells are changed in the presence of the virus. Understanding these changes is key to developing therapeutics and in furthering the development of vaccines to protect against infection.

"I want to know what a protective immune response looks like, because that's what we want to mimic with a vaccine," said Dr. Blish. "But I also want to know what inflammatory mediators we need to block to dampen severe, pathologic disease."

She began her studies using a virus obtained from the first identified patient in Seattle, Washington. She initially lacked a suitable space for her work, as Stanford did not have the needed laboratory facilities. Because the coronavirus is airborne and highly infectious, the federal Centers for Disease Control and Prevention requires a Biosafety Level 3 (BSL-3) lab for COVID-19 research.

Dr. Blish had been using a campus BSL-3 lab for her TB studies, which she shared with chemist Carolyn Bertozzi, Ph.D., the Anne T. and Robert M. Bass Professor in the School of Humanities and Sciences, the Baker Family Director of Chem-H, and a Howard Hughes Medical Institute investigator. But that lab was tiny and outdated. So Dr. Blish became the driving force in the construction of a new, 4,000-square-foot BSL-3 lab, which opened in September 2020. The state-of-the-art facility, which was completed in record time, has separate spaces for work on SARS-CoV-2 and TB. "It's really well-equipped and beautiful," she said.

Dr. Blish's initial SARS-CoV-2 research focus was on the cells that make up the front-line of defense in the immune system—neutrophils, the common white blood cells that are the first to arrive on the scene, and monocytes, which chew up harmful pathogens.

"Those first responders are just incredibly changed in the setting of coronavirus infection," she said.

Monocytes normally secrete cytokines, which help immune cells communicate and direct an attack, but they appear to become dysfunctional when the virus is present. "They are kind of paralyzed in the blood," she said. "They are not making cytokines. We know these cytokines are present, but they are not being produced by these monocytes."

Neutrophils, which are a huge part of the immune response to COVID-19, also appear to undergo unusual changes. Dr. Blish and her colleagues saw immature



CATHERINE BLISH, M.D., PH.D.

Professor of Medicine (Infectious Diseases)

and mature neutrophils become highly inflammatory and express markers for NETosis, a form of cell death. During this process, the cells explode and form a large net that can capture harmful bacteria. But the nets are too small to trap viruses and are highly correlated with poor outcomes during viral infection, she said.

"We think the neutrophils are causing hyper-inflammation and a huge number of problems," Dr. Blish

said. "When that neutrophil population is present, it predicts a fatal outcome 80 percent of the time."

"It's possible that this is one of the many potential pathways to turn back this hyper-inflammatory clock."

— Catherine Blish, M.D., Ph.D.

Dr. Blish and Dr. Bertozzi have identified a protein, called Siglec-9,

that could potentially interfere with this inflammatory response. They incubated healthy neutrophils with plasma from COVID-19 patients to induce NETosis. When they added an agonist Siglec-9 to the mix, it completely inhibited the process, they found.

"It's possible that this is one of the many potential pathways to turn back this hyper-inflammatory clock," said Dr. Blish.

Dr. Blish is also working with Calvin Kuo, M.D., Ph.D., the Maureen Lyles D'Ambrogio Professor, who has created a lung organoid, a mini ball of lung cells that arrange themselves somewhat like a human organ. The researchers infected the cells with the SARS-CoV-2 virus and found it impacted the alveolar type 2 cells, which was expected, but also club cells, which was surprising, she said. Club cells are involved in creating surfactant, a substance that lines the surface of lung cells and prevents them from collapsing during breathing.

"This could potentially explain some of the inflammatory conditions," Dr. Blish said. "We may be messing up the secretions that made the lung cells happy."

She said the researchers hope to use the organoids to test new therapeutics. "Like a mini human lung, we

can figure out which drugs have promise before we do expensive clinical trials," she said.

SARS-COV-2'S ASSAULT ON THE LUNGS

Dr. Blish has also been collaborating with molecular biologist Mark A. Krasnow, M.D., Ph.D., the Paul and Mildred Berg Professor and a Howard Hughes Medical Institute investigator, who has been studying the structure and function of the lungs for decades.

Three years ago, Dr. Krasnow had a bittersweet experience. His friend and Beckman colleague, James Spudich, Ph.D., the Douglass M. and Nola Leishman Professor of Cardiovascular Disease, showed up at his office with the news that he had been diagnosed with lung cancer and would have a portion of his lung removed the next day. Dr. Spudich asked if Dr. Krasnow might be interested in studying his cells.

Dr. Krasnow's team sprang into action the day of the surgery, transporting tissue from the surgical suite to the lab, where they separated out cells from the healthy part of Dr. Spudich's lung. They then used single-cell RNA sequencing technology to break down the RNA and determine what genes were being turned on and off in each of the isolated cells. Dr. Krasnow's collaborator, Stephen Quake, Ph.D., the Lee Otterson Professor and co-president of the Chan Zuckerberg Biohub, helped develop the sequencing technology 10 years ago and provided access to it across Stanford as part of the Biohub.

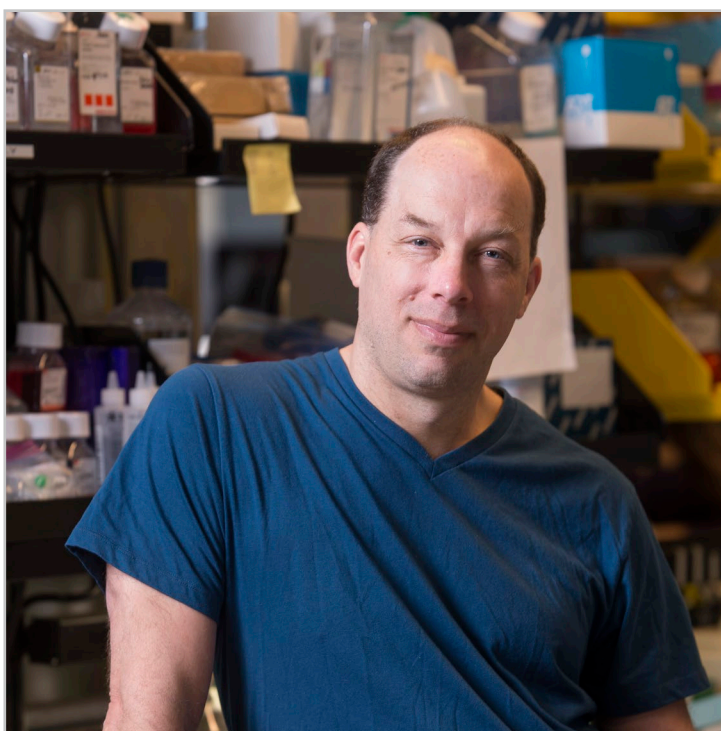
The result was a stunning accomplishment. The researchers produced a complete atlas of the human lung and its 58 types of cells, including 14 that had never been previously identified. Their findings were published in the journal *Nature*.

Then the COVID-19 pandemic struck.

“We didn’t have any inkling that these newly identified cell types were going to be at the site where the virus is doing its deadly damage and whose activity is important in preventing it from getting out of control,” Dr. Krasnow said.



MARK A. KRASNOW, M.D., PH.D.
Paul and Mildred Berg Professor
Howard Hughes Medical Institute Investigator



STEPHEN QUAKE, PH.D.
Lee Otterson Professor
Co-President, Chan Zuckerberg Biohub

Dr. Krasnow expanded his collaboration with Dr. Blish and Dr. Quake to create a system where, for the first time, scientists could study how a virus gains access to human lung tissue and observe the process of viral infection at the level of single-cell resolution. The researchers obtained additional lung tissues from donor patients and exposed exquisitely thin lung slices to the SARS-CoV-2 virus in the lab. The work was done in the new BSL-3 facility, with Dr. Quake carting his single-cell RNA sequencing instrument into the space.

“Because we have almost all of the cell types of the human lung in that tissue, we found which cell types are infected, what the virus life cycle looks like, and how it takes over the infected cells,” Dr. Krasnow said. In studying the initial effects of the disease, he said, he hopes to find therapeutics to intervene and stop the process.

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— *Mark A. Krasnow, M.D., Ph.D.*

Their work has yielded some remarkable results, with the scientists effectively tracing the path of viral destruction. Dr. Krasnow said the virus targets at least half a dozen cell types, including the alveolar cells, key functional structures deep in the lung that facilitate the process of gas exchange. As these cells begin to falter, alveoli epithelial stem cells—which were newly identified by Dr. Krasnow’s team—work to replenish the dying

cells. But if the virus gains the upper hand, it can infect the underlying capillaries and stromal cells to cause leakage into the alveolar space. The alveoli then start to drown in the fluid and the barrier to the bloodstream begins to break down.

“Once the virus gets through the air-blood barrier, it spreads through the body and causes not just the devastating pneumonia but systemic disease, with all the problems in different organs patients have suffered,” Dr. Krasnow said.

In a surprising finding, the researchers discovered that alveolar macrophages also are heavily impacted by the virus, something not seen before. These are the immune cells in the alveoli that serve as sentries, constantly on the lookout for microbes so they can eat them up and destroy them. But these guardians have become traitors to the cause, giving way to the virus.

“The virus is getting in, infecting and converting these sentries into viral terrorists by helping the virus replicate,” Dr. Krasnow said. “They move around and may be spreading the disease in the lung, the exact opposite of what you want your guardians to do.”

In another striking finding, the scientists discovered that the alveolar macrophages don’t express the ACE-2 (angiotensin-converting enzyme 2) receptor, the protein on the surface of many cells through which the SARS-CoV-2 virus is thought to gain entry. Current vaccines and therapeutics are all designed around use of the ACE-2 receptor, which binds to the virus’s spike protein.

“The way it’s getting into these macrophages seems to be molecularly distinct from the way everyone believes the virus gets into other cells, like the alveolar type 2 cell,” Dr. Krasnow said.

“We’re exploring right now—what is the mechanism and the molecules that the virus uses to get into the macrophages? The question is, if the virus is using a different mechanism to get in, is that mechanism being blocked by the current antibodies and vaccines? If not, the virus might still have access to this cell reservoir deep within our alveoli, which it can infect and propagate in.”

Dr. Krasnow is now working with Dr. Kim and his colleagues, who have developed a method to block SARS-CoV-2 in macrophages, to see if this might point the way to a new therapeutic.

A NOVEL PREVENTION METHOD

A pandemic calls for out-of-the-box solutions, and that is what Daria Mochly-Rosen, Ph.D., the George D. Smith Professor in Translational Medicine, is pursuing. Her team at Stanford’s SPARK Program in Translational Research has created a simple, cheap preventative based on an unusual approach: chicken antibodies sprayed in the nose as a temporary barrier to the virus. With one application, a user may be immediately protected for several hours, a hedge against infection that could be particularly useful in settings where a vaccine may not be available.

“It can be made locally and cheaply, so it can help stop the epidemic,” said Dr. Mochly-Rosen. “We now see how difficult it is to distribute the vaccine. There are many low- and middle-income countries where there is zero percent vaccine. If we can show this is efficacious, people in these countries will have some protection while they wait for the vaccine.”

Dr. Mochly-Rosen’s group already has tested the nasal spray in a Phase 1 trial, which showed it to be safe. She is now preparing for a Phase 2 trial to see if it’s as effective as she hopes.



DARIA MOCHLEY-ROSEN, PH.D.

George D. Smith Professor in Translational Medicine

The idea emerged out of SPARK, her nonprofit organization that connects academics around the world in an effort to find rapid answers to emerging medical problems. When the pandemic struck, one of her country directors in Australia, who had tested chicken antibodies in a mouse model of flu, suggested it as a possible response to SARS-CoV-2.

As she scanned the literature, Dr. Mochly-Rosen learned that chicken antibodies in protective nasal sprays had been used for a number of conditions in both animals and humans.

"It has been shown to be effective against influenza, H1N1, so I definitely think it could be used for airborne viruses," Dr. Mochly-Rosen said. "It has been shown in animals to be effective for ulcers caused by bacteria and for various coronaviruses. And it's been shown to be effective against some gut bacteria and gingivitis in humans. It's really a simple procedure that can be effective against a lot of threats, but most importantly against epidemics."

The process is straightforward. Researchers inject the spike protein of the coronavirus into the breast of hens. The animals respond by producing large quantities of antibodies, a chicken version known as immunoglobulin Y (IgY). These antibodies are found in high concentrations in chicken yolk. The researchers harvest these antibodies from eggs laid by immunized hens and then purify them into nasal drops.

The antibodies are considered safe for humans; in fact, we are exposed to them when we eat eggs, Dr. Mochly-Rosen said. "However, they are usually not snorted," she noted with a smile, which is why they need to be rigorously tested.

The approach has many advantages. For one, it provides immediate protection. "It's not like a vaccine where you have to wait three or four weeks," Dr. Mochly-Rosen said. "Here, the moment you put in the nose drops, the antibodies capture the virus before it gets into the body."

The spray doesn't trigger an immune response, like a vaccine, because it's not injected into the bloodstream. Because it's not systemic, it has potential to be used

"We now see how difficult it is to distribute the vaccine. There are many low- and middle-income countries where there is zero percent vaccine. If we can show this is efficacious, people in these countries will have some protection while they wait for the vaccine."

— Daria Mochly-Rosen, Ph.D.



in people who are immunocompromised, in children, and in pregnant women, she said.

The protection is temporary, as the nose naturally clears material in a matter of hours. Dr. Mochly-Rosen believes users would have to reapply the nose drops every four hours when they are at risk of exposure to the virus.

But the nasal spray is easy and cheap to make. Each egg contains between 10 and 100 doses. Hens lay eggs every day, so there is a plentiful supply available. Dr. Mochly-Rosen believes the spray could be produced for as little as \$1 per daily dose.

It may even be possible for people to make the spray themselves at home. She's now working with a group of Stanford undergraduates to see if it could be easily developed in a home setting. She said people anywhere could use it as an extra measure of protection in addition to a face mask.

Dr. Mochly-Rosen believes so strongly in the concept that she has used every resource she has, including most of her SPARK budget, her endowed faculty funds, and various small grants, to test the product. Members of her SPARK network around the world, including researchers in Australia, Norway, Belgium, Canada, Brazil, and South Africa, also have contributed know-how and advice to the project, she said.

Ultimately, she believes the approach will serve as a blueprint for protective nasal sprays that could be applied against other pathogens, such as Ebola. "Within three to four weeks, we could have a product produced in a country when a new epidemic begins," she said.

In that respect, Dr. Mochly-Rosen is like many Beckman scientists whose work will not only benefit human health now, but also help protect us well into the future.