LEAP
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Division of Rheumatology
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SEEN FOR THE FIRST TIME

THE UNEXPECTED

AT THE EDGE OF TECHNOLOGY

UKUTHULA
Alice in Wonderland may come to mind as you read this issue of LEAP, because we are moving from the large to the small, and back again: A rheumatic disease is big, and its effect can be devastating. However, a disease such as rheumatoid arthritis, scleroderma, or Sjögren’s is, in fact, multiple diseases, each slightly different. Each subgroup has a different trajectory of illness, and may respond better or worse to various forms of treatment. But it gets smaller still. One of our Greene Scholars, Sergi Regot, is studying individual cells that are molecularly and genetically identical; they should all behave in the exact same way, but they don’t. This is because of countless tiny factors that we are now beginning to explore.

I love our cover for this issue, because it perfectly captures our excitement as we are seeing new clues peeking out of tissue samples. They are visible now because we have looked for them in completely new ways.

In Sjögren’s syndrome, because our investigators were intrigued by strange filamentous forms of a protein called IFI-16, found in salivary gland biopsies from patients, we have glimpsed the molecular events that drive the relentless cycle of autoimmunity in this disease. In scleroderma research, Greene Scholar Steven Hsu has pinpointed a molecular mechanism underlying cardiac dysfunction — taking our perspective from large to small and back again. In other work led by Thomas Grader-Beck, we are using new approaches — collecting data from patients and their lives as their disease evolves over time — to build a framework to discover ever more precise patient subgroups, at scale, and help us refine our care even further. This is precision medicine at its finest: now we can analyze thousands of points of data to find patterns and tailor our diagnosis and treatment for each subgroup — instead of unsuccessfully treating a very heterogeneous group of people as if they were all the same.

And then, there is our choir — taking us, on our Alice-in-Wonderland journey, to a much larger scale. My ongoing goal for the Division of Rheumatology is for us all to work together in respect and — well, harmony: in this case, that harmony is soprano, alto, tenor, and bass. We chose a Zulu hymn of peace, called “Ukuthula,” and after many hours of practice, our all-volunteer choir has sung it several times. What an amazing experience! Out of many diverse voices — people from all walks of life, people who didn’t know each other very well, people of different religions and ethnicities, and from different countries of origin — we became one voice. In the process, we learned about each other, we laughed together, and we enjoyed it so much that we have kept on going.

As individuals, we have become part of something much bigger. To me, the harmony that we create with our voices is a beautiful representation of the harmony of scale and approach that our Division is pursuing with precision medicine — our patient-reported data, molecular and cell biology — as we work together across all boundaries to accomplish something that is truly unique.

Antony Rosen, M.D.
Director, Division of Rheumatology
Vice Dean for Research
They are creepy little strings, long ropes of proteins in the salivary glands.

Our scientists – rheumatologist Brendan Antiochos, M.D., biophysicist Jungsan “Jay” Sohn, Ph.D., postdoctoral fellow Mariusz Matyszewski, cell biologist Livia Casciola-Rosen, Ph.D., and Director Antony Rosen, M.D., – have captured them in groundbreaking photographs.
If this were a story about a terrible crime, IFI-16 would be the suspect arrested, of whom neighbors might say, “He always kept to himself,” or “He was quiet, but seemed perfectly nice.”

But in very specific tissues and susceptible individuals, this nice veneer cracks: in the salivary gland tissues of people who develop SS, IFI-16 misbehaves. Because these scientists looked at this tissue through a specific filter — focusing on IFI-16 to see what it does — they were able to see what no one had seen before, and now we can see it happening, as well.

Similar pictures can also be taken in salivary cells in culture dishes exposed to interferons and exogenous (foreign) DNA. They are the images from the epicenter of a tiny, terrible earthquake. In a paper recently published in *JCI Insight*, the scientists have connected the dots of the driving events of SS, reconstructed in these tissue cultures of salivary epithelial cells. “The events that we have seen in the biopsy tissue are a snapshot,” says Rosen, “of the forward and reverse pathways — damage, inflammation, and healing — that are happening all the time in people with SS.”

IFI-16 is turned on by interferons, immune system proteins released by the body to fight off a virus or infection. “Interferons are expressed at high levels in the salivary tissues in SS,” explains Antiochos, lead author of the paper. An offending piece of DNA — maybe a virus or piece of foreign bacteria — gets recognized by the immune system. IFI-16 proteins come in and stick to it, and keep right on sticking to each other, as well. “When IFI-16 is activated, it goes from a single molecule to large collections of molecules in long lines that manifest as filaments.”

In people who develop SS, there is something irritating about these strands, or filaments, in this particular tissue. Their very presence seems to stimulate the immune system. “The immune response in Sjögren’s predominately recognizes the filamentous form,” says Antiochos.

Now, we pass briefly out of the sunlight of evidence and into the murky waters of motive and opportunity: How does a long filament inside these salivary cells come into contact with the immune system? “It is particularly interesting,” comments Rosen, “that the specific type of inflammation we see in the salivary gland in SS involves white blood cells called lymphocytes, which are activated to make holes in the membrane of cells in this tissue.” The lymphocytes are soldiers, and their job is to kill; they are “cytotoxic.”

For the first time, says Antiochos, “we show that these cytotoxic cells cause IFI-16 filaments to leak out of the salivary epithelial cells,” and this is likely what drives the immune response.

What happens next is the unfortunate cycle of autoimmunity: Cytotoxic lymphocytes come in to punch more holes in the cell membranes (see main story), killing cell-killing lymphocytes to tiny, “first-line” lymphocytes, and this is likely what drives the immune response. “The immune response itself is a body’s best friend, the superhero that homepage everything that’s not normal, the immune response can turn its attention against itself and start attacking normal tissue,” says Antiochos. “It may be that, just as IFI-16 turned out to be an incredibly effective filter to highlight a key element in the pathogenesis of SS, that there’s another filter we can use to look at these cells that will let us see much more. Right now, our investigators are seeing a fight that erupts outside a bar, but they can’t see what started it inside the building — maybe a spilled drink, or an argument at the pool table.”

Antiochos is trying other filters, too; some of them focus on defining the nucleic acids that might activate IFI-16, others on the machinery that normally degrades the filaments; and still others that highlight the unusual distribution of the filaments in only one type of cell, in the salivary gland in SS.

Another huge question is the event that caused the initial immune response in SS the same thing that sustains it? There are no tissue samples available to show Sjögren’s syndrome actually starting, and there are no action shots of the antibodies first being made; scientists never arrive on the scene until much later. That’s why these types of studies are so important: they allow us to observe the situation in the actual diseased tissues, and then to recreate the crime by doing what Agatha Christie would do: putting the suspects together. In this case, the scientists are adding various combinations of the key stimuli to salivary cells in vitro. “Hardly anyone with SS syndrome gets it diagnosed right away,” says Antiochos, “because the symptoms are so insidious.” For example, there are a lot of people who have dry eyes, and the vast majority of them don’t have SS. “Many of my patients, by the time they come to see me in the clinic, have had symptoms for 10 years that were initially misdiagnosed.”

Four years ago (see LEAP 2015), Jay Sohn and colleagues found that the IFI-16 proteins themselves comprise three parts; one of them is called PYD, and it seems to be the glue that holds the filaments together. When the scientists broke up the PYD, the IFI-16 proteins did not connect. Since these filaments are the targets of the immune response in Sjögren’s, therapies that target PYD may suggest a promising avenue of treatment. It may be that developing a PYD-busting agent, and delivering it to target tissues, could arrest the cycle of damage.
AT THE EDGE OF TECHNOLOGY

RHEUMATOLOGIST THOMAS GRADER-BECK, M.D., IS AT THE FOREFRONT OF A MEDICAL REVOLUTION. HE’S NOT DEVELOPING A BETTER DRUG OR TREATMENT, BUT WHO KNOWS? HIS WORK MAY LEAD TO BOTH.
It’s Not Easy

There are inevitable growing pains and creaks as our health care system tries to adapt to the technology. In record numbers, physicians nationwide are suffering from burnout; EMRs are right at the top of the list of reasons why. Many doctors already feel like they spend more time talking to the top of the physician’s head, as answers are being typed in, than making eye contact. It’s “constant documentation,” says Grader-Beck. Nobody likes this. (Actually, he notes, physician notes in the U.S. are about four times as long as they are in many countries, “all because of certain requirements specific to our health record system.”)

No one thinks that adding more to our physicians’ already large workload is the way to go. So the trick—and Grader-Beck is leading the effort to figure this out—will be to find a way for our patients to be our partners in research and treatment, for us to capture and incorporate all of the relevant data in such a way that will actually enhance our face-to-face time as we work together to manage their very complicated illnesses, “so the doctor-patient visit can be much more effective,” says Rosen.

This work started three years ago, with electronic questionnaires on tablets and iPads, as part of a pilot project (see LEAP 2016). “So many things have sprung off from that beginning,” Grader-Beck says. “The next step allowed us to assist researchers within Rheumatology to build new projects for their clinical research using EPIC.” In a larger pilot study of 400 patients, “we found that using the tablets actually was very efficient, that it took less time for patients to complete these questionnaires than it previously took on paper, and that patients were overall very happy with them.” Grader-Beck presented these and other results at the annual American College of Rheumatology’s meeting in October 2018.

There’s a lot of technological and medical know-how required to make these questionnaires truly patient- and physician-friendly. A lot of “if, then” algorithms to put into the computer—like, “if someone has dry eyes, then we move to these questions.” Multiply that many times for different diseases. Then start thinking like a basic scientist: how can these results help with our research into the causes and search for better treatments? And could new findings then be turned around and used to improve care even more?

“You know, because of the data, we’ve actually changed the way we ask patients about their disease,” says Grader-Beck. In rheumatoid arthritis, patients are asked about such aspects of their lives as physical function, ability to participate in certain activities, social life, sleep, depression, and anxiety. “These questions have become the standard of care; they add greatly to the old measures of disease activity.” There are 10 tablets in use now instead of the original four, with plans to expand to other Rheumatology clinics.

Got an App for That?

‘The idea of an app for your phone or iPad is another exciting possibility for communication and data-capturing. In a recent Hopkins project with multiple sclerosis patients, tablet apps were used to capture a large amount of historical data and even measures of dexterity, “greatly improving the opportunities for patient and physician interaction and decision-making.”

This is all still so new. “We’re at the point where we have so much information, but it’s really not knowledge yet,” says Grader-Beck. “We have a lot of data, but what does it mean? Eventually, more dots will be connected: between blood, tissue, imaging and other tests, and signs and symptoms the patient is experiencing, in large enough numbers, over enough time.” Then, he predicts, “we will be using our findings to help treat the next generation of patients. It will be a long, hard way to get there, but the possibilities are amazing.”
ONE CELL AT A TIME

Picture, if you will, a herd of zebras. At first, maybe you think, “How do they tell each other apart? They’re all the same!” But if you look a little closer, you might start to notice some differences: this one has a scar, and that one’s a little heavier. Although most of them are eating grass, a few are asleep; and two seem to be on guard duty, ready to send out the alarm at the first sign of danger. Though silent, they’re communicating with each other, in subtle movements of the ears and tails. There’s actually a lot going on here.

Now, you could watch this entire herd, and see how it acts as a unit – like a school of fish or flock of birds. Or, you could do what Greene Scholar Sergi Regot, Ph.D., is doing on a much smaller scale, and focus on just one at a time. Unlike many scientists who study thousands of cells simultaneously, Regot, in the Department of Molecular Biology & Genetics, uses a dynamic, single-cell approach: he makes microscopic movies, recording events at the molecular level for in-depth investigation.

Using a bioluminescent, jellyfish-made protein, Regot creates fluorescent biosensors. These glowing points of light are molecular beacons, revealing the activity of certain proteins in real time. “We want to understand biology at the level of the individual cell,” he says. “With single-cell resolution, we can tell cell A apart from cell B, from cell C.” And with funding from the Jerome L. Greene Foundation, he is watching how these individual cells behave in the midst of an immune response.

In particular, he is looking at natural killer (NK) cells. Normally, he explains, these cells patrol tissues, “and when they identify a cancer cell, or a cell infected with a virus, they assassinate it,” by pumping a toxic protein called granzyme B into their target. In Regot’s movies – each is taken over a period of 24 hours – he hopes to see whether granzyme B is always activated, “or if some encounters are not successful,” and the NK cell does not eliminate the unwanted cell. Pioneering work on granzyme B, which makes an unwelcome appearance in many autoimmune diseases, has been done over the last two decades by Antony Rosen, M.D., and Livia Casciola-Rosen, Ph.D., who showed that granzyme B doesn’t always cause cell death; it can also lead to tissue dysfunction. When NK cells contact their targets, says Regot, there is a lot of unpredictability: “There are always cells that are quiescent and cells that are more active. There are differences that shouldn’t be there – because single cells of the same type are supposed to be all the same. But even though they may be molecularly and genetically identical, they aren’t all the same.”

One reason for this variability might be the individual cell’s life history and battle scars. In other words, this dysfunction may be a microscopic example of “nature vs. nurture.” Maybe the events that cells encounter change their “immunological memory,” or exhaust them, or otherwise affect their ability to function. For example, identical twins often do not develop the same illnesses, even though they have the same genes; maybe one twin exercises more, or one has a terrible diet, or smokes.

In his tiny arena, Regot is dealing with some of the same issues that haunt epidemiologists who do large population studies. “We need to understand the molecular mechanisms that explain why individual cells behave differently, and how these seemingly random behaviors contribute to the development of autoimmune disease.”

Cells may be molecularly and genetically identical, but they don’t behave in exactly the same way. “There are differences that shouldn’t be there.”

With support from the Jerome L. Greene Foundation, these highly creative faculty are establishing their research careers, making the transition to independent NIH support.
WEAKNESS IN THE HEART

Not all scleroderma patients have problems in the heart and lungs. But some do, and these complications are serious.

When people with scleroderma develop idiopathic pulmonary arterial hypertension (PAH) — high blood pressure in the lungs — the usual treatments aren’t nearly as effective. “People with scleroderma tend to fare a lot worse in terms of how they feel, how well they respond to treatment, and how long they live,” says cardiologist Steven Hsu, M.D.

With support from the Jerome L. Greene Foundation, Hsu has pinpointed a structural cellular defect that seems to be the source of the problem — and that offers new hope for treating it.

In one way, Hsu notes, the fact that he sees so many scleroderma patients — who come from all over the world to Hopkins’ world-class Scleroderma Center — is a sign of progress in the care of this very complicated illness. “With ongoing advances, we’ve gotten a lot better at treating kidney disease and preventing complications from renal crisis in scleroderma. Now the lungs have emerged as a major challenge for this population.”

When all is well, blood flows through the heart and lungs like water through a garden hose: nice and steady, and strong. But in PAH, it’s as if somebody’s standing on the hose. Current therapies, vasodilators, work to reopen the hose. “Another way to get the blood flow back up,” explains Hsu, “would be to somehow push harder.” To compensate, the right heart muscle needs to bulk up like a weight lifter. If it doesn’t, the blood flow through the diseased lungs becomes sluggish.

For many people with scleroderma, the right heart can’t get stronger, and the vasodilators that help others with PAH do not directly improve contraction of the heart. There is an inherent weakness in the right ventricle in people with scleroderma, even if they don’t have PAH. “Even on a good day,” says Hsu, “their heart probably has a harder time sending blood to the lungs.” PAH just makes it much worse.

PAH is diagnosed by cardiac catheterization, which Hsu performs. In a study funded by the Greene Scholarship, he is doing extra tests in scleroderma patients who come in for catheterization: performing more sophisticated pressure and volume measurements, and taking biopsy samples of the right ventricle.

“This has allowed us to study in an in-depth way how the right ventricle is working,” he says. “We can look at the tissue and study how the cells and different molecules are behaving in response to PAH.” He and colleagues have found that the right ventricle in scleroderma PAH patients is not only weaker; it also becomes weaker faster. “We can see this weakness when the patients get their catheterization, and we can see it in the tissue when we analyze how the muscle cells are squeezing.”

Hsu has discovered a critical problem at the molecular level within the sarcomere, the structure within heart muscle tissue responsible for contraction: “a cellular defect, which causes the sarcomers in the right ventricle not to contract as well.”

Hsu’s research bridges the disciplines of cardiology, pulmonology, and rheumatology, with the goal of being “as translational as it can be,” he says, and he is hopeful: “There are drugs already in development for people with congestive heart failure on the left side of the heart. Some of these boost sarcomeres’ function. Nobody’s thinking about this for the right side of the heart. But some of these preferentially boost sarcomeres that are weak on the left side: they could potentially be beneficial in boosting weak sarcomeres on the right side.”

Hsu started this research with the goal “to drill down and figure out what’s different about this population. We have a target now.”
It doesn’t; it’s way more complicated than that. Among very many intricate components, there are tiny molecules called checkpoints that lash down enemy-fighting cells, putting them in straitjackets so they can’t fight off disease. And then there are powerful cells such as lymphocytes, that once activated (see story on page 2) can cause significant collateral damage – even if they successfully control the virus or cancer that originally turned them on.

Rheumatologist and Greene Scholar Brittany Adler, M.D., is studying the immune system at the nexus of cancer immunity and autoimmunity. She is looking at certain checkpoints – molecules on lymphocytes that are known to be upregulated in inflammation and cancer. “I am working in the lab with Antony Rosen and Livia Casciola-Rosen, on identifying novel autoantibodies against immune checkpoints that may regulate this immune balance,” she says. “Our goal is to help patients who have disease for years, who are unable to tone down the immune response without using immunosuppressive medications.” Learning what these antibodies do – how they fit into the disease process, and also how they may help protect the body from other disease – is critical, says Antony Rosen, M.D., Director of Rheumatology: “Checkpoint autoantibodies could be a form of natural immune regulation. Or, equally, they could be potent natural cancer-fighters.” Adler is working to address both sides of this powerful coin. What sparks the immune system to develop immunity against itself is one riddle. Here’s another: why do specific diseases affect particular tissues, and not all? And here is still another: “What drives the immune system to be in a perpetual ‘on’ state?” says Adler. Could checkpoint autoantibodies somehow control the immune response? Do they send out a signal, in effect, to keep the foot pushing on the gas pedal? “If we can confirm the function of some of these autoantibodies, we might be able to explain why some patients have continual immune problems, and others, more mild disease. This may provide insights into natural regulation of the factors that drive disease forward, which may be different from the triggers that cause the initial immune response.”

And here again, we’re back at the intersection of autoimmune disease and cancer: some of the most exciting research in cancer right now is in trying to turn up the immune system – which is tricked by cancer into locking up T cells, lymphocytes, and other cells that could kill diseased cells. If checkpoints are like handcuffs, checkpoint inhibitors are new classes of drugs that unlock them, so the T cells can come out swinging. This is such an exciting development, in fact, that the scientist who discovered immune checkpoints, James Allison, just shared the 2018 Nobel Prize for this contribution to medicine. These drugs – ipilimumab, for example – are still very new, and far from perfect; they don’t work in everyone, and they can cause immune system-related collateral damage in other tissues. Adler was first author of a study published in the Journal of Internal Medicine this year that characterized some of the features of ipilimumab-associated colitis, an immune-mediated colitis that mimics inflammatory bowel disease.

In cancer research, scientists are trying to unlock the checkpoints; in rheumatology research, scientists are working to do the opposite: perhaps to selectively strengthen the restraints on the immune response. “The fact that there are autoantibodies against these checkpoints,” says Rosen, “may be an important clue. They may be telling us about a mechanism which could be harnessed therapeutically in the future either for cancer or autoimmunity, depending on what we find.”
Some of them had never sung before; others, like soloist Zahira Clark, Clinic Coordinator, sing all the time – “in the car, and to my kids; I’m always singing,” she says. Some of them couldn’t read music; others, like Mecoli – the son of an orchestra conductor, he started playing the piano before he went to preschool – are accomplished musicians. Together, in four-part harmony, they make one beautiful sound.

They are singing a hymn, written in Zulu, called “Ukuthula,” and it has a message of peace (see Antony Rosen’s Letter on Page 1).

“At first, Clark says, “I was nervous” about singing with people she did not know very well. But this didn’t last long: “We bonded together. Not only did our relationships change, it gave us an opportunity to become a real family. Now we see each other in the halls, stop, hug each other, and actually care about each other’s day.”

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To overcome the language barrier, Mecoli began with phonetics. The choir learned the correct pronunciation of the words with the help of Google Translator. Using his piano at home, Mecoli “experimented with a couple different keys,” to find one that wasn’t too high or low for the singers’ voices, and recorded each individual part. He layered the parts together and emailed them to the group in various forms – so that a soprano, for example, could sing just the soprano part, or sing her part together with an alto melody to practice harmonizing.

“It worked out really well,” says Mecoli. “We have to put a part of yourself behind to be able to blend and be part of a larger group; we all became more than just the sum of our parts.” The first time the group sang the soprano, alto, tenor and bass parts together, “it was a very special moment. I could see everyone’s face light up as people appreciated the contrasting paths that the melodies were taking and how that culminated in this rich harmony.”

The choir has performed the song several times, and has plans to learn more music to sing together.

You can hear our choir sing “Ukuthula” at: https://youtu.be/OMthRYmC1XU
“There is no prescribed route to follow to arrive at a new idea. You have to make the intuitive leap.”

— Stephen Hawking