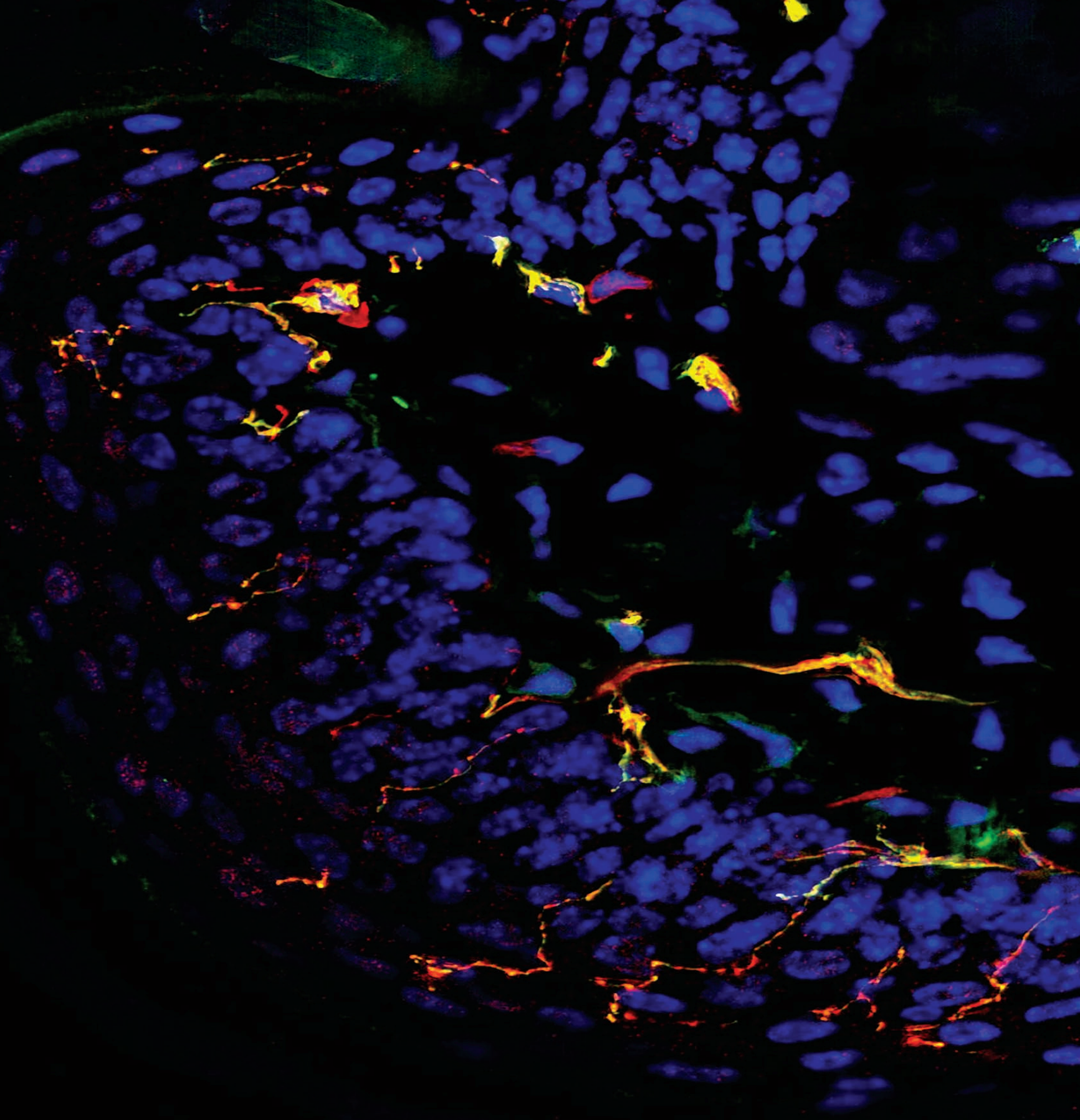

A Pitt team demonstrated that pain-sensing nerves help fight skin infections and prevent their spread, suggesting a previously unknown type of immunity. This image shows dermal and epidermal expression of ion channels (green) that allow pain to reach the brain from the skin. Here we can also see antibody expression shown in nerve fibers (red) and cell nuclei (blue).

SOME NERVE

PAIN-SENSING NEURONS ALSO FIGHT INFECTION IN THE SKIN | **BY GAVIN JENKINS**

On a sunny spring day, two kindergarteners are roughhousing on the playground. One boy pinches the other on the underside of his arm, twisting the skin hard. The boy being nipped cries in agony instantly. The nervous system is that fast. As his skin is tightly squeezed and turned, specialized nerve endings in his epidermis shoot a pain signal up his spinal cord to his brain and back to his arm in a millisecond.

While the nervous system serves as the body's information superhighway, the immune system acts like its missile defense operation. Defined by how it identifies and attacks pathogens, the immune system launches lymphocytes (B cells and T cells) and leukocytes (white blood cells) at viruses, bacteria and parasites.

And now scientists are beginning to realize just how closely these systems work together when someone is pinched, scratched, breaking out in a rash or experiencing a psoriasis flare-up. At least when there's skin in the game, so to speak, the systems work together.

When he started medical school at Washington University, Daniel Kaplan didn't know much about immunology, he says. Raised by virologists who worked in a lab together at Vanderbilt University, Kaplan fell in love with the immune system's "elegance" during his first course on the subject. He was captivated by the competition between cells and how they are widely distributed.

In particular, Kaplan became enchanted by the T cell: Its genetic shuffling that generates receptors to certain antigens. The highly choreographed process that weeds out and kills the T cells that can't detect pathogens. The suppression and removal of T cells that are reactive to proteins. A small slice of T cells left behind can detect a universe of troublemaking antigens.

"It's evolutionary theory writ small," says Kaplan, an MD/PhD professor of immunology and dermatology at the University of Pittsburgh.

Kaplan used to think the nervous system and the immune system hardly intermingled. He and other experts in the field weren't aware that neurons in the skin played an important role in integrating inflammatory signals and regulating inflammation. Then, in 2014, researchers at Harvard University published a study showing that when the nerves in the skin of an animal model of psoriasis are chemically removed, senses of pain and heat from inflammation don't arise.

At the time, Kaplan was working on a Candida infection project at the University of Minnesota. Reading the study, he realized that the Harvard researchers had used the same inflammatory pathway for their model of psoriasis as he was using on the models for his lab's study. Thanks to the Harvard study, Kaplan and his team soon concluded that they had overlooked pain-sensing neurons.

"We were like: *Oh, the nerves are one step upstream in the immune response to infection*," Kaplan says.

He and his team chemically ablated the neurons and showed that these particular neurons are required for an immune response to Candida albicans, a fungus that causes candidiasis, commonly known as thrush.

In 2015, shortly after finishing the Candida study, Kaplan left Minnesota for Pitt Med. But he continued to think about how the neurons were able to detect Candida. When he got his lab set up in Pittsburgh, he wanted to know: Is activation of the nerve alone sufficient to generate inflammation?

"To be honest, I didn't think it would be," Kaplan says. "It seemed unlikely that just neuropeptides could generate inflammation."

But Kaplan was wrong. And in collaboration with Kathryn Albers and Brian Davis, who are professors in Pitt's Department of Neurobiology, he and his team of researchers demonstrated that pain-sensing nerves—the same ones that tell you that you're in pain if you get pinched—help fight skin infections and prevent their spread. The finding, based on studies in mouse models and published in 2019 in Cell, suggests a type of immunity no one realized existed. That pain-sensing nerves can detect pathogens was known, but they showed that nerve activation alone was sufficient to trigger an immune response and also signaled protective immunity to sites adjacent to infection.

"It's a different way of thinking about the skin," Kaplan says. "It looks like the neurons in the skin are actually playing a pretty important role in integrating inflammatory signals and regulating inflammation."

Stretching across a total area of roughly 22 square feet for the average adult, skin is the largest organ in the human body. It regulates heat, contains nerve endings that react to hot and cold temperatures and acts as an anatomical barrier against an array of pathogens.

Kaplan, 52, is fascinated by the progression of skin diseases and by how immune cells interact with one another in the organ's layers. But what does he love most about studying skin immunology? The abundance of diseases for study it affords him.

"There are so many skin diseases that have dysregulated immune responses as part of their pathogenesis that we may have a real chance to have a therapeutic impact on patients," Kaplan says.

And he got into dermatology by accident. As a PhD student, Kaplan studied cancer immunology at a time when few believed the field had much promise. He spent years measuring the size of tumors on the flanks of mice. "It was something at which I was quite proficient," he says, dryly.

Like Pitt, Washington University's MD/PhD program was broken down by two years in the med school, followed by three years earning a PhD, and then ending with two more years in the medical program.

Kaplan assumed that he'd focus on hematology/oncology when he returned to medical school. But one day, a dermatology fellow who worked in a neighboring lab asked Kaplan to join a dermatology clinic. Kaplan went and was blown away by the diversity of skin diseases.

"Most of them had an immunologic basis, and I felt quite ignorant since clinical dermatology was entirely new to me," he says.

A resident asked Kaplan to help with an excision of skin cancer on a patient. She wanted him to measure the tumor size before excision, perhaps his only clinically useful skill at the time, he points out. "I decided right then and there to match into dermatology," he says.

Kaplan's passion for skin immunology has yielded results since he moved to Pittsburgh. His work has revealed the importance of TGF-ß (transforming growth factor beta)—a cytokine that controls proliferation in most cells—in maintaining resident memory T cells in the skin. His team showed that, after a skin infection, antigen-specific CD8+ T cells, which are white blood cells that kill damaged cells, including cancerous ones, migrate into the skin.

Kaplan calls his lab's research on the role of pain-sensing nerves in the skin's immunology, which he refers to as "the nerve project," the most exciting work he's done so far at Pitt. Why? *Lasers!* There are other reasons, of course, like revising how we think about our skin and the potential for therapies.

But Kaplan says optogenetics—the method of using a laser to control the activities of individual neurons in living tissue—made working in the lab fun.

Whenever Kathryn Albers looks up and sees Kaplan standing in her office doorway, he's usually smiling and energetic. "It's like: 'Okay, what exciting conversation are we going to have now?'"

JOHN ALTDORFER

From left: Kathryn Albers, Dan Kaplan and Jonathan Cohen. (Shown before the pandemic came to Pittsburgh.) Brian Davis is another key scientific collaborator.

Albers, a member of the Pittsburgh Center for Pain Research (PCPR) who became a fellow of the American Association for the Advancement of Science recently, met with Kaplan during his interview process at Pitt Med. When they spoke, Kaplan expressed interest in the center's work, and the two discussed how great it would be to collaborate if he moved to Pittsburgh.

He didn't wait long to take Albers up on the idea. On one of Kaplan's first days at the Thomas E. Starzl Biomedical Science Tower, he bumped into her in the hallway, and they discussed possible collaborative projects. Kaplan mentioned the Candida infection study he published before leaving Minnesota, and Albers told him that she and Brian Davis, a visceral pain expert at PCPR, had activated nerve fibers in mouse models using optogenetics.

They knew that the skin cells, immune cells and nerve cells were communicating. The cells have the same molecules and similar receptors; when one cell type is activated, the others respond in some way. But the team wanted to know how that response occurs and what the ramifications are in terms of the system. A project to find out seemed like a perfect extension of Kaplan's Candida study.

"When [Kaplan] came to Pittsburgh, it was an opportunity to really delve into the skin side of things," Albers says. "And also look into the immune system. Before [Kaplan] arrived, we really didn't have the expertise on board. He filled an incredibly valuable niche for us."

Like Kaplan's parents at Vanderbilt years ago, Albers and Davis are a married couple who share a lab. Davis' work examines how the peripheral nervous system (nerves and ganglia outside of the brain and spinal cord) changes and adapts.

Kaplan would soon discover that working with a neuroscientist would come in handy.

"She would point us in the right direction and tell us when our ideas were just plain wrong," Kaplan says.

Albers was uniquely qualified to work with Kaplan. At the PCPR, she uses optogenetics to study how the epithelial lining communicates with nerves. She focuses on how pain, which includes itching, she says, is processed and signaled from the skin.

When "the nerve project" began, Kaplan and Jonathan Cohen, an MD/PhD student in his lab, collaborated with Albers and Davis to develop an optogenetic mouse model where pain-sensing neurons in the skin could be activated by shining a blue laser light. Cohen, a Long Island, N.Y., native who attended Swarthmore College as an undergrad, became known around the lab as "the laser king."

Using a 473-nanometer laser, Cohen beamed a blue light onto the models while they were anesthetized. The light was set to a pulse similar to the frequency at which the neurons in the skin's nerves fire.

"Optogenetics was the best system for doing this," Cohen says. "It's common in neuroscience, but it's very new in immunology."

First, Cohen activated the neurons with the blue laser, releasing a protein called CGRP, which recruited different types of immune cells to the site on the skin. The reaction suggested that neurons can jump-start an immune response even before sentry immune cells can.

"We thought: *This is really cool*," Kaplan says.

When Kaplan's team analyzed the skin on the models, they saw cytokines, which are a characteristic of an immune response. After a couple of days of stimulation, they noticed white blood cells were being recruited to the skin, as well.

"I was surprised that just activating this subset of neuron is sufficient to generate what appeared to be a classic [immune] response in the skin," Kaplan says. "So, we did a lot of work to validate all of that."

Kaplan always uses the word "we" when describing lab work. He's quick to point out that the breakthrough wouldn't have happened without a list of researchers that includes Tara Edwards, Andrew Liu, Toshiro Hirai, Marsha Ritter Jones, Yao Li, Shiqun Zhang, Jonhan Ho and Jianing Wu, as well as Albers, Davis and their lab team.

Next, they infected the same mouse models with either Candida albicans or Staphylococcus aureus, a common bacterium that can turn deadly under certain conditions. Using optogenetics and chemical nerve blockers on the skin, researchers in Kaplan's lab showed that when the fungus infected the models at one location, the nerves not only detected and initiated an immune response to fight the infection, but also sent a signal toward the spinal cord. Those signals then boomeranged back to the skin around the infection to activate immune defenses in anticipation, preventing the infection from spreading.

Kaplan calls the nerve-driven protective mechanism "anticipatory immunity" because the nervous system can communicate information in milliseconds, compared to hours or days for the immune cells to do the same function.

What are the clinical implications of the breakthrough? Kaplan doesn't know for sure, but he says it could have implications for autoimmune diseases of barrier tissues like the skin or gut.

"Understanding this really new type of immunity raises the intriguing question of whether we could develop a drug to selectively suppress excessive autoimmune inflammation in specific tissues, avoiding the negative side effects that come with using a broad immunosuppressant that affects the entire body," he says.

That said, Kaplan did not get started on this work to find a specific cure. He's driven to understand how the body works, specifically the skin.

"I would say not enough people think about it." ■