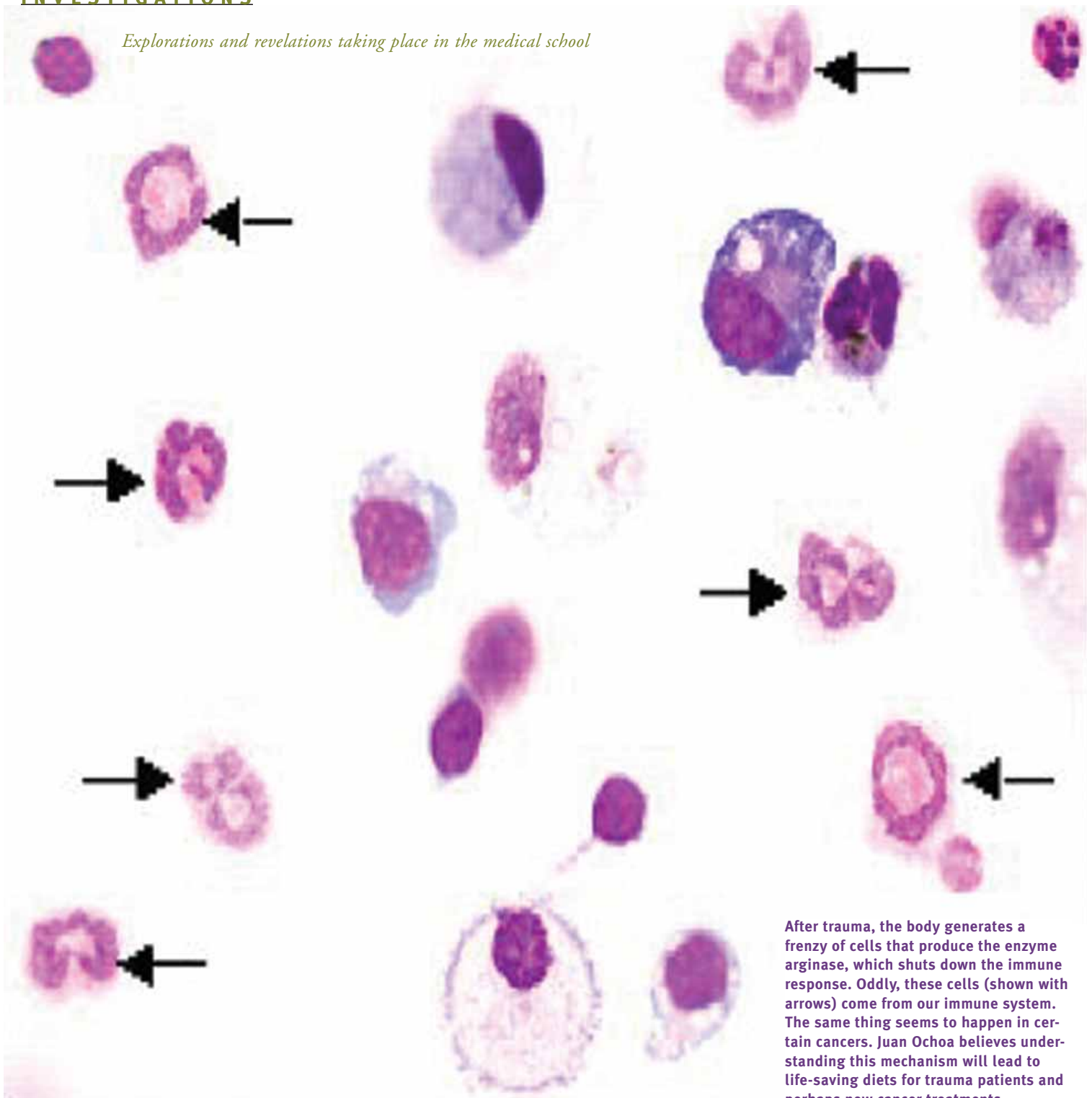


INVESTIGATIONS

Explorations and revelations taking place in the medical school



After trauma, the body generates a frenzy of cells that produce the enzyme arginase, which shuts down the immune response. Oddly, these cells (shown with arrows) come from our immune system. The same thing seems to happen in certain cancers. Juan Ochoa believes understanding this mechanism will lead to life-saving diets for trauma patients and perhaps new cancer treatments.

FEEDING TIME

A DIET THAT MAY HELP TRAUMA
AND CANCER PATIENTS

BY ERICA LLOYD

Juan Ochoa started his doctoring career as a general practitioner with a bent toward preventative medicine in the 1980s. That was in the 8,000-foot-high mountain town of Santa Barbara, Colombia, a few hours' drive from Medellín, where he'd spent his formative years. He even had a weekend radio show, "The Doctor at Your Home." But Ochoa was fresh out of medical school, a bit naive, he says, and less effective than he expected to be. He would encourage women to breast-feed, for example, not realizing that practice was stigmatized in the rural area. Even the doctor on the radio wasn't going to change people's minds.

"I went there naively thinking I was going to be a missionary. And I left thinking, 'This is far more complex than I ever thought.'"

Ochoa decided to specialize. Today, as a trauma surgeon and basic scientist, he is still a creature of prevention.

The questions the University of Pittsburgh professor of surgery and critical care medicine pursues now to save and improve lives are not so much about how to get people to change their behaviors, but how to convince cells to do so.

Since he first came to Pitt for clinical and research training in 1991, Ochoa has wondered: What do you feed trauma patients? Especially those who can't feed themselves? Many surgeons wait a few days after surgery, until the bowels are functioning again, before feeding. Ochoa believed that the right diet, given earlier, would strengthen patients, helping them survive and stave off infection.

Studies have since suggested Ochoa was right. But feeding trauma patients is not a simple matter of inserting a tube and turning on a few monitors. The science behind nutrition after trauma is at least as complex as the social taboos of Santa Barbara. Our bodies are sometimes better able to cope with a little starvation than force-feeding, notes Ochoa. Trauma patients, in particular, are highly susceptible to infection. More than 50 percent with severe injuries are likely to end up with infections. And feeding someone the wrong formula can actually make it more likely the patient ends up on the unfortunate end of those statistics.

"For some reason, the injury process creates a state of immune suppression," Ochoa says.

Many surgeons began using what's called an

"immune-enhancing" diet for surgical patients developed several years ago. That was on the heels of medical science's realization that nitric oxide, the toxic gas your car coughs out of its exhaust pipe, is also manufactured by our bodies as part of the immune response. The diet includes, among other things, the amino acid arginine, a precursor to nitric oxide.

Ochoa believes the diet is probably appropriate for trauma patients and has tested safe for most other surgical patients. But surgeons aren't in agreement about that. There's no protocol in the United States—use of the diet differs from doctor to doctor and case to case. (The European society concerned with nutrition among surgical patients recommends using such diets before elective surgery.)

Now Ochoa believes that doctors are on the cusp of understanding how an arginine diet works and why it may be helpful for some patients and not others. His family ties have helped him make sense of the science.

His brother, Augusto Ochoa, is interim director of Louisiana State University's Stanley S. Scott Cancer Center. When Augusto Ochoa

Juan Ochoa had learned from his own investigations that arginase, an enzyme that metabolizes arginine, is present at elevated levels in the tissue of trauma patients. Patients with more arginase don't do well. If they survive, they are sicker and stay in the hospital longer. The high levels of arginase somehow inhibit the ability of the injured body to keep the needed arginine in store.

The Ochoa brothers began exploring whether the immune system is dampened the same way in cancer patients. They found that arginase is elevated in the tissue of cancer patients, much like it is in trauma patients.

The brothers also learned what arginase was up to: At the heart of the immune response, it robs the arginine necessary to build the zeta chain. That leaves the T cell unable to proliferate to help fight off intruders and leaves the patient unprotected.

Augusto Ochoa and collaborators have since injected arginine into the tumor of a mouse. That therapy stopped the tumor from growing further.

Juan Ochoa believes that immune-enhanc-

What do you feed trauma patients who can't feed themselves?

was a researcher at the National Cancer Institute in the '90s, he began to answer a question that perplexed many cancer researchers: Why don't our immune systems put up a better fight against cancer cells?

"For many years," Juan Ochoa explains, "doctors have been trying to boost the immune system to make it reject the tumor. But in a good number of patients, those lymphocytes that have been recruited to get rid of the tumor just don't seem to work."

In the early '90s, cancer researchers, notably Augusto Ochoa, recognized that a peptide called the "zeta chain" was missing in lymphocytes (specifically T cells) of cancer patients.

"People didn't know why it was lost, but they knew it was important," says Juan Ochoa. T cells play a vital role in defending cells from viruses, foreign tissue, and other unwelcome guests.

Years later, in 2001, the Ochoa brothers began publishing reports showing that arginine was essential to maintaining the zeta chain. Around the same time, a Japanese scientist showed that trauma patients were missing the zeta chain.

ing diets work similarly: A high concentration of arginine restores the zeta chain, allowing the T cell to do its job to fight off infection.

"It's not proven. But we're closing the loop on that," he says.

He's anxious to start refining a diet for precommercial testing in trauma patients. (Arginine, for example, doesn't taste great and can have adverse effects. Juan Ochoa is looking toward substituting it with citrulline, a more palatable amino acid found abundantly in watermelon. Citrulline works the same way and seems to be safer.) "Now that we know the mechanism, we can make better diets," he says.

He's optimistic about the possibilities: "So if we could block the [process that cuts out the zeta chain], we could potentially have a treatment for cancer. We could also have a potential treatment for infection in trauma."

He suspects that the immune system is dampened the same way in leprosy and tuberculosis and wonders whether those diseases could be managed through diet as well. ■

Jessica Mesman contributed to this article.



Birds have it. Bees have it. Even yeast have ubiquitin (shown here). In humans, it helps us keep going by tagging defective proteins for the proteasome, which chews them up into their constituent amino acids.

COURTESY CORNILESCU, G., MARQUARDT, J.L., OTTIGER, M., BAX, A.
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HEAVILY ENGAGED

**A FORMERLY HIDDEN PROTEIN
SHOWS UP IN JUST ABOUT
EVERY LIFE-FORM AND TONS
OF IMPORTANT PROCESSES
BY JAMES SWYERS**

In the 1970s, American researchers were looking for clues to the cause of the human autoimmune disease myasthenia gravis. They found a protein that appeared to have some of the properties that could make it a potential cause of MG. When researchers added the protein to certain types of human immune cells, those cells became more specialized. Investigators also were intrigued by the fact the protein was very good at attaching itself to other proteins, suggesting it had some immune properties.

Unfortunately, their candidate protein flunked the final, and most crucial, test. Researchers believed that for the protein to be involved in MG, it needed to be unique to the thymus gland, which is where immune cells get their marching orders. But when they looked for the protein in other kinds of cow tissue, the medium of their first

experiments, it was in every one. Then they found it in every type of tissue they looked at. The stuff was in pigs, guinea pigs, mice, plants, insects, people, even yeast. The only place it didn't show up was in a few primitive microorganisms.

In 1974, the scientists introduced their new protein—aptly named “ubiquitous immunopoietic polypeptide” (later shorthand to “ubiquitin”)—in a paper in the *Proceedings of the National Academy of Sciences*. Then they promptly abandoned it because it wasn't the thymic hormone they sought. An aside at the end of the *PNAS* paper noted that the protein occurs so universally it must be an “integral feature” of nearly all living things.

Ubiquitin would not resurface as a research topic until five years later when two Israeli scientists, collaborating with an investigator at the University of California, Irvine, set out to find the explanation for a recently discovered phenomenon known as “energy dependent protein degradation.”

Researchers in the 1970s had noted that whenever there was a burst of amino acids released inside cells, there was a corresponding consumption of energy. Looking for what was using the energy—figuring it was chewing up proteins and releasing amino acids in the process—the investigators turned up what they thought was a newly discovered protein. It seemed to be able to degrade other proteins under certain conditions. Upon talking to their colleagues and searching the scientific literature, they realized what they'd come upon was ubiquitin.

In a flurry of studies in the early 1980s, researchers showed that ubiquitin does not degrade proteins directly but rather flags them for demolition by a long, hollow molecule called a proteasome. It takes at least four ubiquitin tags for a protein to be targeted for destruction. As the protein is degraded by the proteasome, the ubiquitin molecules are liberated, free to search out potential new target proteins for disposal.

And scientists made another important discovery: Ubiquitin mostly tags abnormal proteins for destruction. Unfortunately, it took a few decades before the full implication of this latter finding was appreciated. As one retelling of those events recently noted, ubiquitin research “was little more than a backwater of biochemistry studied by a handful of laboratories” for many years to come.

Ubiquitin's status as a topic of research interest did not change dramatically until

2003, when the Israelis and one of their American collaborators were given the Lasker Award for their work on the ubiquitin-proteasome pathway. A year later, the two Israelis and another American colleague were awarded the Nobel Prize for Chemistry.

Scientists now recognize ubiquitin as a master regulator of a broad host of cellular processes. They suspect that alterations in the ubiquitin-proteasome protein-disposal system factor into a number of diseases, including neurological conditions, liver diseases, eye diseases, and a variety of cancers.

Just as the greater scientific community was beginning to recognize the importance of the ubiquitin-proteasome pathway, Yong Wan, now a PhD assistant professor of cell biology and physiology at the University of Pittsburgh School of Medicine, was arriving at Pitt after completing his postdoctoral training at Harvard University.

At Harvard, Wan had worked for cell biologist Marc Kirschner. One of the first to understand the importance of the pathway for normal cell functions in the mid-1990s, Kirschner and his lab had discovered a ubiquitin

ligase known as anaphase-promoting complex (APC), which is involved in making sure that cells divide properly. Kirschner assigned Wan to work on APC, and Wan brought that project with him to Pitt in 2003, where he was one of just a handful of investigators (including the medical school's dean, Arthur S. Levine) interested in this relatively new area of inquiry.

Today, Wan's lab is one of several dozen at Pitt delving into some aspect of this multifaceted enzyme. Others are investigating ubiquitin's role in neurological conditions, including Alzheimer's, as well as xeroderma pigmentosa, an inherited disease that makes people extremely sensitive to natural sunlight while putting them at high risk for skin cancer. The disease can inhibit the effects of radiation therapy and chemotherapy on tumors, promote the growth and proliferation of cancers, and influence circadian rhythms.

Wan has several large grants from the National Cancer Institute and the American Cancer Society to not only investigate APC's role in normal cell division but also to look at

whether environmental factors, such as chemicals or radiation, might damage APC or cause other alterations in its activity.

“The major role of APC is to control the separation of chromosomes during cell division by ungluing the chromosome strands. Deregulation of this separation process usually results in catastrophic alterations in the shape and number of chromosomes in a cell, which is a hallmark for many types of tumor cells,” Wan explains.

His laboratory, based at the University of Pittsburgh Cancer Institute, also looks into ubiquitin's role in stem cell maturation. Wan believes the ubiquitin-proteasome system acts like a licensing agency, allowing stem cells to become specific types of cells, such as muscle or liver cells, by orchestrating the degradation of all but one type of signal.

Others at Pitt have suggested that ubiquitin influences how we perceive the world.

The laboratory of Yong Tae Kwon, a PhD assistant professor in the Center for Pharmacogenetics in the School of Pharmacy, explores a pathway in the ubiquitin-proteasome system called the N-end rule pathway.

Wan believes the system acts like a licensing agency, allowing stem cells to become specific types of cells.

Takafumi Tasaki, a PhD and a member of the Kwon lab, has uncovered indications that the pathway is involved in regulating sensations in mice. “It is only a hypothesis right now, but we think it may be important in regulating synaptic transmission in sensory systems, including smell, taste, vision, and hearing,” he says.

Others in the Kwon lab have shown that the enzymes in the N-end rule pathway play essential roles in heart formation, blood vessel differentiation, male reproduction, chromosome stability, neural tube development, muscle protein degradation, fat metabolism, and blood development.

Although it is hard to predict where all of this research will lead and when it will turn into treatments for major diseases, it is safe to say that ubiquitin research is likely to become much more, well, ubiquitous in the coming years at Pitt.

“When I arrived here a little more than four years ago, there was not a lot of research in ubiquitin going on,” recalls Wan. “But now, research in this area is booming.” ■