

INVESTIGATIONS

Explorations and revelations taking place in the medical school



Acute infections in the gut can throw the immune system out of whack. Timothy Hand and colleagues are learning ways to reboot the system in mice.

REBOOT AND REBUILD

THE IMMUNE SYSTEM'S RESET BUTTON

BY SHARON TREGASKIS

Public health experts have known since the '60s that kids who are chronically malnourished don't mount the same vigorous immune response to vaccinations as their peers. This limitation is especially troubling in the developing world: Children most in need of protection from an array of infectious diseases get the least benefit from vaccines.

Timothy Hand, a PhD assistant professor of immunology and of pediatrics at the University of Pittsburgh, has devoted his research career to understanding the relationship between the immune system and the microbiome—the rich ecosystem of bacteria, fungi, and viruses that live largely in the gut. His current investigations explore how “immunological scarring” in the wake of a single acute intestinal infection could explain incomplete vaccination. This scarring may also play a role in a host of chronic inflammatory conditions such as ulcerative colitis and Crohn's disease.

Like soldiers in urban warfare, immune cells patrolling the gut must somehow differentiate dangerous pathogens from the thousands of microbial species that aid in our digestion and immune function. Further complicating the job: Though beneficial at certain levels, microbes can wreak havoc if their numbers grow out of control. Says Hand, “Infection in the intestinal tract presents a special problem for the immune system.”

In a 2012 paper published in *Science*,

Hand and Yasmine Belkaid, his mentor at the National Institutes of Health, where he was a research fellow at the time, demonstrated that during an infection—the fog of war—immune cells in the gut don't discriminate; friendly fire is rampant as the immune system blasts beneficial and hazardous microbes alike. But once the infection clears, immune cells that had previously attacked beneficial microbes stand down.

“They're sensitized, and they can be reactivated very easily, but they're ignoring [the beneficial microbes],” Hand says.

“As long as the barrier of the gut is maintained, and as long as the cells have the correct regulatory mechanisms in place, they don't become activated.”

Hand and his NIH colleagues had a hunch that acute infection might throw those regulatory systems out of whack. To find out, they exposed mice to a particularly vicious foodborne pathogen. In a paper published last October in *Cell*, Hand and his coauthors revealed how more than nine months later—nearly 25 years in human terms—the gut tissue and immune function among most of those experimental mice remained altered, though the infection was long gone.

Abscesses had formed in the murine lymph nodes, which were populated with dangerous levels of microbes congregating where they didn't belong. And like a road pockmarked by winter weather, the linings of their guts had been transformed. The once-tidy structure of the fatty tissue sandwiched between

the intestinal lining and lymph nodes had become so cluttered that motility of the cells vital to immune communication and function was drastically impaired.

The team then administered a series of vaccines to the mice and found that they triggered both hypersensitivity to food antigens and relatively weak responses to infectious agents.

Finally, the scientists effectively hit a reset button in their subjects' immune systems—by giving the mice a massive dose of broad-spectrum antibiotics.

“The chronic problems in the mouse model were caused by a broken interaction between the immune system and the lymphatic cells that carry immune cells,” says Hand, who cautions that, in humans, using antibiotics might be overkill.

“We need to think of new ways to fix that relationship,” he says. A systemwide reset might be accomplished instead with comparatively gentle interventions such as probiotics or dietary changes.

In his ongoing work—which includes collaborations with investigators at Children's Hospital of Pittsburgh of UPMC—Hand is investigating the particular host- and pathogen-related factors that can trigger permanent damage.

“I'm very, very interested in the vaccination defect,” he says.

He notes there's a big effort under way to determine what it is that makes a vaccination work or not work. ■

WEATHERING THE STORM

**PNEUMONIA DRUGS
TO TURN THE TIDE**
BY NANCY AVERETT



POBYTOV/GETTY IMAGES

Of the more than 1 million Americans who are hospitalized for pneumonia each year, about 5 percent die. Their demise is often caused by severe inflammation brought on by what experts call a “cytokine storm,” during which the immune system—called to duty by cytokine messenger proteins—overreacts and sends too many white blood cells to fight the infection. The white blood cells release more cytokines, which in turn signal the release of more white blood cells. This positive feedback loop eventually causes excess inflammation and damages healthy tissues and organs.

Treatment presents a delicate dilemma. “You don’t want to shut down inflammation, because inflammation itself is important for healing and normal repair after infection,” says Rama Mallampalli, Pitt MD professor of medicine, chief of the Division of Pulmonary, Allergy, and Critical Care Medicine, and director of the Acute Lung Injury Center of Excellence at the University of Pittsburgh. “You want to temper [inflammation], so it’s more controlled and doesn’t become hyperinflammatory.”

In May 2013, Mallampalli published a *Nature Immunology* paper with Bill Chen, a PhD and Mallampalli’s codirector at the center, Bryan McVerry, Pitt MD assistant professor of medicine and environmental and

occupational health, and Frank Sciruba, the MD director of Pitt’s Emphysema COPD Research Center as well as the Pulmonary Function Exercise Physiology Laboratory. They demonstrated that a particular protein, Fbxo3, which is activated during severe infection and pneumonia, stimulates cytokine secretion from human inflammatory cells. Fbxo3 is “very, very pro-inflammatory,” Mallampalli says. The team has since designed, synthesized, and tested a class of small molecules known as F box inhibitors that could block Fbxo3’s actions. The drug candidate is an attractive alternative to corticosteroids such as prednisone, which are used to treat severe inflammation but are not tolerated well by some patients. In January, Mallampalli, a National Institutes of Health–funded investigator, received \$100,000 in new support for his F box inhibitor work when he was named a Harrington Scholar-Innovator—an award that recognizes physician-scientists who have “the potential to change standard of care.”

“With this and other support, we should be able to move [this research] to first-in-human studies; that’s our goal,” Mallampalli says. “We’ve already done large- and small-animal studies and numerous efficacy studies.”

There’s more news from Mallampalli on the perfect storm that is pneumonia.

In addition to perilous inflammation, the

disease, which strikes between 5 and 10 million Americans a year, also often causes epithelial cell death in the lung, for reasons that are not clear.

In October 2015, Mallampalli, Chen, and Chunbin Zou, an MD/PhD research assistant professor of medicine, published a paper in *Science Translational Medicine* demonstrating that a particular strain of pneumonia-causing bacteria, *Pseudomonas aeruginosa*, activates a protein called Morf411, “which acts like a death toxin that kills the lung cells,” Mallampalli says. Normally, another F box protein, Fbxl18, destabilizes Morf411 so that it does not build up in epithelial cells; however, the bacteria induce a process that protects them from Fbxl18.

Mallampalli and his colleagues screened thousands of small-molecule drugs for one that would bind to Morf411 and block its actions in animal models. They found what they were looking for in argatroban, a drug previously approved by the FDA as an anticoagulant. Their next step: a hard look at previous UPMC patients’ outcomes to see whether those who took argatroban differ from controls when it comes to pneumonia incidence and severity.

P. aeruginosa is antibiotic resistant. If the new use for argatroban proves successful, the drug would become a promising nonantibiotic treatment for bacterial pneumonia—good news indeed. ■



TIM GROEN

TRAUMA'S IMMUNOLOGICAL IMPACT

BY ALLA KATSNELSON

Say you're in a bad car crash: broken bones, head wound, internal bleeding, the whole nine yards. Your blood pressure tanks, depriving the cells in your body of oxygen. It's not just the trauma and the bleeding that are so destructive—it's also their immunological aftermath.

According to the textbook understanding of immunology, microbial pathogens or other infectious invaders are what spur the innate immune system into action. But in the past 15 or 20 years, scientists have realized that noninfectious events—heavy blood loss, tissue damage, a broken arm, or even a planned surgery that piques some parallel danger-sensing system—can also ignite inflammation that *itself* can bang tissues around.

"It's only recently been appreciated that such injuries can activate all the same pathways of the innate immune system that infections can," says Melanie Scott, an MD/PhD research assistant professor of surgery at Pitt. "We're only starting to understand how that works."

Scott is at the forefront of this effort. Shortly after arriving at Pitt in 2003 for a postdoc in the lab of surgery department chair Timothy Billiar, an MD and the George V. Foster Professor of Surgery, she began exploring the mechanism of sterile inflammation—that is, inflammation that occurs without an infection—in mouse models of trauma, severe

blood loss, and tissue oxygen starvation (or hypoxia).

Inflammation—whether sterile or not—is activated when immune cells like macrophages release certain immunoreactive substances. Among them is the inflammasome, a recently discovered cluster of molecules that forms in response to signals from receptors that sense infection or danger. The inflammasome's task is to rouse the enzyme caspase 1, which then cranks up production of molecules called cytokines. But what increasingly perplexed Scott was that the inflammasome activated caspase 1 not just in immune cells, but also in other types of cells—such as liver cells—that don't produce cytokines.

"This was strange," she says, "so we asked, *Well, what are these cells really doing with this inflammatory molecule?*" When it came time to launch her own lab in 2007, Scott stayed on at Pitt, where, three years ago, she snagged an R01 grant to explore this question.

In mice engineered to lack caspase 1 in liver cells, she and her colleagues found that hemorrhagic shock and hypoxia were more damaging than in normal animals, suggesting that caspase 1's activation was protective.

Liver cells—which have high-energy jobs such as making proteins for other parts of the body—are especially rich in mitochondria, the organelles that act as cellular powerpacks. But hypoxia damages mitochondria, causing

them to release what are called reactive oxygen species, which damage cells. In a paper published in the *Journal of Biological Chemistry* in 2013, Scott's lab suggested that activation of the inflammasome and caspase 1 turns on a pathway that chomps up the damaged organelles, essentially recycling them for spare parts. "That protective response is what's removing the production of reactive oxygen species," Scott says.

Her lab is trying to work out the details of how this pathway is regulated. Scott believes that it's DNA from the struggling mitochondria that sound the danger signal activating the inflammasome, and that its activation is graded, depending on the amount of reactive oxygen species the damaged mitochondria produce. That's interesting, she says, because inflammasome activation has generally been thought to be all or nothing.

Ultimately, hammering out the details of inflammasome activation in its many permutations will help scientists understand many disease processes, beyond trauma and hypoxia, says Scott, such as autoimmune diseases and inflammation associated with obesity. Her work also underscores the fact that the effects of revving up or stalling this activation may vary by cell type.

"We're not really there yet," she says, "but the idea is to find some way to regulate these different pathways in a beneficial manner." ■