Explorations and revelations taking place in the medical school

Recently, Pitt researchers uncovered how omega-3 fatty acids—which are found in fish and other foods—help to ebb the tides of inflammation that contribute to cardiovascular disease.

BIG CATCH

REELING IN THE SECRETS OF OMEGA-3 FATTY ACIDS

BY JOE MIKSCH

On the rivers, there are fishermen. In the Bible, there are fishers of men. In Bruce Freeman's lab, there are fishers of molecules. And he and his crew of investigators have nabbed a compelling catch.

Freeman, a PhD and the UPMC/Irwin Fridovich Professor and Chair of the Department of Pharmacology and Chemical Biology at the University of Pittsburgh, has recently developed an interest in the anti-inflammatory properties of omega-3 fatty acids, which are found in fish, some plant oils, and nuts. Research shows that those whose diets are rich in omega-3s have, as Freeman puts it, "a significant decrease in stroke, heart attack, and heart failure and other inflammatory disorders."

We know that much. What we didn't know—until Freeman, Chiara Cipollina, Alison Goeger, and Francisco Schopfer, among others, published a study in *Nature Chemical Biology* in May—was how omega-3s do their work.

To find out, Cipollina went fishing.

Cipollina, a PhD, spent the last two years at Pitt working in Freeman's lab as a Ri.MED fellow. The Ri.MED Foundation, founded in 2006, is a collaboration between the University of Pittsburgh, the Italian government, UPMC, and Sicily intended to improve public health in Sicily and worldwide.

Before going fishing, you might make sure the waterways are stocked. For this, Freeman's team employed activated macrophages—immune cells present in inflamed tissue. Macrophages use omega-3 fatty acids as signaling mediators.

Next, using the chemical compound beta-mercaptoethanol (BME) as bait, Cipollina "hooked" the omega-3 derivatives produced by the macrophages. "We always knew when 'fishing' was going on in the lab," says Freeman. "[BME] has a lot of chemical similarities to the odorant of skunks."

The researchers learned that the omega-3 derivatives are chemically modified by the active macrophages. They become electrophilic fatty acid oxidation products (EFOX)—metabolic byproducts that are attracted to electrons and react with important molecular targets in myriad cell types. The Freeman lab found that as EFOX binds to protein residues—such as those found in the BME bait—it stimulates antioxidant and anti-inflammatory responses, the actions that contribute to the beneficial outcomes of an omega-3-rich diet.

The Freeman lab's *Nature Chemical Biology* paper also elucidates how an enzyme called cyclooxygenase-2 (COX-2), the molecular bull's-eye for aspirin and similar drugs, manages the transformation of omega-3s to EFOX. Aspirin, it turns out, enhances the production of EFOX. Hence less inflammation.

"One of the beautiful symmetries of [Cipollina and Schopfer's] discovery is that the source of these activated omega-3 fatty acids is a pro-inflammatory enzyme [the aforementioned COX-2]," Freeman says. "What she and the others discovered is that when aspirin inhibits COX's inflammatory properties, it also stimulates COX's ability to add oxygen to omega-3 fatty acids."

Freeman, Cipollina, et al., think that understanding these mechanisms presents enormous potential for drug discovery, notably for new approaches to mediating inflammation. The omega-3 anti-inflammatory process works well enough in nature, Freeman says, given that its cardioprotective effects have been proven. But understanding how these benefits are arrived at opens up grand possibilities for designing molecules that can do the job even faster and more efficiently.

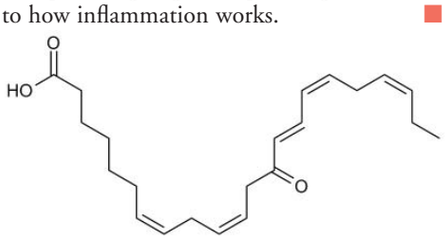
"Natural isn't always best," says Freeman.

This new EFOX insight could lead to new organic-synthetic therapies that improve on Mother Nature, Freeman says, "so that you can have the same clinical benefit from much lower concentrations given less frequently." Cipollina says that this work might, in fact, lead to completely different ways of treating inflammation.

"What we do now is kind of a negative approach in that we're trying to shut down or stop the inflammatory process with drug therapies," she says. Curtailing inflammation entirely, however, can have unpleasant consequences—inflammation is the body's vanguard against injury and infection.

But, she says, knowing the molecular ins and outs of inflammation might permit the crafting of drugs that can target individual parts of the process, taming inflammation when necessary and letting it do its thing in other cases.

Although Cipollina has completed her fellowship and is back in Italy, Freeman and Schopfer are going to keep fishing for clues as to how inflammation works. ■



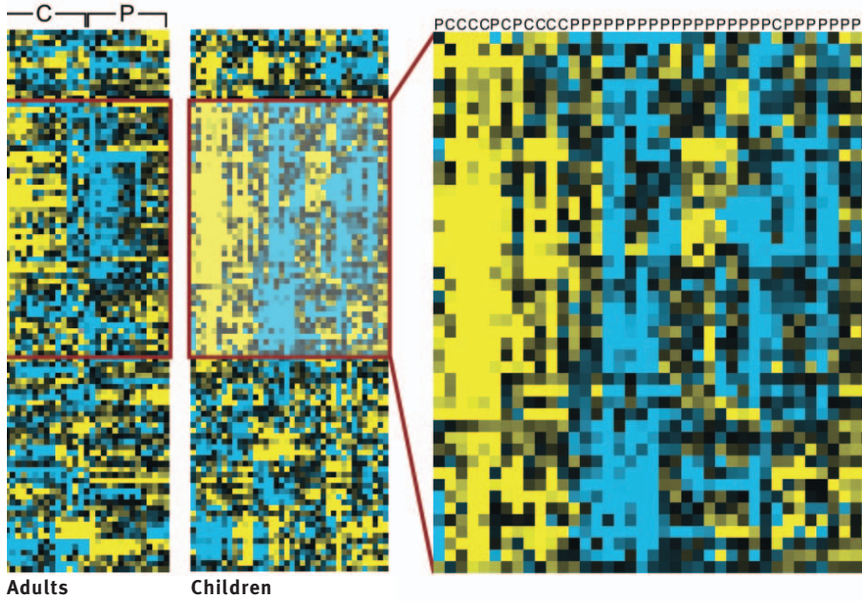
EFOX, shown here, is the key to tapping the anti-inflammatory potential of omega-3 fatty acids.

GETTY IMAGES

DNA DAMAGE PEGGED

NEW BLOOD ASSAYS FOR A DEVASTATING DISEASE

BY CHUCK STARESINIC



LEFT COLUMNS: Heat maps of adults and children reveal gene expression in FRDA patients (P) and controls (C). RIGHT COLUMN: A computer algorithm has sorted the patients based solely on gene expression. A detail of 46 genes known to be associated with one another suggests that the genes are relevant to the disease, as well, with patients and controls mostly clustering together on opposite sides of the column. Yellow represents higher gene expression and blue lower expression.

COURTESY *PLOS GENETICS*, JANUARY 2010, VOL. 6, ISSUE 1

"Bench to bedside" is one of the catch phrases in medicine these days. Another way to think of it is "yeast to human." That's the leap that was made by Ben Van Houten and Astrid Haugen at the National Institute of Environmental Health Sciences in 2005.

Van Houten, a PhD molecular biologist who is now the Richard M. Cyert Professor of Molecular Oncology and a professor of pharmacology and chemical biology in the University of Pittsburgh School of Medicine, had long had an interest in Friedreich's ataxia (FRDA), a neurodegenerative disease marked by low levels of an essential cellular protein. He had knocked out the gene for the protein in yeast to study the resultant DNA damage. A technician in his lab, Haugen, had the expertise to study gene expression in cells—whether human, mouse, or yeast. When the National Institutes of Health introduced a bench-to-bedside award, Haugen and Van Houten got the idea to expand this research to humans.

Friedreich's ataxia is a devastating disease both for those who suffer from it and for their families. It typically appears out of nowhere in young people between the ages of 5 and 15. Parents might notice that their child has trouble running in a straight line or is just not running as well as he or she used to. This is ataxia—an inability to coordinate voluntary muscular movements. Most FRDA patients use a wheelchair eventually. They develop speech problems. Their vision deteriorates. Heart problems begin in the teens. They develop cardiomyopathy and enlarged hearts. FRDA is uniformly fatal, with cardiac arrest often causing death in middle age. There is no effective treatment.

The disease results from a mutation to a gene called frataxin. In this type of mutation—triplet repeat expansion—a trio of nucleotides abnormally repeats several times within the gene. People who inherit this rare mutation from both parents are unable to produce the proper amount of the frataxin protein. Produced in the nucleus, frataxin is transported to the mitochondria, where it plays a key role in the creation of iron-sulfur centers—important components of a host of proteins and processes that begin in the mitochondria. Without frataxin or sufficient iron-sulfur centers, the cell sends out signals to take up more iron, too much of which can be destructive to a cell by damaging macromolecules like DNA.

"If you cut through the heart, you can see that there are large iron deposits," says Van Houten as he pulls up a pathology slide from a deceased patient with FRDA. "So there is this iron overload in the heart and other tissue because the protein frataxin is actually an iron shuttle. It takes iron and puts it into the iron-sulfur centers."

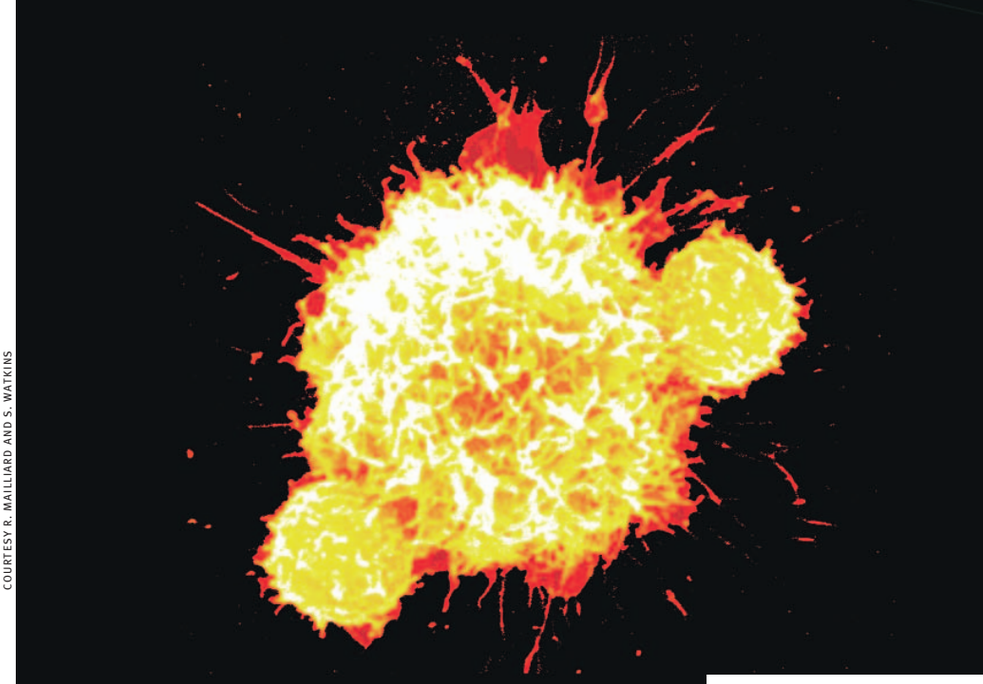
Haugen planned the experiments with Van Houten. "Astrid is a thinker and a doer," he says. "She drove this project." (Van Houten calls her a "supertech.") The researchers took blood samples from children with FRDA and compared the gene expression patterns with samples from young, healthy blood donors. They identified sets of genes that were very differently expressed in FRDA patients, including several sets associated with DNA damage. It was a very clear signal—and a novel finding—that FRDA patients were accumulating DNA damage.

In another first, the group used an assay developed in Van Houten's lab, called QPCR (quantitative polymerase chain reaction) to identify specific regions of DNA that were damaged. Using just a fingerprick of blood ("All we need is 5 nanograms," says Van Houten), the group compared FRDA patients and controls and found clear evidence associating FRDA with DNA damage—especially in the mitochondria. Their findings were published in *PLoS Genetics* in January.

These discoveries suggest the intriguing possibility that scientists could, in the future, routinely identify specific types of DNA damage in an FRDA patient with a single drop of blood. Further research along these lines could usher in the day when doctors evaluate a detailed list of biomarkers found in that tiny sample, then sit down at the bedside for a discussion of the disease mechanisms at work, prognosis, and therapeutic options tailored to the patient. ■

THERAPEUTIC HIV VACCINE TO GET THE IMMUNE SYSTEM FIGHTING AGAIN

BY SARA GOUDARZI



COURTESY R. MAILLIARD AND S. WATKINS

A dendritic cell (center) activates two T cells to tackle a foreign invader.

BACK IN THE RING

HIV is more persistent and evasive than most invaders of the human body. Without medications to suppress the virus, in about eight years, a person with chronic HIV infection will develop AIDS, and her immune system will fail. But what if there were a way to arm the immune system to take up the fight again and eventually eliminate the virus? That's the goal of MD Bernard Macatangay, assistant director of the University of Pittsburgh Immunology Specialty Laboratory.

Researchers are currently working to develop two types of vaccines for HIV, Macatangay explains: prophylactic and therapeutic. HIV prophylactic vaccines are designed to prevent uninfected persons from acquiring the virus; HIV therapeutic vaccines are used as part of a treatment regimen. Macatangay's group concentrates on the latter.

"When someone has cancer, they use chemotherapeutic agents," he says, "but at the same time, there are immunotherapeutic agents allowing the body's immune system to fight the cancer. That's what we're doing now in HIV."

Macatangay came to Pitt in 2006 on an infectious disease fellowship. In 2008, he began a second fellowship to study HIV/AIDS, joining the lab of Charles Rinaldo, a PhD and chair of the Graduate School of Public Health's Department of Infectious Diseases and Microbiology, who also has an appointment in the Department of Pathology in the medical school. When Macatangay arrived, Rinaldo and others—including Sharon Riddler (MD associate professor of medicine) and Theresa Whiteside (PhD professor of pathology)—had finished clinical trials of a therapeutic dendritic cell–based HIV vaccine in 2006 and had just published their results.

Dendritic cells are immune cells that capture foreign antigens and present them to killer T cells. In so doing, the T cells become activated and target the foreign antigen.

The treatment met with moderate and fleeting results. The 18 patients enrolled in Rinaldo's clinical trial responded, but only modestly. The reason, he and Macatangay discovered, involved regulatory T cells (Treg)—cells that keep the immune system balanced so it doesn't go haywire and attack the body. Once an infection is controlled, Treg shut off the killer T cell response.

For the patients involved in the trial, Treg were shutting things off too early and actually suppressing the immune system.

"We need to investigate what particular mechanisms these Treg are employing to suppress the immune response," Macatangay says. "By blocking their actions, we may be able to boost the immune response and control HIV."

Shutting off Treg is not without complications, however. Treg have the ability to control the immune response to infection. For instance, while wiping out harmful microbes, the immune system can also trigger chronic inflammation. Treg can rein in this inflammation, preventing the tissue damage that inflammation can cause when left unchecked.

If a drug completely blocked Treg action, patients could develop severe autoimmune symptoms.

The researchers are now conducting a second clinical trial of a vaccine. This time, each dendritic cell is loaded with the subject's own inactivated virus—instead of with HIV peptides (proteins thought to increase immune response), as in the previous trial.

Unlike prophylactic vaccines, which can be commercially manufactured and given to an entire population, the therapeutic vaccine is tailored to each patient.

So far, four patients have completed clinical trials for the vaccine and four are currently enrolled in the study. This new study is one of the most intensive and time consuming of the clinical studies conducted at Pitt; however, all subjects have tolerated the vaccine and its mild side effects.

"Most of these patients say that even if [this study] doesn't help them," says Macatangay, "if somehow what we're doing helps the next group of people, they would gladly go through it."

Macatangay and Rinaldo expect to have results to report by early 2012. ■