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Nervous System Rallies Immune System Forces Against Invading Pathogens

Researchers have discovered a communications hot line that lets a worm's nervous system dial the immune system to help coordinate the response to infectious pathogens.

The new research is the first to identify direct evidence that specific cells in the nervous system coordinate initial defenses against toxic bacteria. Those first responders are part of the innate immune system, a kind of sixth sense that is hard-wired and fends off invading microbes until the adaptive immune response is mobilized.

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It has been recognized for at least 20 years that there must be bidirectional communication between the nervous and the immune systems, says Alejandro Aballay at Duke University Medical Center. But because of the complexity of the communication network it has been very difficult to prove this connection conclusively. The complexity of the nervous and immune systems of mammals, including humans, makes sorting out neural-immune communications a daunting task.

To cut through this complexity, Aballay and his colleagues turned to the simple roundworm, *C. elegans*. It proved to be an ideal model for dissecting those elusive connections—and for bringing together a diverse research team whose only connection was a signaling protein known primarily for its effect on the social life of worms. The research team included Aballay, Howard Hughes Medical Institute (HHMI) investigator Cornelia Bargmann at the

Rockefeller University, as well as Sarah Steele, an undergraduate research student funded by an HHMI science education grant to Duke. The research is reported in the September 18, 2008 edition of *Science Express*, which provides electronic publication of selected *Science* papers.

Aballay is a long-time translator for HHMI's Spanish research news. But in his day job as an assistant professor of molecular genetics and microbiology, he studies the innate immune system using his model organism of choice, the roundworm *C. elegans*. As part of this work, Aballay examined roundworms to find out which ones thrived and which died in an environment containing infectious bacteria. Only one of the 40 varieties he tested succumbed rapidly to bacterial infection. That particular worm had a mutation in a gene called *npr-1*.

Bargmann had first identified *npr-1* years earlier. Her research showed that the gene coded for a receptor found in at least 20 neurons that control whether worms prefer to feed alone or in groups. But Aballay's work suggested that *npr-1* might also be involved in responding to the presence of infectious bacteria.

Aballay's and Bargmann's groups began to work together to find out whether Aballay's theory was correct. His graduate student, Katie Styer, went to Bargmann's lab in New York City to learn new research techniques that would permit her to see how the worms responded under varying oxygen conditions. Styer wanted to understand whether the mutant gene was simply making the animals die sooner than normal or whether it really was dampening the worms' ability to mount an immune response to bacteria.

At about this time, Aballay's lab welcomed Steele, an undergraduate biology major and a member of the Howard Hughes Research Fellows program at Duke. The program, which was started in 1991, is supported by an HHMI grant to Trinity College at Duke. It is one of the university's longest running summer research programs.

Steele was interested in neurobiology and wanted to work on a research project during the summer between her freshman and sophomore year. She joined Aballay's lab as it was beginning a series of experiments to determine which genes were affected by the normal NPR-1 protein and the mutant form that Aballay had identified.

The researchers knew the NPR-1 receptor belonged to a large protein family that in mammals helps govern biological processes ranging from the senses of taste and smell to heart rhythm. But they did not know how the mutant form of *npr-1* was related to the immune system or how it changed the worm's response to infectious bacteria in its environment.

To find out, Styer and Steele used gene expression microarrays to measure the activity level of thousands of genes under differing experimental conditions. The students' experiments showed that the mutant NPR-1 protein affected a small group of genes that were directly involved in the immune system's response to bacteria.

The results of the microarray studies surprised me, Aballay explains. Most of the changes we did observe corresponded to genes already known to be involved in innate immunity.

The researchers found that the genes affected by NPR-1 are expressed in worm tissues such as the gut that are most likely to be in direct contact with bacteria during an infection. These tissues can secrete antimicrobial substances that work—as part of the innate immune system—to protect the animals against infection. Under normal circumstances, there are break-like mechanisms that keep the antimicrobial agents from killing everything around them; NPR-1 releases the breaks so the antimicrobials can kill the offending bacteria. The gut cells inside worms carrying the mutant form of NPR-1 never released the breaks so the antimicrobials—and the immune system—couldn't kill the invaders. This observation suggests that NPR-1 plays a key role the nervous system regulating the immune system, Aballay says.

Bargmann says the finding meshes well with her work on the role of NPR-1 in worms, which showed that the gene controls how the animal responds to various levels of oxygen in the environment. NPR-1 seems to operate at an intersection point between the nervous system and the physiology of the animal, Bargmann notes.

Bargmann explains that many of the things the nervous system is involved in detecting are physiologically relevant to an organism. Appetite is a great example—it combines sensation of a food's taste and smell with a internal sense of hunger, a signal of the body's needs, she says. Similarly, the sense of pain is intimately involved with injury to tissues and protection against different kinds of damage—particularly inflammatory pain, which is generated in response to signals from the immune system. So it makes sense to integrate neural cues with potential infection and the state of the body.

Aballay's lab continues to tease apart the biological chatter between the innate immune system and the nervous system. They are now examining mutant worms that are better able to survive exposure to bacterial pathogens. A better understanding of these pathways could lead to new therapeutic targets for diseases involving an overactive innate immune system, such as arthritis, lupus and Crohn's disease, Aballay says. We know from these autoimmune diseases that when the innate immune system is not tightly controlled, the results can be disastrous.

And Steele, who blogged about her experience in Aballay's lab, continues to value her time spent there. My favorite aspect of the research experience was the excitement at finding results different from what I had predicted, says Steele, who continued to work in the lab part time through her sophomore year. I like how one answered question leads to myriad questions, with the researcher trying to tease apart and understand part of a biological system.

While she sometimes found this endless list of questions frustrating—Because there was so much to discover, it was difficult to feel that I had ever finished a project.—she loved working on something completely new.

Being among the first people to research the neural control of innate immunity was exciting.