QnAs with Jorge Galán

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For more than three decades, Jorge Galán has studied infectious diseases. Initially trained in veterinary medicine, the Argentine microbiologist began investigating bacterial pathogens as a graduate student at Cornell University in 1982. The dual influence instilled an awareness of the importance of infectious diseases in human health and the significance of host–pathogen interactions. As a postdoctoral fellow at Washington University in St. Louis, Galán studied Salmonella, the bacteria that cause typhoid fever, under National Academy of Sciences member Roy Curtis III. Years later, Galán, who now serves as the Lucille P. Markey Professor and Chair of Microbial Pathogenesis and Professor of Cell Biology at the Yale University School of Medicine, discovered that Salmonella enterica subs. Typhimurium, one of the serovars that causes severe gastroenteritis, uses a molecular machine called a type III secretion system (T3SS) to infect and replicate within eukaryotic cells. A number of disease-causing Gram-negative bacteria, including Salmonella, Shigella, Yersinia, and Chlamydia, also deploy T3SSs, which are large, needle-like complexes of more than 30 proteins, making the system a potential therapeutic target for the next generation of antibiotics. PNAS recently spoke to Galán, who was elected to the National Academy of Sciences in 2012, about his current research.

PNAS: Your Inaugural Article (1) details the discovery and characterization of the typhoid toxin, which causes typhoid fever. What prompted your research into the toxin?

Galán: Interestingly enough, the work that led to the discovery of typhoid toxin hasn’t historically been the focus of our laboratory. It started as a detour from our main areas, which are the mechanisms of type III secretion and the pathogenesis of the enteric pathogens Salmonella and Campylobacter jejuni. But certainly the typhoid toxin has been a fruitful detour.

From an infectious disease perspective, typhoid fever continues to be a major public health challenge, resulting in the death of nearly 200,000 people across the world, primarily children in developing countries. Historically, typhoid fever is one of the oldest human diseases that we have written records of in Western literature. It was thought to have caused the “plague of Athens” in 430 B.C.E., and more recently, Salmonella Typhi is intimately linked to the tragic story of Mary Mallon, also known as Typhoid Mary.

As a microbiologist interested in basic mechanisms of pathogenesis, I found S. Typhi’s host restriction very intriguing. Unlike nontyphoidal Salmonella serovars, this bacterium can only infect humans, offering a window into unique mechanisms of host–pathogen interactions.

PNAS: What did your research reveal about S. Typhi’s exclusivity for human hosts?

Galán: Compared with other Salmonella bacteria, S. Typhi’s genome shows little variability and contains a higher than expected number of pseudogenes, which are indications that in the process of adapting to humans, this pathogen has abbreviated its genome.

The discovery of typhoid toxin certainly adds to this story. We know that the toxin’s specificity is crucial to the development of typhoid fever; chimpanzees, for example, can be efficiently infected with S. Typhi, but they develop symptoms more in keeping with other Salmonella infections rather than typhoid fever.

We found that typhoid toxin recognizes glycans linked to a sialic acid moiety that terminates in N-acetyleneuraminic acid. In other primates and most mammals, sialilated...
glycans terminate in N-glycolyneuraminic acid because humans have a mutation in the gene that encodes the enzyme that converts N-acetylneuraminic acid to N-glycolyneuraminic acid, a mutation that it is believed to have occurred after hominids separated from other primates.

PNAS: Researchers believed that S. Typhi caused typhoid fever long before the discovery of the typhoid toxin. Why was the toxin so difficult to identify?

Galán: Typhoid toxin is only expressed when S. Typhi is within human cells, which is why despite close scrutiny for almost a century, microbiologists could not identify it in bacteria grown in culture medium. Our laboratory discovered the toxin by studying the interaction of S. Typhi with cultured human cells. Other eukaryotic cells, even mammalian cells, simply don’t work because the toxin cannot intoxicate nonhuman cells.

Typhoid toxin’s unique specificity, however, is not the only reason S. Typhi only infects humans. We have recently discovered a novel host-defense mechanism that restricts S. Typhi replication in nonhuman hosts. A significant portion of our defenses against pathogens operate at the individual cell level through mechanisms known as cell autonomous defense. We have discovered that S. Typhi cannot infect nonhuman hosts because it has lost the ability to neutralize a cell-autonomous defense pathway, which broad-host Salmonellae can effectively block with two effector proteins of its T3SSs. Remarkably, one of these two effectors is missing from the S. Typhi genome and the other is a pseudogene. We believe that in humans S. Typhi inhabits a compartment within cells where this defense pathway may not be operational.

PNAS: How else does the typhoid toxin make S. Typhi infection unique in humans?

Galán: We have found that typhoid toxin can by itself recapitulate many of the symptoms of typhoid fever. This includes stupor and a profound leukopenia or white blood cell depletion, which is unusual for a bacterial infection; they are almost always associated with an increase in leukocytes in the blood. What is exciting about these findings is that they have very direct implications for human health.

PNAS: What are the prospects for an effective typhoid fever treatment or vaccine?

Galán: Despite receiving antibiotics, which are effective in controlling the bacterial infection, many people die of typhoid fever in a hospital setting. We believe that typhoid toxin has a lot to do with this and that toxin neutralization combined with antibiotic treatment may result in an effective therapy. We also know that the toxin is very immunogenic, making it a natural candidate to develop an effective vaccine.

From a basic science perspective, we have most of the information we need to use typhoid toxin to develop therapeutic and prevention strategies, as well as new diagnostic tools, which are badly needed. There is still more to do, however, to evaluate the feasibility and potential of these approaches, which will require human volunteer studies.

Unfortunately, there has been little commercial interest in a typhoid vaccine. Luckily, the Gates Foundation has recently taken an interest in exploring these possibilities and has stepped in to fill the gap.

PNAS: What other aspects of S. Typhi are you pursuing?

Galán: We’d like to further explore the toxin’s role in S. Typhi infection and learn how it evolved. We’d like to know how the toxin is exported from the cells in which it is produced as well as the cellular and molecular bases for the symptoms that it causes. We are also eager to learn more about how the cell-autonomous defense pathway restricts the replication of S. Typhi in nonhuman hosts. Of course, we hope that the next five years of research in this area will be as exciting as the last five.