PROFILE

Profile of Carolina Barillas-Mury

Paul Gabrielsen
Science Writer

Although vector biologist Carolina Barillas-Mury is annoyed by mosquito bites just as much as anyone else, she has learned to respect the tiny insects. "It's amazing what they can do," she says. "They're like little drones with very simple programs, but they're able to accomplish remarkable things." Her words may sound like surprising praise for a malaria-carrying species once labeled the world's deadliest animal (1), but Barillas-Mury respects mosquitoes' ability to fight off deadly malaria parasites.

Driven by a sense of service and a fascination with mosquito physiology, Barillas-Mury, a researcher at the National Institutes of Health and a recently elected member of the National Academy of Sciences, has delved into the mosquito's gut to uncover the malarial parasite *Plasmodium*'s methods of invasion, as well as the pathways the mosquito's immune system employs to fight infection.

Learning to Serve in Guatemala

Barillas-Mury was born in Guatemala in 1961, the fifth of six children. She attended an all-girls high school operated by American nuns where she learned English and cultivated an interest in chemistry and math. On Fridays, Barillas-Mury and her classmates visited orphans, the elderly, and other members of disadvantaged populations in Guatemala City. The nuns encouraged the students to understand the problems each of these groups faced and to develop solutions. "They wanted to create a generation of women that would be leaders for the country," Barillas-Mury says. These experiences influenced her early career decisions. "Your life should be about finding something you like to do," she says, "but something that will be for the common good." After high school, Barillas-Mury studied biology as preparation for medical school.

Postgraduate opportunities in Guatemala at the time were limited to professional schools, so Barillas-Mury chose medicine to feed her scientific curiosity and to ensure future job security. As part of a rotation at the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City, she performed simple assays using an outdated spectrophotometer. The experience of adding reagents and witnessing chemical reactions in real time proved fascinating.

"You could see with your eyes the reaction happening," she says, "and that was mind-boggling." Rather than spend her career in the clinic, Barillas-Mury wanted to explore biology at a basic molecular level, which would require a PhD. "It's like saying you're going to be an astronaut in a country that has no space program," she says.

She graduated from medical school in 1985, and after 2 years of visiting embassies and applying for fellowships, left Guatemala for the University of Arizona in 1987.

Coming to Arizona

Barillas-Mury found the desert environment of Tucson, AZ, dramatically different from tropical Guatemala. "To me," she says, "everything looked like sticks." With the arrival of the late-summer monsoon, however, the desert plants bloomed, sprouting leaves and flowers. "The desert is a place that grows on you," she says.

She chose to study in the laboratory of biochemist Michael Wells. Barillas-Mury hoped to apply her medical background to study something relevant to human health in tropical areas. Wells suggested mosquitoes, and Barillas-Mury embarked on her PhD studies to describe the enzymatic aspect of mosquito blood meal digestion, developing molecular tools to study mosquitoes.

In January 1991, Wells invited Barillas-Mury to serve as the slide projectionist for a meeting in Tucson. "It's not just any meeting," he told her, and advised her to listen closely. The invitation-only meeting, convened by the World Health Organization and the MacArthur Foundation, explored molecular biology approaches to controlling vector-borne diseases (2). Wells' invitation gave Barillas-Mury a front row seat to presentations by the foremost researchers in the field.

During a coffee break, Barillas-Mury introduced herself to Fotis Kafatos of Harvard University. Kafatos had built his career on *Drosophila melanogaster* molecular biology but had recently become interested in mosquito research. Barillas-Mury's experience matched Kafatos' research needs, so she joined

Barillas-Mury at the European Molecular Biology Laboratory, circa 1997. Image courtesy of Douglas Seeley.

his laboratory at Harvard as a postdoctoral scholar in 1994. Much of her work with Kafatos laid the foundation for future molecular biology studies of the mosquito immune system (3–5).

Modeling Parasite Invasion

Barillas-Mury moved to the European Molecular Biology Laboratory in Heidelberg, Germany, to complete her postdoctoral fellowship. Applying for research positions in the United States while living in Europe proved challenging. She struggled to match her research interests to traditional academic departments. "My research was not medical," she says, "It was more entomology, but I was not an entomologist. I was a biochemist, but I had mostly done molecular biology, and I was working in immunity, but I was not an immunologist." After 49 rejected applications and two interviews, Barillas-Mury secured a position in the pathology department of Colorado State University's College of Veterinary Medicine in 1998. She now retells her experience to discouraged postdocs: "The 49 rejections are not in my CV."

Around the time Barillas-Mury moved to Colorado State, the vector biology community was grappling with the role of the mosquito immune system in malaria transmission.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 1273.

Kafatos' laboratory had shown that mosquitoes produced factors similar to human complement that were key players of the antimalarial response in *Anopheles gambiae* mosquitoes, the main vector of human malaria in Africa (6). But some others had assumed that mosquitoes' transmission of malaria implied an ineffective immune system.

For a malaria parasite to infect a mosquito, the parasite's motile form, called an ookinete, must cross a barrier of epithelial cells in the mosquito's midgut to reach basal lamina cells, where it can multiply in anticipation of infecting the mosquito's next target. Researchers noticed that the invaded cells looked different from their neighbors. A previous model of malaria infection hypothesized that the ookinetes identified and invaded the morphologically different cells as vulnerable entry points.

But Barillas-Mury suspected that the cause-and-effect relationship may have been backward. "They didn't look different because they were a different cell type," she says, "maybe it was because the invaded cells were undergoing cell death."

Barillas-Mury noticed that the invaded cells' genomes had been shredded and that the cells were also producing the enzyme NOS at microbicidal levels. These observations led her to develop a model of parasite invasion (7), featuring a time-sensitive race between cell and parasite.

In 2003, Barillas-Mury took a research position at the National Institutes of Health (NIH). Continuing to develop the model, she proposed a functional link between the mosquito midgut epithelial responses and the mosquito complement-like system (8). Although the complement-like system is the final effector of antiplasmodial immunity, she found the fate of a parasite is largely defined during its crossing of the midgut epithelium.

"Imagine that you're sitting in a room," she says, "and that room is a midgut cell. Then the door opens and a parasite walks in. The minute it opens the door an alarm goes off." That alarm, she says, triggers production of enzymes that can tag the invading parasite like fluorescent paint. Tagged parasites will be detected by the mosquito's complement-like factors and destroyed. "But if the parasite manages to disconnect the alarm system and all these reactions don't happen and it gets out, then the complement system cannot see the parasite."

A. gambiae mosquitoes were able to tag and destroy around 80% of *Plasmodium berghei* ookinetes, which cause malaria in mice. But when Barillas-Mury introduced the human malaria parasite *P. falciparum* into *A. gambiae*, the once-effective immune system fell silent, allowing the parasite to invade and colonize the mosquito midgut. Further, mosquitoes with heightened immune systems were able to largely kill *P. falciparum* parasites from Brazil, whereas African varieties of the parasite passed through undetected (9).

Barillas-Mury and her colleagues conducted extensive genetic analysis to pinpoint *P. falciparum*'s so-called invisibility cloak, well adapted to evade *A. gambiae*'s midgut nitration and complement activation. By screening the descendants of cross-bred Brazilian and African parasites for ookinete survival in mosquitoes, her research team homed in on a single gene, *pfs47*, as the likely culprit (10).

Barillas-Mury's inaugural article (11) further examines the effect of *pfs47* on ookinete activity in the mosquito midgut. When parasites without the gene attack epithelial cells, the JNK cell signaling pathway triggers production of nitration enzymes to tag the parasite (12). But ookinetes with the *pfs47* gene disrupt JNK signaling, preventing midgut nitration. "And then the parasite can get through," Barillas-Mury says. "The cell dies, but it dies quietly."

Immune Memory

Barillas-Mury's move to the NIH provided an ideal fit for her interdisciplinary research program. The NIH is organized by public health issue instead of by academic department. Her collaboration with entomologists, immunologists, and parasitologists, all of whom focus on malaria, has inspired advances in her own work.

While working on ookinete invasion of the midgut, she began exploring the immune memory of mosquitoes. Previous studies had shown that injection of low doses of bacteria in fruit flies protected the flies from a high dose injected later (13). She applied the same principle to mosquitoes, exposing them to *Plasmodium* parasites and then exposing them again alongside naïve mosquitoes. The prechallenged mosquitoes fared much better than naïve mosquitoes in the second exposure.

In follow-up experiments, she and her colleagues treated mosquitoes with antibiotic before a repeat exposure to a *Plasmodium* parasite. Loss of gut bacteria, she says, affected immune memory. "So if you have bacteria but no *Plasmodium*, no memory. If you have *Plasmodium* but no bacteria, no memory. So you need both."

To further investigate immune memory, Barillas-Mury drew on techniques from her medical background. "I thought—Why don't we give mosquitoes a blood count?" she says. In humans, a complete blood count assays the amounts of different cell types in blood, helping physicians discern between bacterial and viral infections. Barillas-Mury hoped that a blood count might reveal the mosquito immune mechanism.

She found elevated levels of granulocytes, phagocytic hemocytes analogous to human macrophages, in mosquitoes previously challenged with *Plasmodium* (14). Further, the hemocytes displayed an altered pattern of surface protein expression and were essential to elicit the enhanced immune response to a second infection with *Plasmodium*.

A Season of Harvest

Barillas-Mury' election to the National Academy of Sciences in 2014 came at a time when decades of work were beginning to bear fruit. She and her colleagues published the discovery of hemocytes as a component of mosquito immune memory in 2010 (14), the role of midgut nitration in complement activaton in 2012 (8), and the identification of the *pfs47* gene in 2013 (10). After more than 20 years of effort, Barillas-Mury says she is enjoying a season of harvest.

During her years of applying for fellowships in Guatemala, she says, the idea of someday becoming a member of the Academy would have made her laugh. But the United States has proved to be her land of opportunity.

"I came with two letters of recommendation and GRE scores," she says. "I had enough money to buy a ticket and that was it. And that was enough."

1 Gates W (2014) The deadliest animal in the world. Available at: www.gatesnotes.com/Health/Most-Lethal-Animal-Mosquito-Week. Accessed November 14, 2014.

2 Beaty BJ, et al. (2009) From Tucson to genomics and transgenics: The vector biology network and the emergence of modern vector biology. *PLoS Negl Trop Dis* 3(3):e343.

3 Barillas-Mury C, et al. (1996) Immune factor Gambif1, a new rel family member from the human malaria vector, *Anopheles gambiae*. *EMBO J* 15(17):4691–4701.

4 Zheng L, Benedict MQ, Cornel AJ, Collins FH, Kafatos FC (1996) An integrated genetic map of the African human malaria vector mosquito, *Anopheles gambiae*. *Genetics* 143(2): 941–952.

5 Zheng L, et al. (1997) Quantitative trait loci for refractoriness of *Anopheles gambiae* to *Plasmodium cynomolgi* B. *Science* 276(5311):425–428.

6 Blandin S, et al. (2004) Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* 116(5):661–670.

7 Han YS, Thompson J, Kafatos FC, Barillas-Mury C (2000) Molecular interactions between *Anopheles stephensi* midgut cells and *Plasmodium berghei*: The time bomb theory of ookinete invasion of mosquitoes. *EMBO J* 19(22):6030–6040, and erratum (2001) 20:1483.

8 Oliveira GdeA, Lieberman J, Barillas-Mury C (2012) Epithelial nitration by a peroxidase/NOX5 system mediates mosquito antiplasmodial immunity. *Science* 335(6070):856–859.

9 Molina-Cruz A, et al. (2012) Some strains of *Plasmodium falciparum*, a human malaria parasite, evade the complement-like system of *Anopheles gambiae* mosquitoes. *Proc Natl Acad Sci USA* 109(28):E1957–E1962.
10 Molina-Cruz A, et al. (2013) The human malaria parasite *Pfs47* gene mediates evasion of the mosquito immune system. *Science* 340(6135):984–987.
11 Ramphul UN, Garver LS, Molina-Cruz A, Canepa GE, Barillas-Mury C (2015) *Plasmodium falciparum* evades mosquito immunity by disrupting JNK-mediated apoptosis of invaded midgut cells. *Proc Natl Acad Sci USA* 112:1273–1280.
12 Garver LS, de Almeida Oliveira G, Barillas-Mury C (2013) The JNK pathway is a key mediator of *Anopheles gambiae* antiplasmodial immunity. *PLoS Pathog* 9(9):e1003622.
13 Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS (2007) A specific primed immune response in Drosophila is dependent on phagocytes. *PLoS Pathog* 3(3):e26.
14 Rodrigues J, Brayner FA, Alves LC, Dixit R, Barillas-Mury C (2010) Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science* 329(5997): 1353–1355.