Profile of Diane E. Griffin

Reflecting on her career, Diane Griffin admits that she never had a grand plan for her scientific path. When she started graduate school, she did not know what kind of doctorate she wanted. "This was a follow-your-nose career; this was a take-advantage-of-the-opportunities career," she says. Currently Professor of Medicine and Neurology at Johns Hopkins University School of Medicine (Baltimore, MD) and Professor and Alfred and Jill Sommer Chair of the Department of Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health, Griffin has made the most of the sometimes serendipitous opportunities that have peppered her life to become one of the leading researchers in infectious virology.

Griffin has studied host immune responses to viral infections since she first arrived at Johns Hopkins in 1970. "It's such a fascinating area where both host and invader can determine what the outcome is, whether an animal lives or dies." Her two primary areas of research include neurovirulence in Sindbis virus and immunosuppression induced by human measles virus. In both areas, Griffin's research has revealed many of the mechanisms by which these viruses interact with their host and cause disease. She has received many accolades for her pioneering work, including elections to both the American Academy of Microbiology and the National Academy of Sciences in 2004.

She now uses her knowledge of the measles virus to develop new vaccines. In her Inaugural Article in this issue of PNAS, Griffin (1) reports on her latest vaccine design using Sindbis virus particles expressing the measles hemagglutinin protein. Her findings highlight the complexity involved in clearing measles virus from the body and may lead to a new vaccine that could be administered to infants in developing countries, where measles still remains a major public health concern.

Choosing the Right Directions

Griffin grew up in Oklahoma City, OK, and attained an early appreciation of science from her father, a geologist who worked for oil companies, including Standard Oil of Ohio (Sohio). "He was very teaching-oriented," she recalls. "Every time you took a hike [with him], you learned about the plants and the rocks." After World War II, Griffin's father took a break from the oil business to teach at his alma mater, Augustana College in Rock Island, IL.

Diane E. Griffin

"Augustana was basically our family college," says Griffin. "Both my parents had gone there, and my sisters both went there as well." In 1958, Griffin continued the family tradition and enrolled in Augustana. "I don't remember that I was given much of a choice, really, but it worked out fine," she says. "They had a great science program tucked in a small liberal arts college. However, when I was ready to go to graduate school I decided that this was my big chance to try out a new part of the country, either the east coast or the west coast." After some deliberating, the west coast won out, and in 1962 Griffin enrolled at Stanford University (Stanford, CA) in a Ph.D. program in microbiology.

When she first arrived at Stanford, though, her interests had not yet fully materialized. "I think I'd always been interested in microbiology and disease," she says, "but I hadn't really considered medicine as a career option, which is why I chose graduate school." Griffin joined Leon Rosenberg's group and began working on immunoglobulins, but shortly after beginning graduate school, she reconsidered her position on medicine. She applied to Stanford's M.D. program and was accepted. At the time, Stanford offered a 5-year medical program that allowed Griffin to spend half her time during the first 3 years to do research. This flexibility allowed her to continue working on her Ph.D. project, even as she started her M.D. program.

After a couple years, however, she almost gave up on her Ph.D. research. "My initial thought was that I could get a master's degree to sort of have something to show for the time I'd spent," she says. However, both Rosenberg and Sydney Raffel, Chair of Stanford's microbiology department, encouraged Griffin to continue with her project. "Leon, in particular, was very insistent that I ought to finish," she says. "I had already taken all my courses and passed my exams, so all that was left was getting the thesis work done."

Griffin followed Rosenberg's and Raffel's advice and stayed in the Ph.D. program. She wrote her dissertation, on antibodies against nitrophenyl haptens, during a month-long vacation while an intern at Stanford Hospital. Griffin soon realized she had made the right choice. "By the time I had finished my internship and 1 year of residency, I was pretty certain that I wanted to focus more on research than clinical medicine," she says. "Clinical medicine was never what I did best; I was always better in the lab."

In addition to her Ph.D., Griffin also received her M.D. in 1968, which she felt was not a wasted experience. "I started becoming interested in viruses during that time at med school, even though my thesis was in immunology and didn't have anything to do with virology." She also met future husband John (Jack) Griffin during medical school, and the two were married in 1965.

Timely Convergence in Baltimore

In 1970, after Griffin had completed her residency at Stanford Hospital, her husband Jack was recruited to the newly formed neurology department at Johns Hopkins. "Guy McKhann, the person heading up the department, was from Stanford and knew Jack, who had always been interested in neurology, and recruited him to do a residency there." As luck would have it, one of the other new faculty members recruited to the same department, a specialist in viral infections of the nervous system named Richard (Dick) Johnson, was seeking a postdoctoral fellow with an immunology background who was interested in virology.

"Dick had an unusual approach to understanding viral pathogenesis," she says. "He brought in people from all different kinds of disciplines into the lab who looked at things from all different

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This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 11581.

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angles. We had neurologists, pathologists, molecular biologists . . . I became the resident immunologist.'' Griffin remembers her time working in this interactive and collaborative environment as a highly formative experience. ''That's where I learned biology, pathogenesis, animal research, et cetera. It was in his laboratory.''

Griffin began studying pathogenesis of the Sindbis virus, a mouse alphavirus that causes encephalitis, though she assisted with Johnson's other projects. ''My first project was trying to understand mechanisms of how the nervous system recovers from infection,'' she says. ''These were the early times of understanding the immune responses to viruses. I mean, people had described very little. They had sort of described neutralizing antibodies but not much beyond that.'' One of her first results was finding that Sindbis virus produces a fairly rapid immune response, where initially the virus replicates quickly but is soon controlled (2). ''I also observed this prompt response for visna virus, a sheep lentivirus (3),'' she notes, ''and eventually this response was recognized for HIV as well.''

Griffin continued studying Sindbis neurovirulence, though her research focus gradually evolved. ''I tried to figure out what the T cells were doing, what the antibodies were doing,'' she says. One question that intrigued Griffin was how these two elements could actually clear virus from the brain. Normally, T cells engulf and digest infected cells to remove them from the body, but because neurons do not reproduce, T cell immunity would irreversibly kill a large number of brain cells. Griffin discovered that antibodies against a Sindbis envelope protein could clear virus from infected brain cells via a noncytolytic mechanism (4). Rather than interact with the virus particles, the antibodies directly interacted with the cells and stopped viral reproduction. Subsequent studies also revealed that viral RNA remained dormant in the brain cells over the long term, and antibodies were essential to prevent viral reactivation (5).

Griffin has recently gained interest in the genetics of Sindbis infection. ''One area we are working hard on is determining genetic susceptibility to Sindbis virus,'' she says. Her group recently mapped a potential Sindbis virus mortality gene to a region on chromosome 2 (6) and are now attempting to pinpoint the exact gene. ''We're also trying to figure out age dependence because this may be linked to both the antibody and T cell responses,'' she says. Griffin notes that all young mice die from Sindbis virus infection, but mice at least 2 to 3 weeks old can survive such infection.

Like her work, Griffin herself has been consistent yet evolving. She gradually moved away from Johnson's laboratory and became independent but remained in Johns Hopkins's neurology department, allowing her to continue collaborating with Johnson and other colleagues. Griffin became an assistant professor in 1973, associate professor in 1979, and full professor in 1986. In 1994, Griffin finally moved on . . . across the street. She accepted the position as Chair of the Molecular Microbiology and Immunology Department at the Johns Hopkins Bloomberg School of Public Health.

A Surprise in Peru

In 1971, shortly after Griffin began working in Johnson's laboratory, Johnson went on a clinician exchange in Lima, Peru. During his hospital visits, Johnson would stumble across patients who would ultimately alter the course of Griffin's career. ''When he was down there in the neurology wards,'' says Griffin, ''he saw a lot of cases of neurology complications arising from measles, a postmeasles encephalitis.''

Postmeasles encephalitis is relatively rare, occurring in approximately 1 in 1,000 patients. Combined with the naturally low occurrence of measles in developed countries, postmeasles encephalitis is extremely difficult to study. At that time in Peru, however, many people were immigrating into Lima from small population isolates. Measles virus requires large populations to sustain it, so the immigrants had never encountered measles in their small villages. ''But now they moved to a big city, and they got exposed,'' she says.

Griffin's findings explained why measles is so dangerous in developing nations.

''We thought that these cases provided a good opportunity to try to understand this condition, so Dick set up a collaboration . . . to ask whether this condition was an autoimmune disease or whether virus was infecting the brain.'' One of Griffin's first studies compared children with postmeasles encephalitis with children with pneumonia, a more common postmeasles complication (7). Looking at the children's immune responses, ''what became apparent was that there weren't any big differences, and that all of these kids had traumatic alterations in the cellular immune response.''

In 1996, Griffin uncovered a large piece of this puzzle when she demonstrated that measles virus could suppress the release of interleukin 12 (IL-12) from monocytes by binding to the cell surface (8). IL-12 loss weakens cell-mediated immunity, one of the two main components of acquired immune response, and forces the immune system to rely solely on antibodies.

Griffin's findings explained why measles is so dangerous in developing nations. Although the virus itself may not kill many people, it leaves infected individuals, especially children, vulnerable to other infectious agents such as pneumonia or malaria. Griffin had already studied immune responses to the live measles vaccine, so she began looking at developing alternate vaccines that could safely immunize young infants.

Although the current measles vaccine is safe and effective, it is not as effective when given to children younger than 9 months of age. After about 5 or 6 months of age, infants begin losing the immunity they received from their mother, leaving a short but critical time window when infants are vulnerable to infection. In developed countries, the need to reduce the age is not critical, but Griffin notes that it would make things easier from a delivery point of view. ''All the other childhood vaccines, like polio or DPT, are complete by 6 months, so if we could deliver measles at the same time, then the kids wouldn't have to come back for it.''

In 2000, Griffin and her team developed a DNA vaccine, encoding the low-variability surface proteins of measles virus, that showed success in immunizing monkeys (9). This vaccine did not produce adverse effects, such as atypical measles, a severe form of the disease that rarely occurs in response to the live virus vaccine. The only problem with the DNA vaccine was a lack of consistency, and occasionally vaccination would not produce enough of an immune response to achieve full protection.

Griffin combined her Sindbis and measles research in an effort to improve upon the DNA vaccine. The newest vaccine created by Griffin and her group is presented in her Inaugural Article (1). The vaccine consists of a Sindbis virus replicon expressing the measles hemagglutinin protein (SIN-H). A single dose of SIN-H was shown to induce a high titer of measles-neutralizing antibody and memory T cells, protecting monkeys against disease symptoms for up to 18 months.

Complete clearance of measles virus was observed to be a lengthy and complex process. Although virus particles were cleared from vaccinated monkeys within 2 weeks, viral RNA remained detectable in the system months after infection, similar to what Griffin had observed with Sindbis infection in the brain. "For a number of virus infections, and measles was a good example, it was thought that you would have this acute phase of the disease, and then you clear out the virus, and that's it," she explains. "But we could now see that even after you could no longer culture virus, there was still a very active interaction between the virus and the immune system."

Griffin notes that because these hybrid vaccines only express one or two measles proteins, they are not likely to be as potent as the live virus vaccine. On the other hand, such newer vaccines could provide an opportunity to examine some biologically important questions. "While we are trying to develop a practical vaccine, we can also in the process try to understand what protective immunity is and what's required for virus clearance," she says. "And we can do that by looking at the different levels of protection offered by these different vaccines."

Unexpected Run-Ins with Malaria and HIV

In addition to examining measles infections in monkeys, Griffin's group currently performs field work in Zambia to study the measles virus in a natural habitat. These recent studies have unexpectedly reconnected Griffin with lentiviruses, a virus family she has not studied since her early days in Johnson's laboratory. When Griffin began enrolling children for her studies on measles immunosuppression, she knew she had to factor in the effect of HIV. "Zambia has a lot of HIV infections, and because HIV is an immunosuppressive virus just like measles, we needed to know whether children enrolled in our study were HIV-positive or not," she says. Not surprisingly, Griffin found that HIV-compromised children had prolonged measles shedding compared with others (10).

"So long as we were doing HIV tests on our measles kids, it was a logical next step to look at the effect of measles on HIV," she says. Logically, Griffin predicted that measles should exacerbate HIV infections as well. "We were totally surprised to find out that the opposite was true, and HIV replication was suppressed. The loads were reduced as much as if they had been treated with a highly active retroviral." (11) Currently, Griffin's laboratory has set up an *in vitro* system to explore the mechanisms behind this unusual viral interaction.

Griffin also received a bit of a surprise back home in May 2001, when an anonymous donor pledged a major donation to Johns Hopkins to establish a malaria research institute. As an expert in infectious diseases, as well as Chair of the Microbiology and Immunology Department, Griffin was tapped to be the Acting Director to help initiate the project. "So I've been involved in recruiting people and helping to get the institute going, which is important since I think malaria is a fascinating disease and one that needs more basic science funding." The Malaria Institute now provides Griffin with one of her most daunting challenges in finding a permanent director: "We keep trying to find somebody else to do this, but until then, I'll keep doing the job."

Nick Zagorski, *Science Writer*

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