

“You could conceive of many mechanisms where the persistence of the virus may lead to long-term symptoms”

An interview with microbiologist Dr. Diane E. Griffin on Long COVID and viral RNA persistence

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8 July 2022

Dr. Diane E. Griffin, M.D. and Ph.D., is the university distinguished service professor and a professor in the Department of Molecular Microbiology and Immunology at the Johns Hopkins Bloomberg School of Public Health, where she has taught for nearly a half-century. She was the department chair from 1994 to 2015 and has been one of the leading researchers in infectious virology for more than five decades, having been trained by the foremost researchers in the infant stage of the rapidly evolving field.

After graduating from Stanford Medical School in 1968, she began doing postdoctoral research in virology at Johns Hopkins University of Medical School and became a faculty member in 1973. She reached the rank of full professor in 1986.

Profiling her career in 2005, science writer Nick Zagorski explained that Griffin has conducted research into host immune responses to viral infections since she first arrived at Johns Hopkins. She stated at the time, “It’s such a fascinating area where both host [the person infected] and invader [pathogen/virus] can determine what the outcome is, whether an animal lives or dies.”

Zagorski wrote, “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 both the National Academy of Medicine and National Academy of Sciences in 2004.”

With the growing awareness of the implication of Post-Acute Sequelae of SARS-CoV-2 Infection (PASC or Long COVID) among adults, Griffin published a timely scientific article in *PLOS Biology* in June that reviews the accumulated knowledge on why viral RNA persists in hosts after they have recovered from acute infections.

She explains that most acute viral infections are caused by RNA viruses, such as SARS-CoV-2, and cause disease for a short time after which the infected person recovers and develops immunity to the pathogen. Given the transient nature of the infections, the virus must then successfully infect others during this period to “avoid dying out.”

Griffin wrote, “The need to understand the pathophysiology of the prolonged symptoms that for many complicate [their] recovery after infection with SARS-CoV-2 ... has recently called attention to the potential role of RNA persistence in causing specific late complications, as well as

in preventing complete recovery from acute infection; consequences are also seen following other acute RNA virus infections [see Table 1 in *PLOS Biology* link]. But how and why does viral RNA persist, often without evidence of infectious virus, and what are the potential consequences of this persistence for human disease? These questions will form the basis of discussions in this Unsolved Mystery.”

First, Griffin addresses where the viral RNA can persist; these include immune-privileged sites such as the brain, eyes and testes, as well as in blood, lymphatic tissue, lungs, gut, and kidneys and joints. Beside Long COVID, there are post-Ebola and post-polio syndromes that include symptoms of headache, fatigue, muscle and joint pains.

She then addresses the various mechanisms through which the viral RNA persists. Though more studies are needed, Griffin explains that RNA persistence in infected cells may be related to how these infected cells avoid elimination through various immune mechanisms that prevent their death and by default, the persistence of the viral RNA. For instance, non-replicating critical neurons are long-lived cells that may accept infection to protect themselves against destruction by the immune cells. Other short-lived cells, such as the epithelial cells of the respiratory tract may allow the transfer of viral material between each other without their release from the cell surface.

She concludes her review with a discussion on the consequences of RNA persistence that can lead to innate immune responses and chronic inflammation. As she notes, “Consequences of chronic immune stimulation associated with persistent RNA are dependent on the site of persistence... Determining the importance of RNA persistence is of particular relevance for understanding the failure to fully recover from acute infections such as occurs after SARS-CoV-2 infection and Ebola virus disease. PASC [Long COVID] afflicts 30 to 50 percent of those recovering from COVID and encompasses a variety of symptoms that affect organ systems including fatigue, brain fog, muscle weakness, gastrointestinal distress, cough, and shortness of breath.”

Griffin then remarks, “Infectious virions in blood (viremia) have not been documented, but viral RNA in blood (RNAemia) is found in those with more severe disease, suggesting systemic spread of infection, and is predictive of PASC. Those with persistent symptoms at three months after acute disease are more likely to have increased levels of pro-inflammatory cytokines and chemokines [proteins released by infected cells to signal the immune system], as well as factors associated with vascular injury...The importance of persistent viral RNA relative to inflammation,

autoimmunity, or reactivation of latent infection with other viruses (e.g., Epstein-Barr virus) in the pathogenesis of PASC remains to be determined, but PASC is likely to be more than one disease with multiple contributing factors.”

Dr. Griffin recently spoke with the WWS on the pandemic, as well as her life-long work on viruses and their interplay in human diseases.

Interview with Dr. Diane E Griffin

Benjamin Mateus (BM): Good afternoon, Dr. Griffin.

Diane Griffin (DG): How are you?

BM: I'm well, thank you for taking the call. I read your most recent article on the persistence of viral RNA after an acute viral infection and thought it was a very important contribution to what's going on with the pandemic and the issue of Long COVID. Could we begin by having you first provide some background about yourself and your research on viruses.

DG: I have a long history of studying viruses and I'm clinically trained in infectious diseases. I've done research on viral pathogenesis. And really most of what I do is research with animal models, but also with people that are infected with these various agents. I got interested in viruses early on and find the intersection of viruses with the host and the immune response to be a fascinating area that really determines disease and recovering from disease and protection from disease if we're talking about vaccines. So, understanding those interactions is what I've done for essentially most of my career and which has been at Johns Hopkins primarily.

BM: Your work spans several decades in an era where much of the tools that we're employing today were just being discovered. You have also worked at institutions with great distinction in research. Given the breadth of your experience, maybe as an initial question, can you put the COVID pandemic into a historical context?

DG: Obviously we've had other pandemics, and if one looks at history, we've had new viruses that have appeared quite regularly in the human population. Viruses that can spread by the respiratory route are much more likely to cause widespread disease. A lot of the viruses that I study are transmitted by insects or mosquitoes. And so, they're much more restricted in their geographic distribution and their abilities to be able to cause large outbreaks.

Mostly our experiences as everybody knows is with influenza where the population already has background immunity in general though it may not be to the specific strains that cause pandemics. But I think the ability to respond to a pandemic is vastly different today.

With the 1918 influenza pandemic, which is probably the closest thing to the SARS-CoV-2 pandemic, they didn't even know it was a virus then let alone have any mechanism for being able to develop interventions that were useful. They relied on the basics, isolation and quarantine and that sort of thing, which did help. But these also apply to COVID.

But it's my first experience with a virus that has sent everybody home, including shutting down our laboratories and that sort of thing. The COVID pandemic had a much bigger impact on the population than the usual influenza outbreaks that we've experienced before.

BM: Many scientists and infectious disease experts had spoken about the impending pandemic, and it wasn't a surprise that the COVID pandemic did eventually come. And despite our ability to innovate therapeutics and vaccines we were caught woefully unprepared. And we remain unprepared despite the vast experience we have accumulated. I was interested in your thoughts on these issues.

DG: I think there are a couple of things that have led to our lack of preparedness, and which continue to be a problem, some more than others. Coronaviruses have been suspected as the likely causes of pandemics for quite a while. And that first manifestation was really with SARS, which was rapidly controlled by isolating people. I think that it gave people the impression that we would be able to do this again if there were another

outbreak.

But SARS was not very infectious until people were sick and SARS-CoV-2 is obviously infectious even when people aren't sick [presymptomatic and asymptomatic infections]. This made it very difficult to control through isolation and quarantine because you don't know who had it. Certainly, there was a lack of testing early on and the lack of appreciation of how infectious people could be when they weren't already sick.

The other thing that has become very clear that we need, and we still could use more of, is surveillance to have a better idea of what's happening in the world. For instance, when people show up sick at the emergency room it would be important to understand what pathogen they might be harboring. The technology certainly exists to address these issues, but it's only happening at academic medical centers where they will try to identify the causes of the diseases that appear to be infectious.

The ability to have a better, more representative approach to surveillance is certainly something that the Centers for Disease Control and Prevention (CDC) is aware of, but it takes money, and it takes organization and an infrastructure to put that into place. There is the network for surveillance of influenza which they have used during coronavirus, but that could be certainly strengthened.

[The Global Influenza Surveillance and Response System (GISRS) is a World Health Organization-sponsored global network of laboratories to track the spread of influenza. The surveillance network was established in 1952 with the current capacity of testing 2 million specimens annually across a network of 150 laboratories across 114 countries.]

BM: We have had tremendous success at developing our understanding through research, but translating the research and the experience at research institutions into a global public health initiative has been woefully lacking. Would you agree with that?

DG: I think that's true although I think that we're doing better. We're trying harder. And there's more recognition of what needs to be done. These initiatives take leadership and somebody with a vision that can move the agenda forward and get things enacted. It isn't that people don't necessarily know what needs to be done and how to apply that knowledge. It's the will to do it.

BM: Because of our better understanding of the airborne nature of these respiratory pathogens, their transmission via aerosols, one area that we need to work on is investment and improvement in HVAC in public spaces and the careful monitoring of the air quality indoors. Yet, we aren't seeing these initiatives come to fruition.

DG: That's certainly something that has reached attention with this virus, particularly within schools, that air quality is important. And it turns out that just opening the windows helps a lot, but you can't always do that in the middle of the winter. None of these things hurt. However, when things are very contagious, like the recent Omicron variants of coronavirus, whether such measures will be sufficient or not is another question.

BM: Jumping to the next topic, could you define Long COVID? And maybe also speak on post-viral syndrome with other pathogens like SARS, MERS and Ebola? The Russian flu in the late 19th century, probably a coronavirus pandemic, anecdotally, caused those infected to develop a post-viral syndrome. What do we understand about the post-viral process and how do these experiences inform us about Long COVID and its impact globally?

DG: These many other diseases that you mentioned, it was recognized that people didn't always recover fully from those diseases. The pathogenesis or the mechanisms by which that happens have not been and still aren't understood.

It's our thoughts, and these are not unknown hypotheses, is that it's somehow difficult to totally eradicate these organisms from all areas of the body. And they can continue to stimulate the immune system over

time. And that it probably varies between individuals and how effectively they clear all evidence of a virus after infection. That is particularly true for viruses that spread systemically. If a virus were to remain say in the upper respiratory tract or even the lower respiratory tract, then that may be less of a problem.

As to Long COVID... How should I put it? It's been most controversial with chronic fatigue syndrome. We don't have a good understanding of the infectious etiologies that trigger that syndrome which has probably been most debilitating for those experiencing it. I'm hopeful that we will figure out what these lingering symptoms of post-COVID complications are after the apparent recovery and clearance of the virus.

[Chronic Fatigue Syndrome (CFS) is also known as myalgic encephalomyelitis, a debilitating chronic medical condition whose cause and mechanism of disease remains poorly understood. Patients with CFS suffer from exacerbations and flare-ups after routine physical or mental activity and major sleep disturbances. It impacts about 1 percent of primary-care patients. The incidence has been estimated to be between 835,000 to 2.5 million affecting primarily adults between 40 and 60, with women affected at nearly twice the rate compared to men. Infectious etiologies for the syndrome have been linked to Epstein-Barr virus, mononucleosis and dengue fever. Immunological dysregulation has been observed in those with CFS.]

What causes that? We probably have the best chance to figure this out with SARS-CoV-2 because we have so many people that are infected, we know what they're infected with, and we know when they got infected. The ability to be able to compare people who develop these problems versus those who don't gives us a better chance of figuring out what's causing it and therefore what to do about it than we did with Ebola or any of these diseases that led to far fewer cases and often in places that made it difficult to study. You can now subject these people to very sophisticated batteries of tests.

BM: There was a recent article in *Science* that summarized the three leading theories of Long COVID. The first was presence of micro-clots caused by the vascular injury to the blood vessel from the infection, the second was immune dysregulation, and the last was viral RNA persistence, which was the subject of your recent article. Could you explain what persistence of viral RNA means?

DG: So, getting rid of a virus is a complicated thing to have happen. Either the virus must kill the cell that it's infecting, which is often the case in tissue culture, but not as often the case in the host, in an in-vivo model, and many cells don't die when they are infected. Then it's up to the immune response to get rid of that infected cell and it does it by killing it [the cell]. It is the recognized way.

But there are ways of suppressing virus replication in these cells which would mean the immune system wouldn't attack these infected cells. But it also means leaving some of the virus in that infected cell. For instance, long-lived neuron cells are not easily replaced, and it would not be advantageous for the immune system to destroy these cells. They are important for the host. But it could lead to the dysfunction of those neurons.

There are other examples of the virus causing cellular dysfunction—it no longer works as well as it did. The best examples are cardiac myocytes [cells that make up the heart]. So, you could envision that would be true with neurons and other cells. You could conceive of many mechanisms where the persistence of the virus may lead to long-term symptoms.

The cell not working as well as it used to work is one thing. But also, this constant stimulation of the immune system by both the innate immune system—interferon, cytokines, etc.—and the adaptive immune system—making more antibodies, activating T cells, etc.—all of which lead to inflammation or production of various immune factors that can make you feel as miserable as they do during the acute phase of the disease.

[Animals utilize two main immune strategies against viruses and other

pathogens. The function of the innate immune system is to act as a first defense and begin recruiting immune cells to infection sites. They also activate the adaptive immune system which are involved in the process of developing antibodies against the intruder and recruit T-cells that help the immune system develop long lasting memory to the pathogen.]

BM: What I am gathering from what you are saying, is that the virus can adapt itself to multiply at very low levels and hide itself in cells so that the immune system can't see it. You also mentioned that the host cells can adapt themselves to prevent the immune system from attacking them because now the virus and the cell are somewhat joined at the hips?

DG: In cahoots.

BM: The cell doesn't want to die, and the virus says if you don't want to die then you'd better do something or we're both going to bite the dust. Is that kind of an elemental summation?

DG: That basically sums it up.

BM: Another brief question, are these mechanisms that are being employed by viruses and hosts like what we see in cancer cells that attempt to evade the immune system?

DG: That's an interesting question. Not necessarily because cancer cells, usually the kinds of mechanisms that they are employing... there are certainly ways being employed to prevent them from being killed. But they usually employ proliferating mechanism meaning they grow and divide unregulated. There is no appropriate control over the proliferation. I think the cancer cell issues are different than the virus infected cell issues, although certainly some viruses lead to cancers, which is another question.

[Research in immune therapies is a major area of interest in the understanding of the mechanisms of tumor biology and the development of new treatments that can relieve tumor-induced immune suppression. One area being investigated is cancer immune editing, a process which can both constrain and promote tumor development. In a review published in Seminars in Cancer Biology, the authors wrote, "There are a number of factors that contribute to tumor persistence despite having a normal host immune system. Immune editing is one of the key aspects why tumors evade surveillance causing the tumors to lie dormant in patients for years through 'equilibrium' and 'senescence' before re-emerging."]

BM: if viral persistence is occurring, what causes the virus to wake up later if it's in a quiet state?

DG: The situations in which that has been recognized, and these are mostly seen in experimental systems, are when the immune system is decreased at some later time when the immune system was keeping things in check. The best examples are with herpes zoster and varicella when you go decades without any issues and then suddenly you have shingles. And that is usually related to the gradual decrease of the immune response to that virus or the initiation of a treatment that suppresses the immune system.

One of the viruses that we study is measles and that is associated with a very late complication, eight or 10 years after infection, with the central nervous system. In that situation, it seems that the virus has just been replicating and spreading very slowly and eventually it infects enough cells that it causes a disease.

Maybe one of the more interesting questions is what's going on with the late sexual transmission that occurs with Ebola and Zika where viruses seem like they periodically are reactivated to start producing more infectious virus, meaning you produce enough viruses that you can infect another person.

However, we don't have a good idea as to the regulation that takes place there between the host and the virus. Certainly, the control of virus replication in certain organs like the testes is different or more restricted than it is in other organs, the liver, or the lungs, etc. There's a lot of specific understanding of what's going on with the cells and then within the tissues, that's important for eventually understanding the questions

that you asked about Long COVID.

BM: You've done extensive research on measles now. Perhaps you could speak to where we are in eradicating measles and what is the current impact of measles on the global population?

DG: We're in deep trouble for eradicating measles and for controlling it right now. There's no secret as to how to do this. You need to vaccinate a high proportion of the population [to achieve herd immunity]. That means 95% of the population needs to be immune to measles to prevent outbreaks.

What we're seeing now, which was absolutely predicted, is that the decrease in routine health care and routine vaccination that occurred during the COVID pandemic is now coming to bear fruit, meaning a very large proportion of the population has not received their routine vaccination. I was just on a call yesterday with an investigator in Mali and over the last two or three years they have had huge outbreaks of measles that they're trying to control but have had a hard time. Again, that's a respiratory transmitted virus which is very efficiently transmitted. You need high levels of high-quality immunity to be able to control it. It's biologically possible to do it, but it's going to be hard.

[The World Health Organization reported that in the first two months of 2022 measles cases globally were up 79 percent compared to the previous year. In 2019, more than 207,500 deaths were reported from measles, up from 140,000 in 2018 and a 60 percent rise from 2016 when just under 90,000 deaths were reported. During 2020, more than 22 million infants missed their first dose of measles vaccine, a near 15 percent rise from 2019, the largest increase in two decades. This is occurring while measles surveillance is also deteriorating, leading to the recent large-scale outbreaks being reported this year.]

Dr. Kevin Cain, CDC's Global Immunization Director noted, "Large numbers of unvaccinated children, outbreaks of measles, and disease detection and diagnostics diverted to support COVID-19 response are factors that increase the likelihood of measles-related deaths and serious complications in children." The case fatality rate in the US between 1987 and 2002 was around 0.3 percent. In low-income nations due to high rates of malnutrition, fatality rates can reach 28 percent. In immunocompromised people the fatality rate can be as high as 30 percent.]

BM: returning to our discussion on COVID, the FDA and CDC recently signed off on COVID vaccines for children six months to four years of age. However, the approach to the pandemic is "learn to live with it" and the vaccines are being used as the main mode of treatment control. But we're still seeing new variants evolving. SARS-CoV-2 has been selected to be a very fit and immune-evading virus. And the main problem is that immunity is not long-lasting like with measles vaccines. From your perspective, what concerns do these issues raise with the vaccine-only strategy?

DG: It is encouraging that so many kinds of vaccines were and are being developed for COVID. The mRNA vaccines were fantastic because they gave us a very fast approach to immunization, but they haven't turned out to be very durable with regards to the immunity that they've induced.

We have a lot of other vaccines to compare to and perhaps we will find one with a more long-lasting immune response that we might convert over to or a combination of different vaccines [mucosal vaccines or pan-coronavirus vaccines]. And as you point out, the ability of the virus to mutate and to be selected in the face of vaccine induced immunity or natural infection induced immunity is considerable.

I think a better understanding of what we mean by protective immunity is necessary. We went for the obvious, which is a spike protein, and it worked. But it's a partial solution and it may be that the vaccines need to be more complex than just targeting a single protein and that we need to focus on a more broadly reactive immune response that will cover other variants, something like what people are working on and have been

working on for influenza for quite a while. Thus far not successfully, but there is progress there.

BM: Where are we with regards to intranasal or mucosal vaccines? If we were able to employ them combined with the systemic vaccines, we could stop the infection at its source by having mucosal immunity?

DG: That's a good question and a good approach. Influenza is an example where we have a vaccine that we give intranasally. Amongst the 150 different companies and biotech firms that develop vaccines, people are certainly working on this issue for COVID. I think that's one that we'll see come forward. Scientists are working on recombinant protein-based vaccines that could be given nasally or even by the respiratory route with an inhalation approach.

[Novavax's COVID-19 vaccine is sold under the names Nuvaxovid and Covovax and currently undergoing FDA review to gain acceptance as the fourth shot available in the US. It uses a nanoparticle technology made up of proteins from the surface of SARS-CoV-2. However, it is given as an injection of two doses and has similar efficacy against older variants as do the mRNA vaccines. A recent animal model study has been published by researchers from UNC-Chapel Hill and Duke University who have created an inhalable COVID-19 vaccine that is stable at room temperature for up to three months. The researchers noted that the vaccine can be delivered via an inhaler and appears to be more effective at evading the lung's mucosal lining than the mRNA-based technology.]

BM: Regarding the current global monkeypox outbreak, a question a lot of people are asking is about the human-to-human transmission and whether it is an airborne disease. It's not as contagious as SARS-CoV-2, but the presentation of disease and the period you are infective poses certain challenges from a public health aspect meaning you can continue to be contagious even after your lesions have all healed. Can you comment on these issues?

DG: Yes. I think the airborne component is a critical question. I was a little surprised to see it declared to not be an airborne virus. Smallpox certainly had a component of respiratory transmission, though clearly the skin-to-skin transmission is the most efficient. But whether that can't include some respiratory component I don't know.

BM: Finally, what are your thoughts on the news of vaccine-derived poliovirus found in wastewater in England and London sewers?

DG: I'm not surprised to hear that it's being found. I think in Tel Aviv they've known that they've had polio in wastewater for a long time and they've never been able to identify the person that it's coming from. But now COVID wastewater has turned out to be a good way to monitor for that as well. I think that's going to become a much more broadly applied technique in conducting surveillance.

Back to polio. So, polio vaccine is a live virus vaccine. And that group of viruses are RNA viruses. It's very good at constantly mutating and selecting for viruses that replicate better. It also recombines with other viruses like it including other types of polioviruses. There are three types of polioviruses.

Basically, there's a selection process particularly if it's being transmitted in a population. That vaccine virus is constantly being shed from the gastrointestinal tract and in low vaccinated populations where people haven't been vaccinated then you get a lot of transmission.

I think maybe one of the questions that's interesting and I haven't heard about what is happening in the UK but I'm sure the UK has high vaccine coverage for polio, but they use an inactivated vaccine as we do and as many developed countries do and not the live virus vaccine.

But the inactivated vaccine doesn't induce intestinal immunity, meaning you can still get infected even though you don't get sick. The inactivated polio vaccine prevents the virus from going to the brain. And that's the only part of poliovirus infection that anybody's really worried about because of the paralysis. Summing it up, the inactivated vaccine works perfectly well to protect against paralytic polio, but it doesn't protect

against infection.

So, most of the developed countries that are using the inactivated vaccine are susceptible to an introduction of polio through the fecal-oral route or contaminated water or food that then can spread to others. And then if you don't have a highly vaccinated population, you may start getting cases of paralysis.

Surveillance for polio, traditionally, has depended on [the presentation of] paralysis among cases. Even with a completely unvaccinated population with wild type infection, only one in 100 to 200 ever get paralyzed. Most people have asymptomatic infection which means you can have a lot of undetected transmission and spread without recognizing it unless you're doing other kinds of surveillance, like the wastewater surveillance.

BM: Dr. Griffin, that was very clarifying. You've been very kind with your time. Thank you so much.

DG: You are welcome. Have a good day.



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