Dr. Eric Freed
Behind the Mask
December 14, 2020

GB: Good Afternoon. Today is December 14, 2020, and I [Gabrielle Barr] have the pleasure of speaking with Dr. Eric Freed. Dr. Freed is a senior investigator, head of the Virus Cell Interaction Section and Director of the HIV Dynamics and Replication Program in the National Cancer Institute (NCI). Our first question is: Why are interferon-stimulated genes, which you might want to define, why are they so important to understand and why have the vast majority of interferon-stimulated genes not been characterized yet?

EF: Let me start by putting this question in a little bit of perspective. As most of you may know, viruses are very small. They have very small genomes, that is, they don't encode a lot of proteins. So, when they infect a cell, they have to rely very heavily on the cellular machinery in order to replicate, so in that sense they're obligate parasites. On their own they're essentially incapable of doing anything, so they get into a cell and they start essentially hijacking host cell machinery in order to replicate.

But over the course of evolutionary time, cells in turn have acquired a very intricate and complex mechanism of trying to block these invading pathogens. A lot of the machinery that's geared towards blocking viruses is stimulated by a small molecule known as interferon; in fact, “interferon” basically means to interfere. It's a small molecule that's secreted from cells. It binds other cells and triggers the expression of a large number of genes that are known as interferon-stimulated genes.

So essentially what happens when a virus infects a cell, there are components of the virus that look foreign to the cell—for example, protein patterns on the virus particle itself, viral DNA, which normally isn't in the cytoplasm, or double-stranded RNA —so the cell says, “Hey, wait a second, there's suddenly something foreign in my cytoplasm. This must be an invading pathogen. It could be a bacterium, could be a virus.” So-called “sensors” detect these foreign molecules in the cell and this sets off a cascade of events that end up ultimately with the protein interferon being expressed.

This interferon protein is then trafficked to the cell surface and is released from the cell, binds other cells, and that in turn triggers the expression of interferon-stimulated genes in those cells within an remarkably short period of time. It's sort of like the fire alarms going off and the cell says, “Hold on, we’ve got an invasion here. Let's call out the troops.” During this process interferon induces the expression of hundreds, maybe even a thousand, of these interferon-stimulated genes whose role it is to combat in a diverse set of ways the
invading pathogen. What's really fascinating about this is that these interferon-stimulated genes in many cases will do different things to different viruses, so they might block, let's say, an RNA polymerase of one virus, they might block fusion of another virus. In most cases these are antiviral activities but they're very diverse in their manifestations.

As I mentioned, there are large numbers of these genes and you made the point that many of these have not been characterized. Part of this is just the sheer number of these interferon-stimulated genes,

which again number in the hundreds, and we just haven't had a chance to work through all these and understand how they function. Some are very well characterized, very well established, and others are known to be upregulated following interferon stimulation, but their functions are not particularly clear.

GB: That's interesting. Just as a follow-up question: You said that it does it in a rapidly short amount of time. How much time does it take for it to signal for these interferon genes to kind of get into gear?

LF: Usually within a few hours. The expression of interferon and the interferon-stimulated genes begins in the infected cell itself, but subsequently also in the neighboring cells in the sense that the secreted interferon can bind to interferon receptors on other cells around the infected cell and trigger this sort of warning signal in those cells and so on.

GB: That's really interesting.

LF: In fact, as to the genes that we're going to talk about a little bit today, the MARCH proteins [Membrane-associated RING-CH], we've shown that within 30 minutes or so of interferon stimulation the expression of these genes is upregulated.

GB: In lay terms can you please describe your study that is looking at two families of these interferon-stimulated genes. I may not be pronouncing this correctly: the membrane-associated RING-CH E3 ubiquitin ligases, or “MARCH” proteins.

EF: That’s right.

GB: Okay. MARCH proteins and the guanylate-binding proteins and you're looking at how theyantagonize the S-protein of coronavirus.

EF: I'm going to focus most of my comments on the so-called MARCH proteins because those are the ones we've worked on more of the two and these are very interesting proteins. They are E3 ubiquitin ligases which means they ligate or attach a small protein called ubiquitin to their target proteins. Basically, when ubiquitin is attached to a protein it behaves in a different way than it would if it didn't have ubiquitin attached. A very common outcome of ubiquitin attachment to a protein is that protein gets degraded. We'll focus on that because that's the case in the situation with the MARCH proteins.

MARCH protein homologs were originally discovered as viral proteins. They are encoded by a family of herpes viruses, so these viruses express the MARCH protein homologs when they infect cells. These

MARCH protein homologs attach ubiquitin onto cellular proteins that are involved in the immune defenses against the virus. So again, viruses are very clever as they hijacked these proteins so that when they infect a cell they can essentially down-regulate the cell's ability to combat the infection. I mentioned earlier [that] cells have these defenses and in turn viruses have evolved countermeasures that try to uncouple or disable these defenses. Because these proteins are E3-ubiquitin ligases, they attach ubiquitin to target cells and in so doing they specifically target membrane proteins, that is, proteins that are expressed on the surface of cells. It turns out that not only cellular proteins but also viral glycoproteins are expressed on the surface of cells and those viral glycoproteins are essential for virus replication because they are packaged onto virus particle and are required for the binding of virus particles to new target cells and on the fusion reaction that allows the virus to enter the target cell. It was known for a few years from previous work that these proteins, these MARCH proteins, have the ability to disable cellular membrane proteins.

Then a few years ago other groups reported that the HIV-1 envelope protein, and also the G-glycoprotein of a virus known as vesicular stomatitis virus are targeted by MARCH proteins. But the mechanism of how this happens is not known, so we set about trying to understand mechanistically how the MARCH proteins target [the] HIV envelope glycoprotein and the envelope proteins of other viruses, including the vesicular stomatitis virus and Ebolavirus.

So, we were in the middle of this study less than a year ago and SARS-CoV-2 arrived on the scene as everyone is aware. We thought, “Well, let's extend the work that we've been doing with HIV and apply it to the spike protein of SARS-CoV-2.” Again, the spike protein of SARS-CoV-2 and the envelope protein of HIV are viral glycoproteins that are involved in membrane binding activity, so they're in many ways analogous to each other. They're different viruses, different proteins, completely different sequences, but they share the function of binding to receptors and allowing entry of the virus into the target cell. So what we basically do in the lab is we over-express the MARCH proteins in cells and then we examine the levels of the glycoprotein, for example, HIV envelope glycoprotein or SARS-CoV-2 spike protein, we measure the infectivity of virus particles produced from those cells, and we also use microscopy techniques to look at the localization of the viral proteins in cells that are expressing the MARCH proteins. What we've discovered is that in the case of both HIV envelope as well as SARS-CoV-2 spike we see that MARCH proteins are able to antagonize or block the function of the viral glycoproteins. So they represent, we think, part of the cellular defense machinery against these viral glycoproteins. It's really cool again that these are really cellular proteins that get rid of viral proteins, but the herpes viruses have essentially stolen these proteins and made them part of their own viral genomes to downregulate proteins that are part of the host immune response. Again, this illustrates the constant battle between the virus and the cell, the pathogen and the host, in terms of one trying to antagonize the other and then the other developing further countermeasures.

GB: Do you worry about that in time with SARS-CoV-2?

EF: These are mechanisms that have been in place over evolutionary time. The viruses and the cells have reached an equilibrium with each other. Because of this co-evolution, what generally happens with

these interferon-stimulated genes, which are often referred to as restriction factors, is that when the virus jumps from one species to another, the cellular machinery is actually more able to defend itself against this new pathogen. Then over time the virus evolves to counteract the cellular defense mechanism. You might be alluding to another question, which is the evolution of SARS-CoV-2 and whether it might impact that evolution, for example, the long-term effectiveness of vaccines or antivirals. This is something maybe we can talk about. That's a bit different from the proteins we're studying but certainly something I'd be happy to talk about.

GB: We can stick to your study for right now. Have you noticed critical differences between the work that you did with these proteins in the HIV envelope proteins and SARS-CoV-2 spike protein?

EF: Well, actually, in many respects what we're discovering with SARS-CoV-2 is very similar to what we found earlier with HIV and specifically, without getting into too much detail, I mentioned earlier that MARCH proteins attach ubiquitin to target proteins and generally they do this by attaching ubiquitin to a part of the protein that's known as a cytoplasmic tail. This is the part of the viral protein that faces the cytoplasm of the infected cell. What we've found with both the HIV envelope glycoprotein and SARS-CoV-2 spike protein is that we can remove the cytoplasmic tail and we retain the ability of the MARCH protein to antagonize these viral glycoproteins. What we believe is happening is that the MARCH proteins are actually antagonizing a cellular protein that itself is involved in the trafficking or processing of the viral glycoprotein, so it's actually a cellular target rather than a viral target. The net result, of course, is that the virus gets antagonized, but it's an indirect mechanism. We find this indirect mechanism to be in common between HIV and SARS-CoV-2.

GB: Interesting, so today have you encountered any challenges with this study and also anything that you have found surprising?

EF: I think what I just mentioned is probably the thing that was both challenging and surprising. We expected, based on what was known about MARCH proteins, that they would target this region of the protein called the cytoplasmic tail as has been described for other MARCH protein targets. That was not the case, so that part was surprising, and the challenge now is that we have to look much further and in more depth at what that target might be and if that target is a cellular protein. We now have potentially thousands of targets to sift through to find which one is relevant for MARCH protein activity but that's the excitement of science. If everything turned out the way you expected the process might become boring, but more often than not unanticipated surprises turn up.

GB: Would you use similar techniques and to what you're doing now in order to do that, the cryo microscopy and things like that?

EF: We're using very similar techniques for the HIV envelope study and the SARS-CoV-2 study. What we will need to do moving forward is to express the MARCH proteins in cells and then look at the cellular proteins that are eliminated as a result of MARCH protein expression. Then once we identify those, we can then look at those specifically for their antagonism by MARCH proteins using a variety of technologies, for example, using CRISPR-Cas9 to knock down the expression of those proteins and evaluating the effect on viral glycoprotein trafficking and incorporation into virus particles.

GB: Do you have any plans in the coming years to look at more families of the interferon-stimulated genes?

EF: There's another protein that we're looking at, as you mentioned in the introduction, called guanylate- binding protein. This is a family of several proteins and guanylate-binding protein 5 has been shown to block an enzyme called furin. This is important because furin is an enzyme in the cell that activates through a proteolytic cleavage a number of viral glycoproteins including those of HIV and SARS-CoV-2.

Without this enzyme cleaving the viral glycoprotein, the viral glycoprotein is not activated and cannot function, so the concept that a cellular protein that's interferon activated might block an enzyme that's critical for the function of a number of viral glycoproteins is something that we're very interested in and planning to pursue. We have some preliminary data supporting the idea that that's the step that is being blocked by this protein GPB5 [guanylate binding protein 5].

GB: That sounds really exciting. So, I was wondering if you could speak a little bit about what your role has been with these studies.

EF: I'm the head of the lab. I work remotely and stay away from the people who actually do the work. This has been a challenge to be essentially kept away from my group. Under normal circumstances I see everybody on a daily basis, and we have conversations in my office, in the hallway, in the lab. It's very interactive. Now that I'm working remotely and people are either at home or in the lab on a rotating schedule, the interactions are a little bit different, a little bit more formalized, in the sense that we have to say, “We need to talk.” I arrange it, “Can I set up a WebEx with you or a Zoom meeting and we'll talk?” We have to schedule it. It's not a simple matter of people just dropping by my office whenever they have something they want to talk about. That has been somewhat challenging.

But you know I've thought often over the past nine months or so what would have happened if this pandemic had occurred maybe 10 years ago when we had much more limited means of communicating remotely than we do today. That would really have been much more devastating in terms of our ability to do our work. We can talk remotely, as you and I are doing now. People in the lab can show me data and they can share their results via Zoom. We can go over the data quite safely. In that sense, we're managing pretty well after a little bit of adjustment to this new paradigm of interacting

remotely. I think if there are any silver linings to this pandemic, it's that the world has gotten much better at interacting virtually.

Certainly, the scientific community is much more interactive now than it was before, in my view at least. In fact, we have a number of seminar series both within our program and NIH-wide, where we're now inviting people from all over the world to give seminars. In the past we tended to invite people to come physically and this was a major, I won't say inconvenience because it's a tremendous amount of fun to travel and visit other institutions, but certainly a big commitment of time and resources to travel from Europe to here or vice versa and so on. Now we can host and attend a great seminar without anybody having to leave their office. So, I think the world has gotten smaller in that sense, resulting from the fact that we've all been forced on short notice to get used to this mode of interacting.

GB: About how many people are you directing with this project?

EF: This project is being run by a couple people in my lab. This is only one of many projects that my lab is undertaking. We've got about 10 people in my lab and the program that I serve as director for, the HIV Dynamics and Replication Program, has about 70 people across nine or so different labs, all doing their own independent research. But our program is focused primarily on HIV, although a number of groups like mine have pivoted a small amount of their effort to SARS-CoV-2 research for obvious reasons.

GB: You've already answered this question slightly but what are some personal opportunities and challenges that you have faced during the pandemic?

EF: The personal challenges—I think all the viewers will be sympathetic with and face similar things. I'm fortunate that nobody in my family has been adversely affected. I have an elderly mother who's living by herself in Pennsylvania and I've not been able to visit her since last February or March. Today's her birthday in fact, and so it's a bit of a sad occasion that I can't be there to celebrate with her. We missed Thanksgiving; we'll miss Christmas. That's a personal challenge.

But if we focus on the more positive things, the silver linings, one thing that I've really been struck by in this pandemic is the degree to which scientists are working together and collaborating to solve this problem. I've been studying HIV for many years, almost since the beginning of the HIV pandemic and, in those early days of HIV research, I found things to be quite different where labs were very competitive with each other, very secretive, and some were not interested in sharing reagents and helping other labs. Now, in contrast, I've been struck by how open people are and how willing people are to share reagents.

I would also say as a virologist, not that anybody would have wished this to happen, but as a virologist it reinforces the importance of virology. More important than that perhaps, is it reinforces the importance of basic research in virology and in other fields. Corona viruses were really not [a] particularly well

studied group of viruses. In fact, before SARS in the early 2000s, corona virology was a rather quiet area of virology. That picked up a little bit with SARS (2002-2003) and then a bit more with MERS coronavirus, but it was still a group of viruses that not many people worked on and the funding was not particularly good. Then suddenly SARS-CoV-2 emerged and has brought the world to its knees.

The lesson for all of us is that basic research is essential because one can never fully anticipate where the next threat is going to come from. It's fortunate that there were people who were trained in corona virology and had the foresight to begin implementing coronavirus vaccines. People, for example at the Vaccine Research Center at the NIH, made possible this incredibly rapid development of RNA vaccines that are showing great efficacy in preventing COVID-19. In a very real sense it was the basic research in virology, immunology, and vaccinology and the foresight of the people working in these areas that allowed us to be where we are now. Without the basic research laying the foundations of knowledge it would have taken years to develop a vaccine.

GB: That's a great thing. Here is a fun question. What are you most hopeful about in 2021?

EF: Like everyone watching I'm hopeful that vaccines will be distributed quickly and efficiently and go to those in need first and not simply those who have political or economic power. It's important that the vaccines help all of mankind and not just those of us in the wealthier countries. The pandemic will hopefully lead to a newfound respect for science and for people working together to solve critical problems.

GB: Is there anything else you would want to share as an NIH scientist but also as a person who's living through this pandemic?

EF: Again, I'm maybe starting to get repetitive, but I would encourage everybody to be supportive of science and to be supportive of scientific education, to be supportive of vaccines as a tremendous benefit to humanity. There are many people out there who for different reasons, many of which are unclear to me, seem to be suspicious of vaccines. Vaccines have tremendous power to save lives and we need to embrace them, and I believe we're about to see the power that vaccines have in human health and saving lives.

GB: Thank you very much. I wish that you and your family continue to stay safe and, of course, your team as well. I wish you all the success in your research.

EF: Thank you very much. It was a pleasure speaking with you.