Dr. Dominic Esposito
Behind the Mask
August 23, 2021

Barr: Today is August 23, 2021. My name is Gabrielle Barr, and I am the archivist at the Office of NIH History and Stetten Museum, and today I have the pleasure of speaking with Dr. Dominic Esposito. Dr. Esposito is the director of the Protein Expression Laboratory in the NCI (National Cancer Institute) RAS initiative, which is part of the Frederick National Laboratory for Cancer Research. Today he is going to be speaking about his many COVID-19 research contributions. Thank you for being with me.

Esposito: Thank you.

Barr: To begin with, can you please talk about your work in optimizing the production of large amounts of spike proteins to supply NIH scientists who are investigating the structure of the SARS CoV-2 virus? That has been really important to you over the past couple of months.

Esposito: Absolutely. I guess back in March, I think it was like March 20th or 22nd of 2020 [March 18], we were approached by a couple in NIH groups to see if we could potentially use our protein production operation in Frederick to help generate these proteins they needed, for what was at that point the very early stage of pandemic research. We had actually shut most of the lab down at that point, and we were trying to figure out how we were going to continue to do teleworking. Suddenly, we had to bring back a good number of people into the lab to work on these projects. Initially, we were approached by a consortium of NIH institutes who were working on this NIH serosurvey project, which was designed to look at the potential number of people who might have been infected with the virus and did not know it at that point, so asymptomatic people. (The researchers) were interested in looking at a couple of different proteins, the spike protein as well as a piece of the spike protein called the receptor binding domain or the RBD. There were not a lot of opportunities to get this protein commercially at that point, and other labs were using it for their own work so basically, the request was, “Hey, can you guys make these proteins, and can you make them in high enough quality for us to develop assays and run these serosurvey projects?”

Barr: How do you go about producing these different proteins, and what is the quantity of spike proteins that you that you produce now?

Esposito: Initially, there was some literature from other labs on how to make forms of these proteins. Unfortunately, the yields of those proteins were really low which means that we would have had to have done a very large-scale production just to get enough material for these projects so, although we initially did take those reagents from other labs and try and generate protein that way, we quickly realized that with the amounts that we needed, that was not going to work. At the same time that we developed these initial proteins, we also went about optimizing our own process to really scale up the production. For us, that was pretty challenging because we make these proteins in mammalian cells, which is not usually a very high yield system, and it requires a lot of hands-on time. Initially, we were generating a few milligrams of spike protein and maybe 20 or 30 milligrams of RBD protein. I think now,

probably in the entire year and a half we have been doing this, I think that we have generated probably two or three hundred milligrams of spike, and probably a gram or two of the RBD by now.

Barr: Why do high quality soluble spike trimers remain a difficult antigen to both express and purify?

Esposito: It is a challenging protein to work with because it has stability issues. These proteins are normally trimers, so they are three molecules combined into one. If you are not careful, those can fall apart, and when those proteins fall apart, they are no longer useful for most of the assays or structural work. There is a lot that goes into trying to really optimize keeping the proteins happy during the production process.

Barr: Interesting. What is the effect of expression temperature and time on spike, on production yield?

Esposito: That was one of the key findings early on in our process. [We found] that dropping the temperature at which we grew the cells that are making the spike protein dramatically improved both the yield and quality of the proteins, so we were actually able, by reducing the temperature and slightly extending the time that we make the proteins, to get somewhere between three and four times the protein yield per liter of culture. That was a really huge deal for us in terms of being able to generate the quantities of protein we needed.

Barr: How do you assess the quality of the proteins that you create for these different assays?

Esposito: We have a sort of a suite of quality control processes in our lab that we use for all the proteins that we make and for the COVID work. We basically used those same technologies. These are pretty standard quality assessments: Is the protein properly folded? Does it look clean and homogeneous? In the case of these proteins, we actually generated assays. We developed ELISA assays along with our collaborators at NIH and use that to validate that the proteins were reproducible from batch to batch and that they gave us the same results all the time.

Barr: How has your process changed since you began last spring to now?

Esposito: It has changed a lot. I mean, we have basically changed every part of that process from the original days. We now use our own DNA constructs to generate these proteins. We have optimized various parts from the very beginning through the cell culture that we are using to generate it to the method we are doing purification. We have continually changed that, I would say, over the first year.
I think at this point, we have a pretty standardized process which is very reproducible, and we have used that over the last six months to generate a number of the different variants of concern to see how those perform as well.

Barr: How do you hope to further perfect your process, or something else that you are looking into in terms of researching how to produce these different proteins?

Esposito: It is a constant attempt to improve it I think, at the levels of protein that we are being asked to make right now. We probably have optimized this system enough that we do not have to do too much. I

think one of the questions is whether some of the variants may have different properties in terms of production, where we may have to sort of tweak the conditions a little, but I think that at this point, we have gone through most of the optimization work, and are pretty happy with where we are at in terms of the production yields now.

Barr: Are the variants much more difficult than the original virus?

Esposito: So far, the SARS CoV-2 variants do not seem to behave a lot differently than the original virus. We have, however, made a lot of other coronavirus spike proteins and there are actually some that are really challenging to make, so there are still, I think, some things to be learned there but for the most part, the variants behave pretty much like the original.

Barr: What was your role in the study that looked at the standardization of enzyme-linked immunosorbent assays (ELISA) for serosurveys of the SARS CoV-2 pandemic using clinical and at-home blood sampling in the beginning of the pandemic?

Esposito: That really was the primary focus of the work in our lab initially, and how we got involved in that project early on was because [researchers] knew that they were going to need a lot of protein to both develop those assays and deploy the actual serosurvey. That project was run by a team of investigators from three different NIH institutes as well as our group, and so really, we were involved from the beginning in sort of the inception of that project: development of the assays, production of the reagents, and deployment of those throughout.

Barr: It was discovered that there is some variability in some of the seroassays out there. Can you talk about what specific properties may play a role in receptor binding domains seropositivity assay performance?

Esposito: That is a great question. One of the first findings of the assay development work which was actually published, I think, in that Nature Communications paper, was that there are two forms of the receptor binding domains that had been previously generated by other labs, and those two performed differently in the assay. One of them gave about a two-fold higher level of sensitivity. It was not really clear to us initially why that was. The proteins had a similar piece of the SARS CoV-2 RBD (Receptor-Binding-Domain), but one of those proteins had an extra tag on the end that was put on for a completely different reason. It turned out through some of the work that we did, and the validation of the assay work, that the tag had a significant impact on the sensitivity of this assay. That [finding] led us to try and look more carefully at exactly what those proteins were and why those pieces were behaving better, which ultimately [enabled] us understand a lot more about the exact amino acid details of these proteins. That turned out to be really useful, and I think it is one of the reasons this assay has proven to be really sensitive for the serosurvey.

Barr: How did you and others go about improving some of these existing versions to be the more accurate in the version that you all created?

Esposito: One of the nice things we did, we were able to really nail down exactly what the sequence of these proteins, because our lab does a lot of quality control using mass spectrometry. A lot of people do not have that technology and so they make the proteins and they look like they are the right protein, but what we discovered is actually that the ends of these proteins when they are made in these mammalian cells can vary, and in the case of RBD (Receptor-Binding-Domain) in particular, some of these RBD proteins had missing amino acids at the amino terminal end of the protein. That may or may not impact the assay, but it does make it a very heterogeneous sample, which is not something that we like to see in an assay that we want repeatable. We spend a lot of time refining what those amino terminal residues were, and then constructing DNA that would ensure that we made only the protein that we wanted, which was basically 100% pure. I think that for the most part we were the first lab to show that some of these proteins that were being used actually did not have the correct amino-terminus so it has actually worked, and that was recently published.

Barr: Why were they missing? Was it because of the tag that they were so different or other reasons?

Esposito: It actually has more to do with the artificial method in which these proteins are being produced in mammalian cells. The receptor binding domain is normally a part of the spike protein, so it is not normally made by itself. When you engineer these proteins to be made by themselves sometimes the machinery of the cell does not process them correctly at one end, and in the case of the RBD protein that we used, it was actually getting cut at a couple different spots, and there was a lot of heterogeneity in that sequence which was really unexpected, and again, may or may not make a significant difference, but from the point of view of having a high quality homogeneous standard it is really nice to have one protein.

Barr: Definitely. So, in this study that looked at undiagnosed SARS CoV-2 seropositivity during the first six months of the COVID-19 team pandemic in the United States, were you surprised at the numbers of undiagnosed infections in some of the other demographic findings that resulted from the study?

Esposito: I do not think I was terribly surprised. I think we all expected that there was going to be a significant number of undiagnosed infections. There had been a lot of numbers thrown around from other serosurveys in different places and the numbers were all over the place. We tried to go into it pretty open-minded about what the expectation would be. What we found was somewhere around four and a half to five times the number of expected cases based on known positives, which I think is interesting. I actually think that the most interesting results from this work will be in the next six months. Right now, we are processing the data from the six-month follow-up and there is also a 12-month follow-up. That will be fascinating because that will be after the first and second big waves of COVID, and that data will be much more interesting from a standpoint of demographics and geographical location.

Barr: Would you and others consider conducting a similar study based on reinfection following vaccines?

Esposito: That is a great question, and it is something that we are definitely considering. We have actually modified the second and third parts of this study to take into account people who have been

vaccinated because that actually has a big impact on the study. Most of this study was designed to look at people who had not been infected or did not know they were infected, but I agree you could easily follow up on a study and look for reinfection for breakthrough infections, things like that, because all of that is easily monitored by this assay.

Barr: In the effort to improve production of SARS CoV-2 spike receptor-binding-domain for serology-assay several questions are asked. One of the first questions is: Why has the receptor-binding-domain production optimization lagged behind creating soluble folding spike proteins?

Esposito: I think that some of the early optimization of spike protein was focused around structural biology work. There were a lot of labs that were interested in the structure of spike and they spent a lot of time looking at the spike protein itself. I think the fact that people were able to produce RBD that worked in ELISA (enzyme-linked immunosorbent assays) assays, and other assays was fine, and those proteins work, and many of the commercial assays actually use those RBDs. I think the key for us was just finding that this one was more sensitive, and if it works people did not really need to do a lot of optimization. I think they felt like they had already had a reagent that worked and that was great. For us, I think it was really important to maximize that sensitivity for our assay in the beginning, and that is why we spent the extra time optimizing that RBD.

Barr: You were part of a study that looked at quantitative and standardized serological and neutralization assays for COVID-19. Will you please mention what were some of the issues with some of the other COVID-19 assays, and what were some of the assays that you and your team created?

Esposito: This was an interesting project that was in collaboration with folks at the National Institute of Standards and Technology (NIST). Their goal was to sort and compare agents across a number of different assay platforms. There were dozens and dozens of ELISA-based assays that were generated pretty quickly at the beginning of the pandemic and a lot of them had really bad results in terms of sensitivity. One of the key questions was: Does reagent quality really impact these various assays, both the particular quality of the spike protein in the RBD protein, but also some of these assays used in other proteins from SARS CoV-2—things like nucleocapsid and these other proteins? We worked with the NIST folks to basically provide them reagents that they could use as sort of gold standards for analyzing those assays and trying to decide which assays used good quality proteins, which did not, and whether they were related to the sensitivity. That was a nice collaboration in the sense that they could get their hands on good, standardized proteins that we had already tested in assays here, and helped them develop their platforms and go out and test all these commercial kits.

Barr: When looking at the effect of the D614G spike variant on the Immunoglobulin G, M, A spike seroassay performance, were you surprised by the cross-response of humoral immunity, and what does that mean for other variations?

Esposito: This was an interesting little sort of side project which we were just playing around with the D614G because, at the time, that was the first significant mutation that was showing up in patient samples. Now all SARS CoV-2 spike proteins have that mutation, of course, but it was really important to look at the different IgGs [immunoglobulin G] because we were concerned in the serosurvey that we

would miss people who may have developed a particular IgG reaction either to IgG or IgA [immunoglobulin A], initially. We really wanted to understand the lay of the land in terms of what immunoglobulins showed up. A lot of this was driven by Kaitlyn Sadtler, from NIBIB [National Institute of Biomedical Imaging and Biomedical Engineering], who was the immunologist that was really driving the development of this assay, and so I do not think there was a lot of surprise. We expected to see this kind of cross-responsive immunity, and we also expected to see things like IgA coming up initially and then transferring to G and M, and when you look at the serosurvey results, you actually do see patients who are positive for one or two or all three of these types of Igs. That work helped us define how we would determine what was positive and what was not in those assays.

Barr: Interesting. As part of the team that looked at SARS CoV-2 seroprevalence in drug use and trauma patients from six sites in the United States, what made you and your team look for at trauma patients in particular, and what is the correlation between type of drugs a person is taking and seropositivity?

Esposito: This study actually developed from folks at the National Highway Safety and Transportation Administration who contacted our collaborators and said: "Hey, we have blood samples from trauma patients, from a bunch of different locations. Would you guys be interested in looking at the seropositivity?" The philosophy here might be that with these trauma patients who many of them were victims in auto accidents, there might be some relationship between, for instance high-risk behaviors, drug use, alcohol use, which might correlate to high-risk potential of getting COVID. That was an interesting study, and the data is pretty interesting. There are clearly some correlations in these populations with certain drug use and with certain types of trauma. The number of patients is not super high in the study so, trying to get the sort of clinical significance of this is challenging, but we are pretty happy so far. This paper has actually not been peer reviewed yet, so we are kind of waiting to see if [it is accepted], but I thought that it was really interesting, there were some interesting findings and clearly much higher seropositivity among some of these groups than we had seen in the nationwide baseline study.

Barr: Yes, it was interesting when they looked at it, depending on what type of drug, [with] the antidote antidepressants there was more of a correlation than with other like opioids, sedatives, and narcotics. There did not seem to make a difference.

Esposito: Yes, and I think that is really fascinating. We clearly need to look at larger populations, and we also need to think about breaking out more of the demographics because these are all in large cities so you are selecting from a certain demographic. There is also some bias in the racial makeup and the ethnic makeup of these groups, but it is really fascinating data, and I think it speaks to the kinds of experiments you can do with these kinds of assays to look at different patient populations.

Barr: I was really surprised because I would have thought that some of the drugs such as like narcotics would have had a higher COVID correlation so, I was just very surprised by that.

Esposito: That actually, was exactly what we expected to see as well, and so it is very surprising that the data [did not show that]. There are a good number of people that are taking those drugs and it

was really interesting to see that there was no correlation there. There is a lot more to be learned from that kind of study, for sure.

Barr: Have you been involved in any other COVID-19 research initiatives both at NIH are outside of NIH?

Esposito: We did a few other things related to COVID-19. We were approached by NIAID early on to assist them in production of spike proteins for the Moderna phase one trials. They needed to get protein at the last minute. They were having problems with getting enough protein, and so we actually were able to supply them a significant amount of spike protein to finish those phase one studies for Moderna, which was really nice. We also have an ongoing relationship with NCATS to work on other COVID-19 projects with them. They have been developing these affinity reagents called nanobodies that bind to the RBD protein and try and block it from being able to bind to its receptor, and we have generated a number of proteins as part of that operation. All of this work combined led us to start up a small group within my lab that is focused entirely on COVID protein product action work and that is in support of NCI's SeroNet program, which is an extramural-facing program, in which the NCI is developing a serology laboratory and interfacing with laboratories in 12 or 13 different universities to do advanced COVID testing and check on assay qualifications, and things like that. We have been doing that now for the last six or seven months and have a couple people that spend most of their time developing COVID reagents.

Barr: Wow!

Esposito: That is the majority of what we have done. We have interacted with some other intramural labs who are interested in studying various aspects of SARS CoV-2 proteins including some structural biology work with the folks that do electron microscopy of Spike.

Barr: Have you resumed any of the kinds of work that you did before the pandemic?

Esposito: We have been back up to speed for, I do not know, probably nine months now, within the lab. That is one of the reasons we were able to hire a couple of new people to focus on the COVID work because we did have to go back to doing the RAS Drug Discovery work that we normally do. It has been a challenge to get that all going at the same time, but I think that in the long run it is great because people are going to be studying this at NIH for some time to come so, I anticipate that there is going to continue to be demand. There has not stopped being demand with every new variant that comes up.

Barr: It is true. What have been some personal opportunities and challenges for you with COVID-19?

Esposito: The main challenge for me was the fact that I been working from home since March of 2020, as part of the group that is teleworking, to allow the lab people to go back and actually do the lab work, so figuring out how to run a group and to carry out this kind of research from home was a challenge, especially in the early days of the pandemic, when time was a factor, and everybody was putting in 16-hour days. It was very difficult for me to be that far away and not have my hands on and stand there in the lab and look at the data. That was a challenge, but frankly, it turned out to be a great opportunity. It made our group more efficient; we learned a lot of new technologies out of this. We had never done

large-scale mammalian cell culture at the level that we needed to do for this project. We had cell culture going seven days a week basically 24/7; we had people in the lab in multiple shifts to pump proteins out. For a lot of the lab folks, it was a very invigorating sort of change for them from their normal process, and some of the technologies we developed, like the cell culture, like the magnetic bead technology we use to purify the proteins, those are now things that we are applying to all of our other projects for NCI so, I think that has been a great success.

Barr: Can you talk a little bit about the magnetic bead purifications in them because it is very cool, and I think it would be interesting to hear more about it?

Esposito: It is a relatively new technology. Pharma has been using it for maybe half a dozen years to purify antibodies. It was always kind of an interesting idea, but it never filtered down to our R&D [research and development] labs. Basically, instead of purifying proteins over columns where you need to run your protein, your cell culture supernatants, which can be liters and liters of protein over these resins in a column which takes in the case of spike protein seven or eight hours sometimes to load, you actually can take the same purification resin but it is attached to magnetic beads and you just drop these magnetic beads into your culture, you shake them for a little while, and then you stick a magnetic rod in there, and basically it pulls out the magnetic beads, which are then attached to your protein, and then you can simply put those into another vessel use a little bit of liquid to elute them off of the beads. You can purify your proteins that way, and it saves an incredible amount of time. It takes an eight-hour process down to an hour maybe, much less likelihood of failure from various components and mechanical things so, for any protein that is produced in the media of a cell culture experiment like spike or RBD, it was revolutionary for us to be able to do that. It works at all scales. You can do it at a tiny little scale of a couple mls [milliliters], up to these three- or four-liter flasks, so it is definitely something we are applying to a lot of other projects at this point.

Barr: That is very good.

Esposito: It is a cool technology. That is for sure.

Barr: Well, is there anything else that you would like to share as a scientist at NIH but also as somebody who is living through the pandemic?

Esposito: It has been a great experience for me. I have told people that this is by far the best collaborative effort I have ever been involved in during my 20 years at NIH. The fact that I got to interact with these folks from NIAID, NIBIB, and NCATS, and that we could all get together to basically pivot our labs almost instantaneously into doing something that actually supported public health like this was an example of what NIH can do if it wants to do that. For me particularly, at the Frederick National Lab, the ability to take the research that we have been doing for the past 20 years and be able to apply it so quickly to a project that is important was really great for me. I think long term, the collaborations that we have had with these groups are going to continue. This is going to be a great way for us to demonstrate that NIH can work together across institutes, across groups, and even across the government/contractor divide at the Frederick National Lab. That to me is a great sign of the sorts of

things that NIH can really do and why we should be doing this. It is a perfect example of government-funded science.

Barr: Definitely. Well, I wish you and your lab continued success and continued health, and I look forward to seeing what you what continue to do.

Esposito: Excellent. Thanks Gabrielle. I appreciate the opportunity to talk about all this. It has been great.