BEHIND THE MASK October 8, 2020

Oral History of Nihal Altan-Bonnet, Ph.D. (NHLBI)

Interviewed by Gabrielle Barr, Archivist, Office of NIH History and Stetten Museum, NIH

G. Barr: Good morning. Today is October 8, 2020 and I have the pleasure of talking to Dr. Nihal Altan-Bonnet who is a senior investigator with the National Heart, Lung and Blood institute. She works at the laboratory of Host Pathogen Dynamics. So, good morning and thank you very much for sharing your research experience with us for our Behind the Mask Project. So, Dr. Altan-Bonnet, recently you received a grant to study how coronaviruses exit the cells. In lay terms can you please describe the premise of your research.

Dr. Altan-Bonnet: Absolutely. Thank you for putting this together. It's a pleasure to be here chatting with you. So, let's start from the very basics. In order for coronaviruses to spread to other hosts, other people, they need to replicate inside a cell and then they need to be able to get out of that cell. There has been a real lack of information or insight into how these viruses get out of cells. So, I started to investigate what might be going on there and what we found was surprising. We found that these viruses use a very unusual pathway to get out of the cell, a pathway unlike those used by other types of viruses that we're familiar with, for instance, hepatitis C or West Nile virus or Dengue virus or Flu virus.

So, this observation was sort of the seed, the foundation, of the grant.

G. Barr: Why is it unusual? What about it makes it different?

Dr. Altan-Bonnet: Good question. The pathway that they are using involves lysosomes. Our cells contain lots of different types of organelles. The nucleus is an organelle where our genetic material resides. The endoplasmic reticulum is a giant organelle in which proteins are synthesized, including viral proteins. There is the Golgi apparatus, which is very important for processing proteins, including viral proteins, and sorting them out and sending them to different parts of the cell. Then the mitochondria—that's the energy center of our cells, and then we have these organelles called lysosomes. These organelles are considered as the trash disposers of our cells but they have a lot of important functions. They recycle cellular material by chopping up those cellular materials back to the basic building blocks and make them available for the cell to reuse or to repurpose to make new proteins, to make new lipids, and so forth.

In order for the lysosomes to do that these organelles have to be really acidic inside. So, the pH of these organelles has to be like the pH of lemon juice, you know pH 4, whereas the pH of water for instance is seven—it's neutral. So, in this highly acidic organelle you have enzymes that do the business of chopping up our cellular material. They absolutely need this acidic pH to do this work.

What we found was that coronaviruses were transporting themselves into these organelles, and then these lysosomes with the coronaviruses inside of them would move out to the periphery of the cell and fuse with the plasma membrane of the cell—that's the limiting membrane of the cell—and, when the lysosomes fused with that membrane, that would result in the viruses that are inside the lysosome to come out of the cell.

When we saw the lysosomes being exploited by coronaviruses to get out of the cell, we were really surprised. How come the viruses were not getting degraded inside the lysosome? because, the lysosomes are normally very acidic organelles and have all those enzymes in them that chop up proteins. So, we asked: Are the lysosomes shuttling the coronaviruses still acidic ? What we found was: no, they were not as acidic. They had a much more basic pH, and then when we looked at the degradative activity of lysosomal enzymes, we found that they were no longer as active, probably because the pH wasn't quite right for them to be active.

So, seeing that made a lot of sense in terms of the virus because we realized that the virus was using this organelle to get out of cells and, in the process, essentially destroying the functions of this organelle. Destroying those functions, we discovered, helps the virus in two ways. The first way is kind of obvious: by not having an acidic environment there, by not having those lysosomal enzymes active, the virus could be protected, it didn't get chopped up.

But the second way was much more sinister. Lysosomes are critical for breaking down viruses and bacteria when cells get infected and pieces of these pathogens become presented on the cell surface. When this happens, it's an alert or a call to action for our immune cells, telling them that there is a viral or bacterial infection in the host. What we discovered, and this part of the study was done in collaboration with my husband Gregoire Altan-Bonnet who is an investigator at the NCI, is that coronaviruses by using lysosomes to get out of the cell, also end up destroying this essential function of the host cell being able to alert the immune system. That initial alert of the immune system is now perturbed and this we think has ramifications for our immune system, potentially handicapping it.

G. Barr: Did you start getting interested in this sort of research with other coronaviruses? I know Covid-19 is very severe and a big one, but you've been studying viruses for a very long time.

Dr. Altan-Bonnet: Yes, so actually we started this work about five years ago. We used at the beginning, and still do, other family members of Covid-19 that are a little bit safer to work with in the laboratory setting, so a lot of our initial experiments are done with those family members. Then we do the key experiments in the biosafety 3 level suite with Covid-19 viruses to demonstrate that they behave the same.

G. Barr: Can you speak a little bit about the technologies and the process that you go through when you're doing this research?

Dr. Altan-Bonnet: A lot of our experiments start out with observation and for observation we use microscopy which is really a very important tool in our lab. From that observation using microscopy we then branch out and go and use all other kinds of tools to test our hypotheses. But really fundamentally most of our findings start out with looking through the microscope and looking at what's going on in the cells. In this case, what's going on with lysosomes—for example their pH changing in the infected cells. After that we use whatever tools we need to test our hypothesis. It can be tools of immunology, tools virology, cell biology, proteomics, lipidomics etc.

G. Barr: What challenges have you experienced over the course of this study? Have there been any hurdles that have come up?

Dr. Altan-Bonnet: Oh yes, so you know, the kernel of this work started five years ago when we observed that these viruses were not using the usual pathways of exit that other viruses I mentioned, such as

hepatitis C and flu, were using. We got stuck and we really didn't know where to go. We didn't know what they were using. We knew that wasn't the classical pathway and so over the last four years until this pandemic hit this was very much a side project in the lab. People who came into the lab kind of worked on it a little bit and then got distracted or frustrated and moved on to other projects not to do with coronaviruses. I usually would be the one left with this project and would try to push it along with summer students who come to NIH, for instance a summer internship program, but really nothing much got done and it wasn't because it was the summer students' fault; it was really because I was distracted working on other projects and this was always sort of a fringe project. Then when the pandemic hit us at the beginning of this year, it seemed like the perfect time to buckle down and try to solve this problem. It was also an excellent opportunity to keep working in the lab; I love working in the lab. But I could not have done it without my wonderful postdoc Sourish Ghosh who also chose to stay and work on solving this problem with me. This pandemic was in some ways a great opportunity for us to keep working in the lab.

G. Barr: That's good. Well, I think that transitions to our next question. During the pandemic have you been on campus and, if so, what has it been like?

Dr. Altan-Bonnet: Absolutely Sourish and I have been coming to lab 7 days a week, putting in 12 hours or more a day. I also want to thank the cleaning and the maintenance crew who were here with us, not only keeping us company but also making sure lab operations and equipment ran smoothly: the garbage was picked up, the autoclaves were working. But other than one or two labs in our building that were also open, everything was shut down, it was very, very quiet, empty and a bit sad on the campus. The only silver lining was we could get a parking space very easily right next to the lab.

G. Barr: Did you feel like it was easier for you to focus or did you miss the extra help, or both?

Dr. Altan-Bonnet: Well, I certainly missed my lab members. But I have to admit that I also loved having the lab space pretty much to myself—no meetings, no commitments—just doing experiments and testing ideas. It was blissful.

G. Barr: That's really good. Personally, I guess, not work-related, what have been some challenges that you've faced with coronavirus and what have been some opportunities?

Dr. Altan-Bonnet: One of the challenges is that I have a son and a daughter. My son graduated from high school this year and my daughter was in her freshman year of high school when pandemic struck. It was difficult to see the kids having to transition to online learning. It was kind of difficult to see my son spending his senior semester at home, not getting to do the fun things that he worked all really hard toward for four years. The good thing was having them around, especially my son, because I knew that he was going away to college in the fall and we wouldn't really be able to spend time as a family together with him anymore. That was the silver lining that all of us got to be together as a family whether they liked it or not—the kids, but for my husband and I it was really nice to have this time to be with them and also kind of kept the kids safe too. Seniors, you know, in their senior semester tend to go wild after the college acceptances are in—parties and beach weeks and this and that. I was happy that he didn't get to go anywhere and do all those things but stayed safe at home. So that was kind of more family time; it definitely was the silver lining in all of this and I'm sure that's true for everybody.

G. Barr: Definitely. One question I always ask: if your Covid experience could be defined by a song which one or ones would it be?

Dr. Altan-Bonnet: Oh, my goodness. I saw this question. I really don't know. I mean I don't have...I listen to a lot of songs. I listen to a lot of music in the lab when I'm working.

G. Barr: So, if you had a superpower to get you through this time, which one would it be?

Dr. Altan-Bonnet: That's all right. "Mama" by Elvis Presley. I love that song. It always puts me in a good mood when I'm feeling down so I guess I would pick that as that's one of my favorite songs.

G. Barr: Okay, that's very good. Well, thank you very much for talking with us today and we wish you the best of luck with the continuation of your research.

Dr. Altan-Bonnet: Thank you so much. It's been a pleasure talking with you.