Dr. Behdad (Ben) Afzali
Behind the Mask
August 2, 2021

Barr: Good afternoon. Today is August 2, 2021. My name is Gabrielle Barr, and I'm the archivist at the Office of NIH History and Stetten Museum. Today I have the pleasure of speaking with Dr. Ben Afzali who is a Stadtman Tenure Track Investigator and the section chief of the Immunoregulation Section of the Kidney Diseases Branch at NIDDK [National Institute of Diabetes and Digestive and Kidney Diseases], and today he is going to speak about his COVID research. Thank you for being with me.

Afzali: My pleasure; thank you for the invitation.

Barr: Absolutely. One of my first questions is: can you please describe the premise behind the study that you were a part of that looked at whether SARS-CoV-2 drives the JAK1/2 —I don't know how to quite pronounce that—dependent local and systemic complement hypoactive activation? It's quite a term.

Afzali: Of course. Towards the beginning of the pandemic, most of us were unable to carry out our regular research, so we looked to see whether there was any way in which we could contribute towards the fight against the pandemic, and one of the things that our laboratory does is that it's very good at distinguishing signals from noise in data that comes from sequencing. What we mean by that is that if you take a cell and you sequence all of the DNA and the RNA inside it, and you compare it to another cell you can see what the differences are between them and that might reveal the kind of effects that have gone on within those cells. We teamed up very early with our great collaborators at Purdue University. This is Majid Kazemian and with a number of people both at the NIH and outside, and I'll name them right now. Claudia Kemper at NHLBI [National Heart, Lung, and Blood Institute], Michail Lionakis in NIAID [National Institute of Allergy and Infectious Diseases], and Christiane Wobus over at the University of Michigan.

What we did was try to source sequencing data, and one of the real success stories of living through a pandemic is that people started generating data from all around the world and making it publicly available without regard to their own publication. So the data was available for many studies before their own papers were published, and we were able to look at some of this sequencing data. We compared infected cells against uninfected cells and by comparing those two we could see some of the effects that SARS-CoV-2 infections, a coronavirus 2 infection, had in those cells compared to uninfected controls, and it didn't really matter what kind of cell types we looked at or patients with diseases or patients who were healthy controls. We found a similar signature in all of them, which was that a system that's normally present within the blood was highly induced within these cells by the virus, and this is cool.

Barr: Can I stop you? I had a question. I saw that you took from two patients with COVID, and then you had a corresponding number of controls for your study. How did you choose the number two because it seemed like such a low number considering some studies have thousands of patients enrolled?

Afzali: Right. These two were the first two that became publicly available. That's why they're in our studies. We gathered all the data that we could find, and we analyzed them all together. Then of course there was single cell RNA sequencing from more patients that is presented further on within the same paper. In terms of just biopsy samples from patients, there were only two available at the time.

As I was saying, we found that the virus really induced this system. That's called complement, and the reason that is called complement is because this is a really ancient system that was discovered a very long time ago that was supposed to complement the rest of the immune system. It's an ancient and highly conserved system among multiple species that recognizes bacteria and viruses and helps to remove them from the body. It's a set of danger signals, and it's usually designed to induce inflammation. Now around 10 years ago, [in] 2013, there were papers that were being produced that were showing that this complement system is not just restricted to the blood, but that other cells produce it. Most of those papers focused on immune cells, and we're part of the consortium of people that published those papers to show that actually cells express their own complement proteins. This is important because circulating complement that's made by the liver is great if you have a blood-borne infection, but inside a tissue, let's say within your skin or something, that complement can't reach that site, so it's advantageous for local cells to be able to make their own. Which begs the question: What are the signals that induces them? What we found was that there were lots of complement proteins being made by lung cells that were infected by the virus, and in fact it was very proportional to the amount of virus infection. The more virus, the more of these proteins that were found.

Barr: That's very interesting. What kind of signals are there? Because there are different kinds of signals so what kind of signals are produced in the lungs for COVID-19 patients?

Afzali: What we found was that if we traced it back—so you take you take all the genes that are induced by the virus and you try to see what the common elements are between them. From that, you can sometimes work out what the signal must have been to have induced all of these genes. We found essentially that by this analysis there are two types of signals: one of which was called STAT1, which is part of the interferon response, which is a classical antiviral response, and the other is an NF-KappaB response, which is common to a lot of activating signals in cells and probably induced by the virus. The most likely thing was that the virus was inducing this antiviral response, the interferons, and they were then signaling into cells through the proteins that you mentioned earlier, JAK1/2 and STAT1, and the good news is that there are drugs that can block this signal.

Barr: So can you speak in more—because part of your study was looking at some possible combinations to help with the situation so can you talk a little bit about that part?

Afzali: Of course. There were two components here. One of these is that because we knew that the regulator of the response was STAT1 and JAK1, there was a very specific inhibitor of JAK1 called Ruxolitinib, and what we found is that in an independent analysis of drug, that the top predicted candidate for blocking the SARS-CoV-2 induced changes in a cell was Ruxolitinib. We also knew that because we had already mapped the likely regulator of these complement components, that

Ruxolitinib is likely to stop these. So then we did some *in vitro* experiments with Christiane Wobus's group in the University of Michigan where they infected cells in the culture plate with the virus and either gave them Ruxolitinib or gave them Ruxolitinib with an antiviral or gave them nothing. What we found was that the amount of complement produced went down with the Ruxolitinib and then went almost to zero if you also had the antiviral. What was very interesting in all of this, is that we teamed up with GSK [GlaxoSmithKline] to also test a very specific inhibitor of complement as well.

Now the second reason why the complement story is important, is that most trials blocking complements are giving people drugs that only work in the circulation. What we had shown was that there's all this complement being made by cells that are infected at these sites. Those won't be blocked by drugs that work in the circulation. They can only be blocked by drugs that penetrate the cells and work inside the cell, so intracellularly. To simplify the complement story, what we found was that two complement components were produced. One of them is an enzyme that activates the other one.

Barr: The C3 on the C5?

Afzali: That is Complement Factor B. Complement Factor B was also being produced in response to the virus and that was processing the C3 to inflammatory subcomponents. We managed to partner with GSK who had a cell permeable inhibitor of Complement Factor B in development, and this paper is the first demonstration of this particular inhibitor blocking complement activation. In the in vitro assays, that drug also did stop complement being produced by the cells, which was great.

Barr: That is really great. What are some next steps that you have done since the paper was published?

Afzali: Although I'm a clinician, it's not really in my roundhouse to do a lot of clinical trials. Most of what I do is work at the basic biology. There have been some trials of JAK inhibitors, and some of them have not fully reported. Some were positive, and we're waiting for more data to come through on the JAK inhibitor story, but our guess is that these drugs, like any other drug in COVID-19, needs to be given at the right time, probably with the right combination. If you give them too early or too late, they might not be as effective, or they could potentially even be detrimental. It's very important to get the right kind of studies done, and but we think that Ruxolitinib together with an antiviral is likely to be beneficial, particularly with more severe disease. When you're really trying to stop the inflammation.

Barr: Do you think some of your basic research into the complement system could be used for other kinds of diseases?

Afzali: Oh absolutely. Our other papers on complement have revolved around immune cells and their production of complement because it's easy to access them. Most of those have been in the context of rheumatic diseases. My own research from my lab is looking at complement being produced by kidney cells in response to injury. Right now we're trying to find out which components of complement,

whether it's good or bad, and how we can manipulate that to improve outcomes from kidney diseases. But on that complement sort of trajectory, we've also done some work on the Vitamin D system, which I think you've also seen, right?

Barr: Yes. So that that will be my next set of questions. Can you talk a little bit about the study that you were part of that looked to see if adjunct therapy with vitamin D in the context of other immunomodulatory drugs could be a beneficial strategy to dampen hyperinflammation in severe COVID-19? And how does vitamin D help dampen hyperinflammation? I guess we could start with that.

Afzali: We could start with that. This paper is not published yet. We're still working on it, but the current version is substantially different probably from the one that is available as a preprint. What we found is that, again talking about complement, obviously the lungs are a complement rich environment as we just said, and also immune cells make their own complement. What happens when an immune cell gets a complement-derived signal, particularly C3b, one of the fragments of C3, is that it starts becoming inflammatory. And if you have that continuous signaling from that receptor, it then starts switching off its inflammatory functions and starts producing anti-inflammatory ones, because that's part of the mechanism of shutting down inflammation. It becomes inflammatory, then it shuts itself down, and starts making factors that help in wound repair. You know, there's been a lot of inflammation now you need to get rid of all of that. We were interested in how this system works, because no one has really ever conclusively demonstrated the mechanisms of T-cell shutdown, if you think about it, right. If you have a lot of inflammation mediated by T-cells, what you really want to do is find a way to shut them down.

We looked, again using sequencing, at cells that were inflammatory or non-inflammatory, and what we found was that complement signaling really induces a system within T-cells that activates vitamin D, which is really quite interesting, because the system not only activates vitamin D, but it also gives the cells the ability to respond to it. It means that within the local environment, they can activate and respond to vitamin D. The rest of the story is about the molecular mechanisms by which vitamin D dampens down the inflammation. If you want to summarize it very quickly, what we found it does is that it changes the architecture of the genome within that cell so some parts of the genome become very accessible. Some places become less accessible. Then it recruits a group of transcription factors, other proteins, that then tell those genes to switch on or switch off. We found three genes, three proteins, and the fourth one was the vitamin D receptor itself. Between the four of them, we can explain about 60% of the genes that are regulated by vitamin D in a cell. That's how, generally speaking, vitamin D works. That's the short version of the story.

Barr: How long does the process take between the vitamin D and then the cells responding to it?

Afzali: Yeah, that's a really good question. We can see changes in the architecture of the genome within 45 minutes of giving vitamin D, but then if you leave it longer, 24, 48, 72 hours, then you start seeing a sort of stabilization of those changes. It takes a little bit of time. Remember the structure of the gene has to change and then the expression of the genes changes. It’s usually sequential.

Barr: So what has been your role in this particular study?

Afzali: Again, it's a collaborative effort. I can't possibly take all the credit for this. They key players are people in my group, Majid Kazemian at Purdue University, Michail Lionakis, and an old colleague called Arian Laurence in London who helped with a lot of it.

Barr: Do you think that vitamin D or vitamin D plus other kinds of therapeutics will be eventually administered to patients with COVID- 19?

Afzali: It already is. There is a very strong association between vitamin D deficiency or insufficiency and the incidence and severity of COVID-19. Across different countries, it seems to hold true, and the larger studies, the meta-analyses, support that, but vitamin D deficiency is implicated in a lot of diseases, and we have not really seen in large trials that vitamin D can reverse things, which has always been disappointing. Now one of the reasons for that is because vitamin D needs to be given, again, at the right time in the right concentrations, and it takes time for it to replenish your vitamin D stores. Also not all of the vitamin D that you give is absorbed. One study that's been published recently has a sizable trial from Spain, I think it was 700 patients or something. That showed that it worked very powerfully actually to prevent people going to ICU. One of the tricks that they use which is actually quite clever is they used an analog of vitamin D which has very high absorption and doesn't rely on the liver to get activated. This thing replenished vitamin D very rapidly, and it was quite helpful. Now in England, where I'm originally from, giving vitamin D and steroids together for patients with COVID-19 is relatively routine. You know, my mother actually had COVID-19, and that's how she was treated: steroids, antivirals, vitamin D, and oxygen, and there aren't many side effects doing it. Doing it is acceptable to patients so even if the evidence base for it isn't 100%, most physicians in a controlled environment like a hospital will replenish someone's Vitamin D stores.

Barr: In addition to these two studies, are you involved in any other COVID research or activities, either at NIH or outside of NIH?

Afzali: Yeah. Actually we had another paper recently. If you remember, there was a publication a few months ago that had suggested that the virus integrates into the human genome—and I have to qualify this. Right at the beginning of when we started doing our research in the sequencing, what happens is you get your sequencing information and all you have is a bunch of nucleic acid sequences. What you have to do is figure out which genes they're from. You align it to the human genome and say, "So these are the human genes and how much they're expressed, but there's a bunch of stuff that doesn't align." You often just throw that away. Obviously in an infection, we said, "Okay, well, we're going to also align this to the virus genome and see what the virus genes are doing." Then we found that there were a third group of RNA molecules which were half-human and half-virus. These "chimeras" in DNA viruses can sometimes indicate that the virus has integrated into the genome and now that part is being expressed, meaning you get half-human half-virus transcripts. Our interpretation of the data was that this was artifactual because this is not the virus that has machinery to be integrating into the genome, and also it doesn't have a life cycle that goes into the nucleus. We'd be asking a lot.

Now another group took a different view: That certain endogenous proteins in the cell are induced which helps the virus to integrate into the genome. And that paper generated a lot of anxiety among the community about the potential, obviously, long-term implications of having the virus but also safety concerns about the vaccine. Unfortunately, a lot of the anti-vaxxer community use this as ammunition to advocate not having the vaccine. At that point we thought, well, we now have to do something about this. We analyzed this data in great depth, and we didn't find any compelling indication that the virus integrates into the genome. We also developed a sort of a new technology for our new method for enriching any nucleic acids that had virus components in them, and in that we also didn't see any evidence of integration. The best thing that we could say is that these all look like artifacts of the sequencing technology themselves, and since our paper was published (it was published in the *Journal of Virology*), another group did long read sequencing, so they looked at really large sections of RNA, and their interpretation, this was in *Cell Reports*, is also that the chimeras, the hybrids, are also artifacts. They're not real. I think that was an important contribution. For us as scientists, it's small debates, but in the context of a pandemic it became a big thing. And we made our contribution to that area as well.

Barr: Yeah. What was your method? You said that you improved upon a method. What did that look like and what did that entail?

Afzali: Oh, it's just—so we took the standard sequencing methods, and we added a couple of steps to it to help us pull out the virus transcript. Think about it this way: if you have RNA from the virus, and these are needles in a haystack, what we did was we managed to get a really strong magnet to pull out the needles, and then sequence those preferentially. So that's what we do.

Barr: Are there other aspects of COVID-19 that you and your lab are eager to either delve deeper into or study from scratch?

Afzali: Yeah. So now that we're back full time to work, we do have a couple of things that we're looking at, but it's not a major focus of my lab now, because we work mostly in the context of immune cells and kidney diseases, and we'll probably have to focus on that. There is another story that we are a part of that will come out in time. The first thing we have to do is to finish the vitamin D story and publish that. Then go from there.

Barr: That's true. So in addition to being a scientist at NIH during the pandemic, you've also been a person who has lived through COVID or is living through COVID-19. So what have been some opportunities and challenges that COVID-19 personally has presented for you?

Afzali: So that's a really good question. I think it's true to say that when you live through historic times, life is going to be difficult or challenging. Most often historic times have involved major depressions or even international wars. Now in some ways we've been lucky that we haven't had to live through that in our lifetimes. This is probably the biggest challenge that most of us have faced in our professional and personal lives to date, and it hasn't been easy, but it's important to contextualize it. We have jobs

that are secure that we could do to an extent from home. We're not necessarily front-line workers, and we've seen that there are people that have lost their jobs, their livelihood, possibly their abodes. In that context, we can't say that it's been particularly difficult, even though it has not been easy for us.

The biggest challenge probably for me personally is the inability to travel and to see my family. I lived in the United Kingdom, and some of my friends and family that have actually contracted COVID and been in the hospital. Thankfully everybody came out of it, but nevertheless that's probably the challenge from a personal perspective. From a professional perspective, obviously there are patients. I'm a doctor; there are patients that need to be looked after, so we did our part to the extent that we could. I run a lab where I have to look after the well-being and the careers of my trainees. Overall, I think we decided very early on that we're a team, and we're going to help and support each other, no matter how bad it gets, and we'll come out of it at the other end. In 10 years' time, we'll be telling people what it was like living through these times, and how we coped. I think overall, everybody in my lab, to their credit—amazing people that they are, came out of it as not only stronger but also as better people.

Barr: Are there any ways in particular that you help support your trainees at this time?

Afzali: Because we could work remotely, the portions of the lab that could perform computational biology worked computationally. Those that were not able to, read papers and wrote or reviewed articles, and some of those that were allowed to perform experiments, were in the laboratory performing experiments. Then, of course, to make sure that everybody's career is on track, once we were allowed to have people back, we went to a shift system in the lab to make sure that we don't exceed maximum occupancy. Everybody complied very stringently actually with COVID guidelines, and we didn't have a single person even have a hint of the sniffles, let alone COVID-19. I think in the end, it's all about teamwork. I think it's important for people to know that in this kind of environment, you too are concerned, worried, and to an extent scared, and that what they're feeling is also what the rest of us are feeling. If a problem's shared it's less of a problem.

Barr: Yes, well is there anything else that you would like to share about either your research or just your situation dealing with COVID-19 as an individual?

Afzali: Yeah. Actually, I think it's important to acknowledge the role played by the NIH and particularly senior leadership. We have to remember that this is a situation where there was considerable panic around the world as the virus was sweeping through country after country, and we had the reports from Italy and in New York of how bad it could be and then I think in Washington state. I think the communication responsiveness of our leadership at NIH was incredibly helpful, and I think we mobilized the troops to do what we could and to help. I think I'm very grateful to be here and to work for and with some of the people that I work with, and I feel privileged and blessed in that respect. So yeah, that's all I have to add.

Barr: Well, that's very good. I wish you and your lab the best with both your COVID research and your kidney research, and I hope that you and your family continue to stay well.

Afzali: Thank you very much. It's been a real pleasure being here and thank you for the invitation. I'm a big fan of oral histories, and at another time, I can tell you some of my experiences of oral histories.