

Dr. Jianliang Xu

Behind the Mask

December 2, 2021

Barr: Good morning. Today is December 2, 2021. My name is Gabrielle Barr. I'm the archivist at the Office of NIH History and Stetten Museum. Today I have a pleasure of speaking with Dr. Jianliang Xu, a research fellow in the Laboratory of Lymphocyte Nuclear Biology at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). Today, he's going to be speaking about his COVID-19 research and experiences. Thank you very much for being with me.

Xu: Thank you, Gabrielle.

Barr: To begin, please speak about your contribution to the mRNA vaccine-elicited antibodies study that looked at SARS-CoV-2 circulating variants.

Xu: Of course. We started our study immediately after the NIH shutdown in March in 2020. At that time, my work was not focusing on the mRNA vaccine antibody study. In doing my study, a vaccine got approved. It became our main concern whether the vaccine was working well or not. My PI [principal investigator], Dr. Rafael Casellas, thought it was a good direction to look into. I personally didn't participate in the start of the project. I helped by processing clinical samples from vaccinated people. These samples were then later sent to Dr. Michel Nussenzweig at the Rockefeller University for analysis. I don't really have much to say about that, but the results turned out really well. This turned out to be one of the first studies showing that the mRNA vaccine can elicit antibodies that can neutralize quite well the SARS-CoV-2 variants of concern.

Barr: One of the takeaways, though, was that vaccines should be updated, and monoclonal antibodies should be looked at periodically to see how well they're still working against each variant. Given how often the SARS-CoV-2 virus is mutating, how often should vaccines be looked at for efficacy, and how often should other therapeutics or monoclonal antibodies should be analyzed?

Xu: I don't know the exact timeline. We named those variants by Greek letters—Alpha, Beta, Gamma, Delta, and now we have Omicron. Over time, SARS-CoV-2 is mutating. From the vaccine antibody study I mentioned, the conclusion was that we have a vaccine which may need to be updated periodically, but so far, we are still using the original version of vaccine. That vaccine is still functioning well against any of the circulating variants. Maybe it'll be ideal to update them, but so far, it's not really necessary—but people are working on that still.

Barr: Can you speak to the difference that this study showed for people who had a natural immunity—that is, people who have had COVID-19—versus those who were given the vaccine? What was the extent of similarities and differences in their immune responses?

Xu: There are some studies comparing immune responses among naturally infected people, infected people who were subsequently vaccinated, and immunized healthy people, and then compared that immune response to SARS-CoV-2. The results show that naturally infected people who were subsequently vaccinated tended to have a better and increased immune response. Their body can better produce more [and better] neutralizing antibody against SARS-CoV-2. The main conclusion would be that vaccination given following natural infection and recovery provides better protection [compared to natural infection alone].

Barr: Let's now discuss the study that you led, which was the development of nanobodies against SARS-CoV-2. Before we get into this study, what are some advantages of nanobodies—which are also known as variable heavy chain domains of heavy chain-only antibodies—over other types of therapies and measures against COVID-19?

Xu: The biggest problem with neutralizing viruses is whether your antibody can gain access to the virus surface protein. That is because the virus surface protein is heavily glycosylated, so it's protected from being attacked by antibodies. The advantage of nanobodies is that they're very small, so they can, in a lot of cases, ignore that glycan shield, penetrating the epitope that is hidden, and can then try to neutralize the virus. A lot of variants, although they mutate, don't change much in some places. If your antibody can target or bind to those regions, and then ignore any mutations, because it's small, it can find and access unchanged places among variants. That's one of the advantages of nanobodies.

Barr: What are some of these areas on the virus that don't change very much?

Xu: Even in the very changeable region that's called the receptor binding domain (RBD) region, there are some unchanged places that are conserved. We found some nanobodies targeting the epitopes of conserved regions. SARS-CoV-2's surface protein is like a crown, and it has a stem at the bottom. The stem region is really conserved. Recently, there have been some discoveries of antibodies targeting that stem. A certain portion of RBD is conserved and the stem region is conserved.

Barr: Will you speak about the process of isolating anti-RBD nanobodies from llamas and mice that your team engineered to produce VHH [variable domain of heavy chain-only antibodies] cloned from alpacas, dromedaries, and Bactrian camels? What were some of the obstacles you and your team encountered?

Xu: Yes, of course. To isolate the anti-RBD antibody, you first need to immunize—or vaccinate—llamas or mice with the target antigen to induce an immune response to that protein you gave them. We give the llamas and the mice spike proteins and RBD proteins. After immunization, at a certain time point, we collect blood from the llamas. For the mice, we collect samples from the spleen, bone marrow, and blood. We use these samples to collect B-cells which can produce antibodies. From B-cells we try to get information on all antibodies and make a library. From the library, we screen them to find RBD specific antibodies. From these antibodies, we test candidates for neutralizing virus. That's how we get these antibodies.

Barr: That sounds very time consuming. What was the time frame to carry out this work?

Xu: For immunizing animals, it's about two months. For immunizing the mice it's a little bit shorter. Then you have to spend time isolating cells, making nanobody libraries, and doing the sequential rounds of selection. Because of our previous experience with other viruses like HIV, we've optimized everything. Those target candidates were accomplished fairly quickly.

Barr: How did you and your team test the nanobodies against the two variants of concern of SARS-CoV-2 that were in circulation at the time, and what were your findings?

Xu: First, when we got our lead nanobody candidates, we tested them on the wild type version of virus, using a pseudo-virus or fake virus. It was around January or February and the circulating viruses at that time were the so-called UK and South African variants, which got people's attention. We wanted to test whether our nanobody could still neutralize them. We set up collaborations with Dr. Michel Nussenzweig at the Rockefeller University, started testing them, and then, very excitingly, found that our nanobodies could neutralize those variants.

Barr: Were you and your team surprised that the nanobodies were increasingly less effective with each new emerging variant? Have you continued to test against variants of concern since your original study?

Xu: We had two types of nanobodies isolated. One type became increasingly less effective against the new strains, especially the South African strain. For some nanobodies, activity was totally abolished. But the [effect of the] other group of nanobodies, isolated from the nanomouse, never got blunted. That told us that the mechanism for neutralizing those variants is different for the two groups of nanobodies. For the group that got affected, we actually figured out that it was because those nanobodies are targeting a very changeable region, so of course you got reduced effect. For the group [isolated] from nanomice, they are targeting the conserved regions. They don't lose effect.

Barr: Could you explain the advantages of multimeric nanobodies, which turned out to be the most effective? What does that mean for people that don't know?

Xu: Nanobodies are very small, and like a Lego, you can assemble them into many repeating pieces. In our case, we'll have a small nanobody, and then we'll add another one, and another one— three copies of them put together tandemly. If one nanobody can bind to a certain virus protein type, then three copies can bind really well. That's one of the mechanisms—triplicate copies of nanobodies can work better.

Barr: As you had such a large library, did you test individual candidates and test as a group?

Xu: We have two different strategies. For a big library, first we screen the library against the RBD protein, and make sure we are looking only at RBD binders to ensure that nanobodies can recognize this SARS-CoV-2 surface protein. Then, from the narrowed down library, we randomly pick about 100 candidates. Then you would see the frequency of different antibodies showing up. This way, we try to pick as many as possible, but you may still lose some candidates. Another strategy was to sequence each and every one of them, and then by bioinformatic

analysis, look at the sequence of nanobodies and cluster them into different groups. We pick some representative leads from the different groups and then test them.

Barr: How do you see your study translating into therapeutics that patients can take?

Xu: Honestly, that will take time. Nanobodies in our case have been isolated from llamas and nanomice. These are something the human body has never seen. Although those nanobodies can neutralize virus, if you put these nanobodies into a human body, they are foreign materials. They will be rejected and cause some trouble. Before you can really use them for therapy, you have to make them less toxic to human beings. That's called "antibody humanization" and that will take a lot of effort. But people are working on that—and we are still working in that direction, but it will take time.

Barr: Have you been involved, or do you plan to be involved, in other COVID-19 initiatives? What are the next steps for both of the studies you have worked on?

Xu: Because we already have very good leads from our work with SARS-CoV-2, the next step would be to humanize the nanobodies [and] to further increase their activity. For example, we can change a few connectors or attach the nanobody to a different conjugate and boost the activity and increase the half-life in the human body. Also, because we were working on RBD-targeting nanobodies, but RBD is only one part of the useful spike protein, I am aiming to isolate nanobodies against some [other] conserved un-changeable regions of the spike protein. Further isolating nanobodies [that] can ignore any further mutations or variants.

Barr: Sounds like a lot of exciting work ahead! Can you speak about how your training and background have prepared you for your work with COVID-19? You said that you've worked before on antibodies and nanobodies against other diseases?

Xu: Looking back, I can say I've been preparing for this for many, many years! Before coming to the NIH, I was doing my first postdoctoral training in Professor Tasuku Honjo's lab at Kyoto University, where I studied antibody diversification mechanisms. With that, I obtained a good understanding of [the mechanism of] antibody structure and maturation. After joining [the laboratory of] Dr. Rafael Casellas at NIH, I continued working on antibodies. One of the critical projects I led was trying to make a mouse that could express nanobodies. This work started in 2016, before any pandemic came. We had a feeling that nanobodies might be a very useful tool for therapeutics. I succeeded in making a mouse model that [we can use to] quickly generate nanobodies. We tested those mice for different targets, including the flu and HIV, and we accumulated a lot of experience. In March 2020, when NIH shut down and all non-essential work was stopped, I was working on HIV, which was not considered essential. We switched to working on SARS-CoV-2 since we had our proven platform.

Barr: Can you speak about your role in the nanobody study? What did it mean to lead this study? What were your responsibilities and what you did for the team?

Xu: I was the one who generated the nanomouse before the pandemic and used it for our HIV project. Then SARS-CoV-2 came, and I was the only one in our lab assigned to be an essential worker. I came back to the lab immediately in April. I was working on my own on the 13th floor for about two months, every day, by myself, preparing antigens—including the spike and RBD proteins—and shipping them to a company for injecting the llamas. I did the immunization of the mice myself on the 9<sup>th</sup> floor. Basically, I did everything myself in the early stages. Later on, I was making nanobody libraries and screening for nanobodies. In June or July, people started coming back and we had more people joining the group.

Barr: Did you like being by yourself and the ability to focus, or was it creepy to be all by yourself every day?

Xu: It felt strange, but I saw it as a privilege. I really appreciate the opportunity that Dr. Rafael Casellas and IAM [Identity and Access Management] provided to continue my research, even though it changed from my original project. I feel really, really privileged for being able to work on this important project. It was just an amazing experience.

Barr: That's great. In addition to being a scientist, you're also a person who's been living through the pandemic. Briefly, what have been some personal challenges and opportunities that have arisen for you because of the pandemic?

Xu: [In terms of] personal challenges, I have a family—my wife and my son. My boy was in the first grade in elementary school at that time, and suddenly every student had to stay at home and learn virtually. Everybody's routine and schedule changed. I had to accommodate everything. I had to come to the lab really, really early—sometimes 3:00AM or 4:00AM—to start working, and then went back home in the early afternoon to help my wife and be with my son. That is the most memorable experience from the COVID time.

Barr: Were there any opportunities? It sounds like at least you got to be with your son more than you might have otherwise.

Xu: I would say that with this pandemic and the different schedule, I get more time with my family—and it's a good thing.

Barr: Definitely. Is there anything else you would like to share about your COVID-19 research or experiences, or just anything you would like to say at all about the pandemic?

Xu: People must have faith. Even though new variants keep coming—and I believe more will come—and the virus is changing, our vaccines have readied our bodies for those variants. Even though the viruses are mutating, and we are still using the first version of vaccine, I believe that it should still be able to protect us well from getting really sick. People must have faith. It will be over.

Barr: Definitely. I wish you and your family all the best, and continued success in your research.

Xu: Thank you very much. I wish you the same.