Dr. Iyadh Douagi
Behind the Mask
November 4, 2022

Barr: Good afternoon. Today is November 4, 2022. My name is Gabrielle Barr, and I'm the archivist at the Office of NIH History and Stetten Museum. And today, I have the pleasure of speaking with Dr. Iyadh Douagi. Dr. Douagi is the Chief of the Flow Cytometry Section and the Research Technologies Branch of the National Institute of Allergy and Infectious Diseases (NIAID). Today, he's going to be speaking about some of his COVID-19 Research and experience. Thank you very much for being with me.

Douagi: Thank you very much for Gabrielle for having me. Absolutely.

Barr: To begin, briefly, will you please describe what the technique of flow cytometry entails, its basic principles, and why it is so often used to rapidly analyze a significant number of cells at a single cell level?

Douagi: Yes, so that's a wonderful question and a very long one. And just to make sure that we don't lose many people here, I think I will take the first task to simplify it. What you're asking about is this technology that is a central technology in biomedical research, also for diagnostics, that helps to interrogate different cells that are, for example, in the body and to say, “How do they look like?” So just to start, take one step back. Flow cytometry, that was the word you use, and that's composed of two words, the flow is the flowing components. There's something that is moving, and everyone is moving with the flow, moving with the flow terminology. Then we have the cytometry, and cytometry could be divided in two parts: the word cyto, which refers to the Latin word of cell, and metry, which is the metrics of what you are measuring. What we're interested to do here is to measure some properties of the cells, “How do they look like? How are they? Are they normal or abnormal? Are they different from each other?” As we said, there is so much diversity; an example, for the human body, [is] that we are very, very interested to dissect this diversity and use the single cell. We are pushing really the boundaries here and to say, “Can we look at the cells one by one and capture this information?” This is not really new because I will need to go back to really centuries back, where the first technologies that has been developed, which for example, typically, when you use microscopy, to look at slides, and you look at the cells from different histopathological sections, and you see “Is this a normal healthy tissue? Is this an abnormal tissue?” [You] can look at cells in the bone marrow, and you can see how they are differentiated at which stage if they are normal or abnormal. What we are trying here to implement is technology that is not new. It has been there for over four decades but has been really a milestone in terms of biomedical research because there is a lot of technique, a lot of areas that have this common interest in characterizing how things are different, and also to quantify this, and I will revisit this a little bit more.

Barr: Yeah, I have a question. I know that you have a background in a lot of different areas, one of them is pharmacology and you've done a lot of different things. How did you get interested in particular in flow cytometry?

Douagi: Yes. Actually, things lead to each other in a very surprising way, often. I knew very early during my studies that I was very curious about these little details. I knew that somehow I was more interested in the little details rather than the bigger pictures, and I knew for that I took different paths. I was very much interested in understanding what you really need to know in order to do research at that level. I quickly realized that it's not only one discipline. Research really needs to integrate multiple facets. It was a tough start, but luckily, I think when [I was] through [with] the education I got, I went to my training in France. Then I actually started by learning a lot about physics, about chemistry, and about biology. That allowed me to really broaden up and take

the time to think, and I think that's something that we would love to see more in the next generation, that people get the time to learn about things and to mature. Then that's where you start to think.

Then the path needed to kind of narrow down at a certain time. Pharmacology was an exciting component because I was very excited about how drugs work, and again, that kind of bounces you back into the [topic of] cells because the drugs, they don't act by themselves. There is a target, and the target cell, that's the receptor for that. In a way, that kind of led me really to [be] more interested in that. Then, more to that, I think the next step has been my interest in immunology. I decided to continue with doctoral studies at the Pasteur Institute in Paris where I did my full training as a PhD student. I was very lucky to be in an environment that was extremely supportive and extremely diverse as well. There again, there was this multidisciplinary approach. [For example,] it was mathematicians working together with cell biologists. And immunology is one of the youngest disciplines, I think it's important to say; there is [still] too little knowledge about the immune system. And as we talk about the theme of our discussion, pandemics and infectious diseases, we know that things had been working very well and we were lucky because we found interventions, typically vaccines, that allowed us to eradicate many of the diseases. But we know that the same approach, the Pasteurian approach, [which] typically took a pathogen and then to cooked it down to neutralize it, [so that it isn't dangerous] anymore. Then you use that to expose it [the pathogen] to the immune system. That's the way to train the immune system to some pathogen, but that pathogen is attenuated or inactivated. That was the vaccinology, [the] whole vaccinology area was built upon that. We were pretty lucky, or very lucky in a way that many, many diseases were actually resolved thanks to that approach, but then we see that they are the top of the iceberg, and diseases still are not solvable, typically, [diseases] like tuberculosis, HIV, influenza, malaria. These are another class of diseases that requires more insight into not the general picture, but we need to understand the details of the immune system. This is a very, very long answer to your question. That's what pushed me into flow cytometry.

As a trainee at that time at the Pasteur Institute, my mentor, Dr. Anna Cumano, was a wonderful mentor. She was hands-on, and she trained me how to use these instruments. There were very few people in the world who could actually do that. That expertise [is rare] because it relies on the interface of, not only biology, [but] you are having lasers, and you have a lot of optics, you have a lot of electronics, you have scopes—there's a lot of things that you need to deal with in addition to the question, the biological question, you are doing. That fascination continued, but I was not very much interested in the technology at that time. At that time, I was more interested in it as a tool to answer the questions that we were asking. The questions we were asking required us to use this because we were very much interested to understand, "How can you program a cell to become a specific phenotype?" That means if you take this cell, can you make it become the cell type that you want it to be?

That approach is, of course, the direct application that we saw [at] that time with HIV. Because if you have HIV, one of the key markers that indicates the disease is the depletion of the CD4 T-cells. You have the CD4 count that goes down. At that time, in the mid-1990s, we're talking about, " Can we regenerate the CD4 cells? Can we push the immune system to make more CD4 to compensate for that loss?" In order to do that, you really need to understand at the very single unit level of each cell, "How does that cell develop? How does it go from one stage to another? How will it look tomorrow? Can you anticipate that?" These questions were pretty early, and we were too early, I think, in asking these questions because the technology was not really available.

To me, [those questions] came on the path, and then that continued with more interest in vaccine research, and I moved to Sweden to carry on with my research there, joining the Karolinska Institute, where I spent a lot of years there trying to dissect the immune system and trying to understand the immune system in concept, from the aspect of our pure vaccine development. Our focus has been HIV as one prototype.

Barr: That's very interesting. So, back to COVID. Will you please discuss your work conducting sorting for a study that looked at whether bispecific antibodies targeting distinct regions of the spike protein could potently neutralize SARS-CoV-2 variants of concern, isolating 216 monoclonal antibodies targeting SARS-CoV-2 from plasmablasts and memory B cells collected from COVID-19 patients?

Douagi: Yes, I will do my best to explain that. So, this is…

Barr: It is a big study that involved a lot of people.

Douagi: Yes, exactly. That's the first thing I wanted to say; this is really a tremendous study that has been involving many, many people, but I want to highlight at least two of them, and that's really Dr. Joshua Tan and Dr. Peter Crompton. Who approached me through [a] phone call. This was very, very early, you can imagine, in the pandemic. We're talking about, I think, in February, March, sorry, I can't remember exactly the dates. But, at this time, it was the first wave where we had some of the patients [who were] already in the New York area. They immediately thought about starting to collect samples from convalescent patients—patients who went through the infection and who recovered and became healthy. This is like an experiment that a lot of immunologists are thinking of, and we do this experimentally, but this is now happening live. This is the first time an immune response is generated for what we call a de novo antigen, assuming that this pathogen has not been circulating before. “What do we do about that?” Dr. Crompton and Dr. Tan are really the experts, and they have a lot of background in the malaria field, where they have done a lot of work in characterizing these B cell responses. And [I] myself had the experience from working in the HIV field, but we also had the common interest in antibody responses [so] that we also share decades of work. When they asked me, "Can we do [it] yet? Can we somehow enrich and try to isolate the cells that are specific for SARS-CoV-2?" Yes, we could.

We have developed some tools to do it. But what was new there was that we are navigating in space [where] there were too many unknowns. There were a lot of things that we didn't know at that time. The technology that we wanted to use was standard technology that's based, as you say, on cell sorting. Cell sorting means that we don't look at the whole body, but we look at the blood drawn. It's often a blood sample that comes from these donors, [and] this blood sample says the donor has been exposed to the virus so there shouldn't be a trace of the virus; they have actually developed successfully an immune response that controls the virus.

What we're after is really taking the leftovers and the fingerprint of that response. To do that, what we developed, and that was a really tight collaborative effort with the team, is what we call high throughput assay. It was not enough to just do it in a standard way. Why? Because these cells were so rare. We needed to look at many, many, many cells, but we needed to do that in a new innovative way. And that was really [where] our collaboration starts. We developed together a combined strategy for what we call high throughput screening and cell isolation of these rare cells. And we succeeded at first. The first attempt was great, but then suddenly—that's how research is—the second attempt totally failed. That was a major drawback for us. This was really in the middle of the time where, you can imagine, where things were slowing down [because of the pandemic]. I want to say this: it's not one single effort. It's not one person. A lot of people are involved, and not only the people who are in the lab. I really want to take this opportunity to say this because there's so many people at the NIH [National Institute of Health] who are working with us, and they are facilitating this research. Typically, it is really the effort that was done through the Division of Intramural Research and the programs that we have at the NIH that has really allowed us to work together between these different teams and [enabled] that coordination to make that happen. Yes, so that's the way it went.

What we successfully did here is really try to isolate these very, very rare cells that had the signature on them to say that we have been exposed to SARS-CoV-2. And then, we started to look among all the cells that were there.

“How can we identify [them]? Are they all similar, or [do] they have different properties?” And that's where a lot of technologies got combined together, including the molecular characterization and the production of what we call monoclonal antibodies, and this could be used for many, many, many, many approaches. One is the basic understanding of how these antibodies look like both [in] sequence, but also structure binding, but also could be used for interventions….. To be clear, but this was done also in an experimental model where we showed that these antibodies have a protective effect.

Barr: Can you talk a little bit about some of the methodologies that you used and some of the technologies that you employed in order to look at the properties of the cells and their signatures?

Douagi: Yes, so that's what I was alluding to a little bit at the very end of my answer. There is a combination of technology that's really the entry point and so our focus has been to implement the flow cytometric approach to isolate the cell; that was the key first step, but then our collaborators took different pieces of that project. Right.

Barr: How do you go about, at what point do you get involved in a project like this? And how do you and your team go about doing it? What's your process like?

Douagi: Yes, so typically, we get involved very early because often, [in] this project, they rely on working with live cells. We are often involved at the stage where we work the closest possible to a sample that is isolated. As soon as the design of the study is done—this was after the collection of the samples, of course, working with the clinicians, etc., and this team. The decision was, let's do this. Often, we are involved in the beginning, and that's where the dialogue works. This is the way we, both my lab and also my other colleagues within the research and technology branch at NIAID, support all research that is available at NIH, and we are here doing it in a collaborative manner because there [are] many, many, many technologies that also cross-link to this.

Barr: Yeah, what are the advantages of bispecific antibodies over other kinds of antibodies?

Douagi: The path we went in there with this work was first, the aim of it, was really to map this antibody. How does a specific response to a SARS-CoV-2 pathogen look like? And there's so many unknowns. Talking about differences, this is pretty much a complex structure that is exposed to the immune system. The immune responses will be directed to different areas. We heard about the spike protein that we will talk a lot about [and] that we see in cartoons, but there's also other parts of that complex. The idea here was a little bit more, "If you target different specificities or parts of that target, would you have a synergy between these two?" [Using hands to motion and express a synergetic reaction between two agents by moving hands together and apart vertically and horizontally] That's the idea of having the bispecificity; that was a tweak that we tried to use, but that [was] based on the antibodies that we have isolated. If you combine two specificities, based on the experimental data, we show that combining specificity A with specificity B [holding up hands, using his right hand to represent specificity A and left to represent specificity B], if you test these two antibodies even together, that is not enough. If you put them in a bispecific context [motions both hands together], what you achieve is sometimes 100 times more efficient. So that [is] what worked in this case. These bispecific antibodies are actually used widely in many approaches where, for example, you want to have target tumors and target the immune system to specific tumors, you could use that. The concept has been there for a long, long, long time, but it was more of having a conceptual idea than any other thing.

Barr: Yeah, will you please discuss your role in the study that took an epitope-agnostic approach to identify six monoclonal antibodies that bind to spike proteins from seven human-infecting coronaviruses?

Douagi: This is really a team effort involving strongly the group of people who are under the flow cytometry

section, and we have wonderful people working, experts in high containment. That means that there is a specific training that you need to do to be able to do this work. I didn't say much about this, but this is also some of the bigger challenges. It's not just a routine thing you do because we need to work safely with this agent, and that was really at a time where we didn't know a lot. We tried to collect all the data and all the knowledge and grow that knowledge in terms of transforming that into good practice. Our contribution, again, remains at that time at really coordinating the key steps from the beginning in order to allow the isolation of these cells, and doing that, this was continued effort from the first study that you refer to, and we continue to explore this. This is really an example where investments that we have [made have] generated a little bit more than one way, right? We want this to be out to the scientific community so that the approach could be taken and also used for the future. Right. So that's what we want. That's, I think, what we really have learned, and [it will help] to get us better prepared for any coming other situations.

Barr: Absolutely. Can you speak a little bit about why you and others in this study chose to look at sites other than the receptor-binding domain that's been heavily studied, but there are other areas to, I guess, target?

Douagi: Yeah, so that's a very good question. I think that kind of the angle that we took there is based on the characterization that we did, globally, right? We didn't only focus on the non-RBD (receptor-binding domain) regions, we did not. The first work we did was actually showing that there's a stronger activity, and then there is a good neutralization that you achieve. But the idea there again is we wanted to broaden that aspect of characterizing the antibody responses, because also there is one aspect that we should be aware of and that's, typically, that we know even if targeting the antibody—antibodies that are binding with the receptor binding site—there's always escaped mutants. There is, as the immune system is fighting against the virus that have a strategy to evade that. The idea here was to really map other sites that would be conserved. Out of this huge screen that Dr. Tan has developed elegantly, that allowed this discovery, to show that actually the fusion peptide in this case could be actually a pretty solid alternative target in that case, especially looking at different coronavirus strains. That's the reason behind this.

Barr: Yeah. Are you aware if any of this research has moved on into the direction of developing particular therapeutics or vaccines that would target those other areas?

Douagi: So I mean...

Barr: It takes a long time, I know.

Douagi: Yes, absolutely. I think none of this is just a kind of a ready-to-go product. That's not what we only want to achieve. This is also working together with other approaches and tremendous effort that's [been] done with the Vaccine Research Center, as you know. When, but the pieces of information that are added, these are kind of small pieces that add together to better understand the immune diversity of the responses and what would be our alternative approaches if this doesn’t work. I think it's a wise way to go, to not play all the cards in one place. Right. So that's the strategy.

Barr: Absolutely. Can you discuss other ways you and your team have used your expertise in flow cytometry to assist others, NYAM (New York Academy of Medicine), NIH, and even outside of NIH with their COVID-19 research and initiative?

Douagi: Yes, and we do that constantly. This is really the beauty of working in a place like the NIH. There's so many brilliant people around, and really this is something that I was fascinated by leaving everything I had and then joining the NIH in 2019, just a couple of months before all this started. It was a tremendous experience,

and that to me was a reason to discover how beautiful it is to collaborate with people, and we try to do it constantly. This is our mission. But in the research and technology branch, we are here, as I said before, really to present the best of the technology at the time where we feel this is ready to address the most challenging biological questions that our researchers here at NIH are ready to answer. And of course, this is all done, again, hands to hand, and continues to be implemented for basic characterization and basic research, but also translational research bridging all what's done in the Clinical Center.

Barr: You've spoken a little bit about your past work with HIV, but will you expound upon how your research on HIV, particularly HIV vaccines in the past, has informed your work in developing next-generation COVID-19 vaccines and therapeutics and other basic research around COVID-19?

Douagi: Yeah, I think the answer from the community is pretty much, [I] would say, aligned in recognizing the fact that without the knowledge that was existing from the HIV [study], which is one of the most studied glycoproteins—the GP (glycoprotein) 120, we know everything about that piece of that protein that is on that virus—[that COVID research would have been more difficult]. We know a lot about [HIV}, but still, we have been failing in really providing us a good solution. I think that training for these decades on this perseverance has helped the field in understanding that this is not an easy game. It could have been worse. I mean, I think we were very lucky in dealing with the situation. I cannot imagine the situation [if it had been otherwise.] For example, the strategy that is used to stabilize the spike protein and the strategy that was used by our colleagues at the Vaccine Research Center, which is a beautiful strategy, would have not been possible without the knowledge that [HIV work provided], and I think many other experts would speak better than me on that. My answer is absolutely yes. I think that's even, I will stretch this later, how this is giving us a lesson for other pathogens and more challenging pathogens; that is where we need to be at.

Barr: Yeah, will you please speak about some of the suggestions you and others have put forth in making the shared laboratory safer, such as in the times of the COVID-19 pandemic? Particularly, you suggested a lot of ideas for [safety] during COVID because you all have to be in person.

Douagi: Yeah. That was part of really our mission, and when this all started it was a difficult time, there is no doubt about it. I think I was really lucky to have a strong team, and really, everyone was willing to work. We do it on purely on a voluntary basis, and everyone answered to that call. Being here with all the challenges and the logistics in [arranging that], but that was the only way to get things done. So that understanding of being there, to getting these first steps towards how to work with this, because we needed to do that. I mean, there was no other way. To us, that was absolutely the critical thing to do because we had a previous experience. That's what we do. We work with human pathogens, and we work with that in a very controlled manner. We have the most wonderful support you can think about, experts working with biosafety. To us, this was again another example of how to forward procedures that will be still working at the highest standard of safety because we do not want to compromise [the] safety of any worker. So that was really working closely with a wonderful team of biosafety experts at the NIH and multiple experts from the [NIAID] Rocky Mountain Laboratories, where they had a lot of insight into the biology of the virus. Dr. Vincent Munster was one of the lead persons. I tried to go to him immediately, asking about the very first data he generated, about the surface stability of the virus, and that helped us to develop these procedures to work with the virus. We saw immediately that the whole world was trying to do the same. Remember, cytometry is the key thing there because we wanted to know what's happening with the immune system but, remember what I started with, that is the way you would do it. How do you do work with cytometry in a safe way [when] working with a pathogen we do not know a lot about? What we have developed, we have shared it broadly with the community; we have done that by sharing our protocols. We have published some articles that allowed description of what we have done and how to do this in a safer manner. And let's continue this effort; [it] is continuing. This is not something that is stagnating, but what I can

tell you is that the procedures we have developed already, by that time already in March, they are still holding, and that's what we are still doing now. For that, I really want to applaud all the people who have been involved in doing that, the whole branch and the whole team that consisted of all these people in making that possible and allowed us to do all this work in a very safe and productive manner.

Barr: Absolutely, definitely. In addition to being a scientist, you're also an individual who's been living through the pandemic the past two and a half years. What have been some personal opportunities and challenges for you that COVID-19 has presented?

Douagi: Yeah, on the personal level, there is, of course, a lot of things that I said to you. First, it cannot belong to the "normal" things when you move from overseas, and then you join a new country, a new workplace, and you have new colleagues, and then you suddenly realize—boom—there's a pandemic, and then you need to deal with that. So again, without really the support I got from my mentors, from my supervisors, from the leadership team at the DIR (Division of Intramural Research), at the RTB [Research Technologies Branch], at the NIH and without all that, it would have not been possible. So, in that, we grow; we all grow by doing this. We learn a lot from the way we have been doing that, but at the end, it has been really a way to, I think, strengthen that internally, [for] me personally because I feel like that I become more, how to say? You will realize that this was something that we were trained for, this was what we needed to do all the time, right. We needed to develop, to find solutions that are scientifically driven to approach a problem. And this was an immunological problem related to a virus. To me, that was personal, that was like the closest and the most relevant it can be, and how I can contribute to that at that time. Now I'm focusing on really providing the support [to] the scientific community here. [The way to do that] was to answer to that call by allowing and facilitating the work that was planned by many [of] my colleagues. Working together, that has been really, the way that has been strengthening for our experience.

The other very good thing is that it allowed me to remind me that I'm very, very active in swimming. I have been swimming all my life, and it was an opportunity to remind me that if the pool is shut down, then that was not a good thing. I needed to keep active. I think that's what I learned as well. That was [a] very good thing to learn the hard way. Since then, we found ways we could book individual times to go to the pool and swim. I'm very, very happy that I could do that and recharge the batteries to come [in] can continue to do the work we do. That's the two sides.

Barr: Absolutely. Is there anything else you'd like to share about your COVID-19 research or experiences?

Douagi: I think we talked a lot about the different aspects. What I think, the way to summarize it is this has been really an experience that challenged all of the humanity. I think I'm very proud as a human being, as a scientist, to see how we have navigated through this. It was [the] true scientific approach. Really the hard work of lots of people across the world, the globe, the collaborative aspect of that in sharing the science and sharing the knowledge, that's why we are still here. I'm really thankful for the whole community, and I'm thankful to be part of it and to get the chance to contribute the little piece of it.

Barr: Well, thank you for all your service, and I wish you and your team only success and health, unfortunately with COVID continuing.

Douagi: Thank you so much Gabrielle. It was really a pleasure talking to you.

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