Willy Albert Flegel, M.D.
Behind the Mask
October 8, 2021

Chief of the Laboratory Services Section, Department of Transfusion Medicine, NIH Clinical Center
Interviewed by Gabrielle Barr, Archivist, Office of NIH History and Stetten Museum, National Institutes of Health

Barr: Good afternoon. Today is October 8, 2021. My name is Gabrielle Barr, and I am the Archivist at the Office of NIH History and Stetten Museum. Today I have the pleasure of speaking with Doctor Willy Flegel. Doctor Flegel is the Chief of the Laboratory Services Section in the Department of Transfusion Medicine at the NIH Clinical Center. Today he is going to be speaking about his COVID-19 research and experiences. Thank you very much for being with me.

Flegel: You're welcome. Thank you.

Barr: To begin with, can you speak a little bit about your background, what led you to NIH, and then some of your responsibilities in your position that are not COVID related?

Flegel: I am the Chief of the Laboratory Services Section within the Department of Transfusion Medicine. So, we are doing all the blood-group typing for the NIH Clinical Center, the cross matching of blood units, and then also supplying those blood units to the floor where they get transfused. The second lab in that section is doing the HLA [human leukocyte antigen] typing mostly for bone marrow transplantation, and that's the routine part. I have also been very much interested in three decades in molecular research on blood groups, characterizing the peptides that are stuck into the membrane of the red [blood] cell surface. That actually is, in the end, also the topic of the assay that we developed.

Barr: Before we discuss the assay, you have a very interesting history. You went to medical school [J W Goethe -University in Frankfurt, Germany] and then you worked at the German Red Cross [German Red Cross Blood Center], and then NIH. Can you talk a little bit about that? What interested you in in blood transfusion in general?

Flegel: I started out in hematology with bone marrow transplantation in the 1980s, and then learned how much blood is needed and how much blood transfusion can contribute to patient care and safety. I went very early in training into transfusion medicine [at the University of Ulm in Ulm, Germany], which is a separate program in Germany. Then, I went on to do a postdoc in the Department of Biology at the University of California in San Diego. Before I then led the immunohematology lab at the University of Ulm in Germany for more than one and a half decades. Then, I came here [in 2009] for research purposes, essentially, to the NIH Clinical Center.

Barr: How long have you been at the Clinical Center?

Flegel: It's now 12 years already here, leading this section for the transfusion services, as well as for the HLA typing.

Barr: That's quite a bit of time. To get into your COVID research in particular, can you talk a little bit about—you worked a lot with an assay that used kodecytes [Kode Technology modified red cells], but a lot of people listening will not know what that means. Can you briefly define what that is?

Flegel: That's a technology that's [been] around for a long time and was actually developed by a good friend of mine who I've known for 20 years. It's a unique technology to attach proteins, or small protein fragments called peptides, to the surface of any cell, as a matter of fact, in this particular case, on the red cell. It essentially becomes something like a blood group of a red cell, but it can be any peptide that can mimic all sorts of peptides that are out there. It is physically linked into this membrane. That distinguishes this assay from any other assay that's available for COVID antibody detection.

Barr: Then it makes each red blood cell unique. How does it make it unique? I thought that was very interesting.

Flegel: Typically, the red cell is not covered by the virus protein. One would attach the proteins of COVID—actually the SARS-CoV-2 virus—to this red cell, and then it mimics being a little bit like a virus. So that the antibodies that the individual develops will bind suddenly to the red cell. Then it will link red cells with each other, which is called agglutination. That is then macroscopically visible—you can see it in an assay—and that determines whether the individual has an antibody to the SARS-CoV-2 virus or not.

Barr: I had a question before we go into the details of your study. There are many assays out there already before you all created your assay, and I was just wondering, what role did you see that was currently on the market for the creation of your assay? What did you think that your creation could add?

Flegel: That's a very valid and important question at the core of the understanding of the assay and why this assay might be needed there. There are more than 200 assays in the field of COVID, and I've read that there are more assays out for COVID than for any other infectious disease, And that might well be true.

Why do we need another assay? The reason is that this is a very special technique that allows the testing for COVID antibodies in settings that may not have the capability to use the other assays. Most assays are called ELISA assays [enzyme-linked immunosorbent assays] and you need special equipment, which is widely available in Western settings but not necessarily in other settings worldwide, such as in Africa or in Asia. But almost every hospital in the world transfuses red cells, and they somehow have to test for blood groups and to match those blood units with the patient. This requires a special technique that is available for the purpose of blood transfusion. This is the exact same technique that is used with this particular assay and not with any other of those 200 and more COVID assays that are out there.

Barr That's very interesting. Can you discuss the design of your study, the methodology you all used, as well as the techniques, that were employed?

Flegel: This lipid-linker technology that [has been] around for a while. I was thinking about using it, but there was not really an application or a specific research question that I wanted to answer for which this technology was fitting. When the COVID pandemic came around, we started thinking whether one could apply this particular technology to address the needs and the urgency of the COVID pandemic. In fact, we thought it might be worthwhile trying this technology to develop an assay at a time when there

weren't that many assays around. A lot of other people tried other technologies. It didn't work for months.

Barr: Oh my goodness. When did you start using it, in April of 2020?

Flegel: That was during the time when the NIH was in shutdown, and actually the research people were doing telework at home. We needed and requested, and eventually got granted, special permission to bring the research folks back to work on that particular question, which was very urgent at the time and still is. Then we started working on the assay. Again, it didn't quite work for months, until finally we came down with an assay that now works very smoothly and is actually very robust.

Barr: Why do you think—well, we can get into that with the challenges—but what types of tools and equipment did you all use with your study, and did that change over the course of your study as you were perfecting it?

Flegel: The beauty of this assay is that it actually doesn't require that many tools beyond what is available in a basic routine blood bank in any hospital worldwide. We do use the usual available equipment—test tubes, vials, centrifuges, and - in this particular case - a so-called commercially available gel cart. This is used on an everyday basis for patient care and nothing additional is needed, except for the specially designed linker.

Barr: Can you speak a little bit about the samples that you all looked at? They were varied from people with COVID and then also people not with COVID, vaccinated people, and they're from different places in the world.

Flegel: Well, we had to show that the assay actually works. Initially this was done with plasma—blood fluid samples from patients who had COVID infection and were recovered. They were donating here on an NIH clinical protocol, the so-called convalescent plasma. They were supposed to be positive and infected. It turned out that they all were positive in that assay. Then we used healthy controls from individuals where we thought that they were not infected or had not seen the virus before and they were negative.

Later, we expanded that study and we included people after vaccination. That was done in collaboration with a European blood bank for the most part. We also got much support by the NIAID [National Institute of Allergy and Infectious Diseases] with samples that were drawn before any COVID was known. These samples were actually from healthy individuals from the year 2008, so they can't be positive for COVID, for the coronavirus.

Barr: Can you speak a little bit about how you went about comparing your assay that you created with other assays that you used as kind of comparison?

Flegel: The convalescent plasma donors here at the NIH Clinical Center have to be tested. That's an FDA regulation with a commercially available and FDA-authorized assays. Two of those assays were run on those samples, though unbeknownst to us, the results were known. We were blinded to that at the time. They were also tested with a virus neutralization assay out in Frederick [Maryland], tested by NIAID. Also, the results were not known to us. Then we did our assay and gathered the results, and then the code was broken, and we could compare the results of our assay with those two commercially

available in routine use, and then with this experimental assay of virus neutralization. The correlation was solid, as expected.

Barr: Can you first define what sensitivity and specificity means when you speak about assays and then talk about how that relates to your assay?

Flegel: These are the two critical parameters, and the FDA requires certain minimal performance in that regard. Otherwise, an assay would not be acceptable. The sensitivity essentially determines that an individual who is supposed to be positive is actually detected positive. The specificity is such that someone who is supposed to be negative because they've never seen the virus, tests negative. Now, ideally both results would be 100%. But there is no assay in the world that yields that if you go into larger numbers. You want to have a certain minimal performance. I have to look that up for the assay.

Barr: It's over 90% if I recall.

Flegel: Yeah, well above 90%. I just don't remember the exact numbers. It was, I believe, 92% sensitivity and 96% specificity. But I actually would have to double check—that is published. That's well above what the FDA would typically expect for an Emergency Use Authorization. We haven't requested this. A lot more data is still required to request such an Emergency Use Authorization, but the numbers are very good. I don't see a reason that we could not generate the required data volume to eventually apply for an Emergency Use Authorization.

Barr: Would you have to test against other assays? You tested against two commercially available assays. Would you have to test against more in order to be considered that you've done all your research that you can do?

Flegel: Yeah. There is a whole process that the various authorities require. Here in the U.S., it would be the FDA. In Europe, there are other requirements. They may differ slightly. Again, we are thinking to apply that in particular to African and Asian settings, and they may have even other requirements. This is more a question to commercialization, and I wouldn't really be involved in that aspect. Again, the rules differ slightly depending on the health care authorities in the respective countries.

Barr: Can you speak about your findings just in general? Were you surprised by your results?

Flegel: We hoped for a viable assay that is easy to apply, stable, robust, inexpensive. That actually, in the end, was accomplished. I wouldn't say that we were surprised. This was, after all, our goal. But when we started out, it was not a given that it would work and would work in such a robust way. The beauty with that assay is that you can use routine red cells that are available anywhere, and one would need a freeze-dried linker that can be shipped around the world under any conditions. It's super stable. This could be used to establish the assay within four hours in any hospital blood bank worldwide.

Within four hours you would have the assay running without requiring any additional equipment that you wouldn't have in your hospital blood bank. It is an accomplishment that this assay was established, and it's not a given that it will work. But in the end, we were not surprised because that was the goal when we started out thinking about that in March of 2020.

Barr: Why was the manual tube serology less sensitive than the column agglutination technology platform?

Flegel: That's a general feature and applies essentially to all serologic tests in the blood bank. The tube technique, which is time honored for 120 years, is a little less sensitive than this gel agglutination technique, but both assays work. If somebody doesn't have the gel agglutination technique available, they might still rely on the tube technique. But there is a difference in sensitivity, and this applies to any blood group typing. As I mentioned at the beginning, this COVID assay with red cells mimics blood group typing.

Barr: Right. How come the gel is not as widely available to places?

Flegel: It is very widely available. It was introduced into the field around the year 1990. It requires a little bit more different equipment than the tube technique.

Barr: Oh, okay.

Flegel: It is a widely available technique, and if that is not available, then we'll still think about using the tube technique. One of those techniques is available. Otherwise, you wouldn't be able to type for a blood group, and you wouldn't be able to match the blood unit to the patient. But you have to do that if you want to do blood transfusion. That's done in very many hospitals of the world.

Barr: What were some of the challenges that you and your team experienced, especially in the early months of the research?

Flegel: It was tricky to figure out how to attach this SARS-CoV-2 protein, and then in the end, the smaller fragments of the proteins, called the peptides, to the membrane. There are various ways of doing it. In the end, when we had to figure out which one is the easiest and the right one, plus then one had to figure out which peptide. There are very many possibilities for the right ones. We tested a lot of them, and very many did not work, until we found two very promising ones. They, in the end, are used now as a mix for this assay that is currently used and published.

Barr: So, it works, but what are some next steps that you all are going to take in this research, or that your team hopes to take?

Flegel: Another interesting feature that's not yet mentioned is that these peptides can be easily changed. That's another special feature of this assay. It's not that difficult to vary the peptides by making them a little bit longer or shifting them along the peptide. This changes the sensitivity and specificity quite a bit. However, it also allows us to test for different antibody specificities, and that can be—we don't have much data in that field—but these are the next questions to be addressed. This might allow us to differentiate between individuals who had the infection and actually seen the virus or who have been vaccinated.

Barr: Wow, that's really specific.

Flegel: Yeah. It may theoretically even be possible to distinguish, to some extent, which vaccine has been used, or which virus variant was the culprit of the infection. Eventually, this assay can be used to check whether the antibody titer for vaccination, or even the disease, is strong enough, or whether the individual needs another booster vaccination. These are all questions that are very important in epidemiology and in the immune monitoring of populations, deciding who needs additional

vaccinations, or who may not need it. That's a potential application. All these things need to be worked out. But it could be potentially done with this assay, and it could be done in places where other assays are not available.

Barr: Have you and your team started working on any of those questions?

Flegel: Currently, the lipid linker or these constructs that are used to label the red cells are modified. That's currently in the making, and we look forward to receiving them in hopefully November 2021. So that is the next step.

Barr: Can you talk about your role in this research, as well as mention some of the other [people] whom you've collaborated with in the research?

Flegel: I collaborated with a researcher in New Zealand who developed the technology of the lipid linker. In the end, this resulted in the assay. We did mostly the clinical evaluation, the initial clinical evaluation, to determine which peptides are the right ones and then what concentration. Within my lab, it was the staff scientist and the postdoc at the time who came actually back from their teleworking and started working on this assay in the lab.

There are chemists involved doing the chemical part. It's a fully synthetic construct. We need chemists to do that. Then my former students and deputies back in German blood banks who are leading those blood banks now collaborated on evaluating the vaccinated group. They had a nice set of approved clinical studies to evaluate vaccinated people. They did this testing with the vaccinated individuals.

Barr: That's quite a lot of people who were part of it.

Flegel: Yeah, and here, of course, at the NIH Clinical Center, the Infectious Disease and the Blood Services chiefs participated in it, as well as, as I said, NIAID who gave us the negative controls from 2008 to prove that they are not falsely positive and non-specifically positive in our assay, as well as an individual from NIAID in Frederick, who did this virus neutralization assay, showing that there is a correlation between the strength in our assay versus the strength in the virus neutralization assay. This is all very important, and they were all super helpful.

Barr: That's really great. Have you been involved in any other COVID-19 protocols or initiatives at NIH or outside of NIH?

Flegel: Yeah. We have this Convalescent Plasma Donor Program. This required recruiting these individuals, collecting the blood, and testing them with the commercially available assays. My section then produced the units, labeled them, froze them. We still have frozen units in my section in case they will be needed.

Barr: Can you talk a little bit about the correlation some scientists and clinicians have made with those with the ABO blood group and other blood characteristics and infectivity with SARS-CoV-2?

Flegel: This was a big story when COVID was first recognized. Almost immediately, there was a publication that it correlates with the ABO blood group, and there are some mechanistic ideas why that could be. By today, we actually have a lot of studies that tested that. In fact, almost the majority of them showed some effect. Interestingly, they are all in one direction. But there may actually be some truth in

it. In early 2020, it was, however, recognized, and I published on that—that the ABO blood group does not protect from infection at least in certain circumstances.

I think that's a very important take-home message: Individuals should not think that they have [one] blood group, and therefore they don't need to be careful in avoiding the infection, or they might not need vaccination. The ABO blood group, unfortunately, does not help much in that regard. If the conditions are right, everyone will get the virus, and therefore everyone needs to be vaccinated to protect one from infection.

Barr: Can you talk a little bit about how COVID-19 has impacted the field of transfusion medicine as a whole and the Department of Transfusion Medicine at NIH. I know this is a very large question to ask, but it is really interesting to know.

Flegel: It impacted medicine in general, very, very much. It did it affect us in Transfusion Medicine, where we are concerned and busy with providing blood units for transfusion purposes to patients. There are many aspects to it. One aspect is that there was actually less need because there was less routine care by standard surgeries, that, in a way, helped. Because on the other side, there was also less blood collection going on. Generally, the good news is that the virus apparently cannot be transmitted by blood transfusion. Though we were prepared to test for the virus and prevent the transmission by testing or by inactivating the virus, it turned out that that's not necessary. That helped. The biology of this virus, in regard to transfusion medicine, made it relatively easy to handle. That was not known at the beginning when the virus showed up for the first time.

Barr: Yeah. Where is the NIH in terms of its supply of blood right now?

Flegel: Well, right now in October 2021, there is actually a nationwide blood shortage. We are struggling to keep up with the blood supply. That's not only affecting the NIH Clinical Center, but again, that's an issue for the whole U.S. Therefore, we can only solicit blood donations. We need blood donors to collect enough blood units to service the needs of the patients. That is right now a bit of a problem.

Barr: Interestingly, you have collaborated with Wuhan University [in China] before the virus for many, many years. Can you speak a little bit about your collaboration?

Flegel: I've actually been a guest professor at the largest university in Wuhan for many years, for a long time before anyone knew of Coronavirus. This dates back to the time when the current chief of transfusion medicine at the University of Wuhan actually did her medical thesis studies in my lab, and then eventually got the MD by research almost 20 years ago. That long is my connection with people in Wuhan.

Barr: What was it like for you when the virus broke out in Wuhan, and what was your reaction and feelings about what was occurring?

Flegel: This was very scary and had a lot of impact right at the beginning when no one really knew what this virus really means for a population. This was very scary. Therefore, it is important to be in touch with the people in Wuhan and China, to exchange ideas and to learn from each other's experience in that field. We have collaborations with the anesthesiologists and intensive care physicians in Wuhan and with transfusion medicine specialists, and we hope to hear about their experience. The pandemic is not over, so who knows what's up next. It's better to keep in touch and to learn from each other both ways.

Barr: Before the pandemic, when you were a guest lecturer, were you ever there in person? Have you been to Wuhan?

Flegel: Yeah, [but] not since the pandemic hit, but I've been to Wuhan. Yes, of course.

Barr: Personally, what challenges and opportunities has COVID-19 presented for you as an individual, not just as a scientist or physician.

Flegel: At the personal level, it was a challenge for the family, some of whom are old and at real risk until we had a vaccination. It is still a challenge, although at the private as well as the professional level, we get, I guess, acquainted with it, and we learn to live with it. I think it has changed and it will come continue to that we have a different approach how we live and work.

A lot of that can actually be also positive. This different way of interacting by video conferences will continue. We are still getting accustomed to the new ways, the new normal in that field. But I think there are positive aspects. Eventually we will be better off than we were before. But for now, we have to still consider that the pandemic is with us, and we have to take the necessary measures to curb that and to limit the negative impacts of the pandemic on us and the people outside of the U.S.

Barr: Yes, for sure. Is there anything else that you would like to share about your COVID-19 research or experiences as a person or at NIH?

Flegel: I think we pretty much covered a lot of topics, and we will still learn a lot and move forward in the future with collaborations. As this example shows very well, it's important to have collaborations. So, who would have thought that eventually this collaboration and friendship with folks in New Zealand would turn out to be so important, and that perhaps the friendship and collaboration with people in Wuhan could have turned out to be of such a relevance.

Barr: You never know. Well, I wish you all the best, and it's very exciting about the assay. We all look forward to hearing how that progresses. I wish you and your lab and everybody all continued success and continued health.

Flegel: Thank you very much. I will forward your regards, and it was a pleasure to have this conversation.

https://pubmed.ncbi.nlm.nih.gov/32640485/
https://pubmed.ncbi.nlm.nih.gov/33256371/
https://pubmed.ncbi.nlm.nih.gov/33590501/
https://pubmed.ncbi.nlm.nih.gov/35464155/
https://pubmed.ncbi.nlm.nih.gov/34878316/
https://pubmed.ncbi.nlm.nih.gov/35969130/