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CellPress

Conversations

Persistent progress

The highly effective and safe mRNA-based severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines draw on decades of painstaking research to overcome the many hurdles for delivering, expressing, and avoiding toxicity of therapeutic mRNA. *Cell* editor Nicole Neuman talked with Dr. Katalin Karikó and Dr. Drew Weissman, recipients of the 2021 Lasker∼DeBakey Clinical Medical Research Award, to learn more about their quest to develop mRNA-based therapeutics, which led them to the crucial discovery that modification of mRNA could prevent toxicity and increase expression. This conversation has been adapted for print below, with editing for clarity, accuracy, and length.

Drew Weissman and Katalin Karikó
University of Pennsylvania; BioNTech

Nicole Neuman: How did each of you first get interested in the idea of mRNA as a therapeutic?

Katalin Karikó: I started at the University of Pennsylvania in '89 and started to get interested in making mRNA coding for therapeutic protein. I have to emphasize that even today at BioNTech, I am responsible for the protein replacement program—mRNA-based protein replacement—and that was always my major interest. And that was when I met Drew, who was interested in vaccines.

Drew Weissman: I did my fellowship at NIH in Tony Fauci's lab. While I was there, I started a new research program studying dendritic cells and their role in HIV pathogenesis. Dendritic cells are really the target for vaccines, but Tony didn't want us doing vaccine research. So when I came to Penn, being

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an impetuous kid, the first thing I wanted to do was vaccine research.

I started to investigate ways of loading dendritic cells with antigen, and many had been described: DNA, RNA, peptide, protein, viruses. I didn't have access to RNA, and I didn't know how to make it, so I looked at everything else. And that's when I met Kati at the copy machine, and we started talking, and she told me about the RNA work she was doing. Then we started working together, and that's led us here.

NN: How did this initial work get started?

KK: Drew gave me the plasmid template encoding HIV gag, I generated the mRNA, and he tested it out. Drew was happy with the outcome—the mRNA was activating all those markers, it was immunogenic, and there was a very high level of protein made from it. We were very excited about that. I have to emphasize that the scientists before us, they did use the messenger RNA for vaccination, but ...

NN: But it wasn't initially adopted, right? What were the hurdles to translating mRNA into an effective vaccine?

DW: There were two groups who had one paper using mRNA as a therapeutic, and then they disappeared. Out of that, people started using it for vaccines, but it was a small number of groups. There was a French group, a Swedish group, and a group at Duke, and that was pretty much it who had sustained interest in making vaccines.

NN: Do you have a sense for why it didn't become popular? It seems, in retrospect, very intuitive. I'm curious to get your thoughts on why this idea didn't catch fire at the time?

KK: The cancer vaccine has its own challenge to figure out what the antigen should be. For the mRNA-based infectious disease vaccine, as we learned from others, it was the toxicity—the immunogenicity of the mRNA was a reason.

DW: What all those groups were doing is they were taking cells, namely dendritic cells, out of an animal, pulsing them with the mRNA, and then giving them back. And they did human phase I clinical trials doing the same thing. Their problem was they couldn't inject the RNA in because it was so inflammatory that it made mice sick. It killed mice, and the assumption was it would have made people sick, and that's not a very good therapeutic.

NN: Tell me about the turning point in your research, where you really had a breakthrough where you thought, "We have something with this."

KK: So actually, why we started to look for how to reduce immunogenicity was that I always wanted to use mRNA for therapeutics. When Drew said how immunogenic the RNA that I gave him was, I went back and tried to change different structural elements on the RNA, altered the cap, and added a longer polyA tail. As a control, we used transfer RNA (tRNA), which happened to not induce any inflammatory molecules. Knowing that the tRNA has a lot of modified nucleosides gave the idea that maybe we had to introduce modification into the mRNA.

And of course, then came the bigger challenge of how to do it, because none of the enzymes that can modify the nucleosides in RNA were available. So, we didn't introduce the modifications enzymatically as nature does it. Rather, we incorporated the modified nucleotides into the RNA during transcription and demonstrated that some of those RNAs are not immunogenic. We also identified that human Toll-like receptors 7 and 8 were responsible for the immunogenicity.

We also isolated RNA from different compartments of human cells and then tested them to see whether they are immunogenic or not. These were the results we published in 2005, demonstrating that nucleoside-modified RNAs were less immunogenic and tRNA was not immunogenic.

DW: What a lot of people don't realize is that the vaccine groups knew RNA was toxic, but they didn't know why. Back then, there was really a limited number of RNA sensors known, and RNA wasn't thought to be a ligand for any of them. So when we saw that the RNA was inflammatory, we spent years figuring out why it was inflammatory, what receptors were involved, what signaling molecules were involved.

We investigated why RNA was inflammatory, and then as part of those investigations, that led us to figure out how to make it noninflammatory. The other groups simply said, "Oh, you can't inject it *in vivo* because it makes the mice sick." And they were vaccine people. They weren't immunologists or molecular biologists who wanted to do the basic science involved.

KK: The first identified RNA sensor was Toll-like receptor 3, and it was discovered in 2001 that it responds double-stranded RNA. But for single-stranded RNA, there was nothing known prior to 2004 when we were doing those experiments.

NN: Tell me about your experience getting this work published in 2005. That was at *Immunity*. Did you have to take it a lot of places first? Was there a lot of interest in the work? And how did reviewers respond to it?

DW: We went to *Nature*, we went to *Science*, we went to *Cell*. All three of them wouldn't even send it out for review. They sent it back and said ... I think what they said is, "Send it to a specialty journal."

KK: I think that they said the phrase "incremental improvement."

DW: Yes.

KK: Because, you remember, I didn't know that word and I had to look it up in the dictionary?

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DW: Right. I mean, they were unimpressed. They were uninterested. So we sent it to *Immunity*, and I think they had four reviewers. One of them liked it, two of them hated it, and one of them was kind of wishy-washy. We kept sending it back to *Immunity* and saying, "You need to ignore these reviewers. They're missing the point of the paper." We kept changing things, we kept adding things, and we finally got it accepted.

And then about maybe two years later, we were at a meeting. I think it was in Germany, but I'm not sure. And one of the reviewers of the paper, who was a big-time RNA researcher, came to us and said, "I reviewed your *Immunity* paper. I fought with the journal to get them to publish it, because I thought it was really important." I think we owe him a lot of respect for reading the paper and seeing what's in it and pushing to get it published.

KK: I know that we didn't like that one of the reviewers wanted us to generate two new sets of cell line expressing all the Toll-like receptors just to prove that it was indeed the human TLR7 and TLR8 that sensed the single-stranded RNA. This was nonsense, but otherwise I liked the nice input the reviewers gave.

NN: What happened after you got that paper out? Did you immediately go and try to translate the work? And what was the experience with that?

KK: Drew told me that, "You will see, we will be invited to give talks," but nothing happened.

DW: Yes. I'm still embarrassed by that. I said to Kati, "Oh, well, now the phone is going to ring off the hook. People are going to ask us to help them with RNA. We're going to get invited to talk." And we sat and stared at the phone, and nothing happened.

KK: I mean, we didn't really stare at the phone, we did a lot of work.

DW: We kept working, of course, but we were ... I mean, we went as far as trying to license it from Penn, we talked to venture capitalists.

KK: We also started a company.

DW: Yeah, we started a company. We weren't sitting on our hands at that point. We were trying our best to move things forward.

KK: We established the company and then tried to purify the mRNA. Drew was very determined to figure it out. He was coming from seeing patients for medical service and would constantly go to the high-performance liquid chromatography machine and try a new column, thinking that maybe this one will be the best one.

We also characterized the translation of the nucleoside-modified mRNA, which we published in 2008. We demonstrated that pseudouridine-containing mRNA translates so much better. We also did a lot of work trying to understand why that is. Finally, we had two, three other papers showing the superiority of the pseudouridine-modified RNA.

In the meantime, we didn't have to spend time giving interviews and lectures, because there was no interest up until 2010.

DW: I would go to conferences. I was also doing HIV pathogenesis work, so I would go to HIV conferences, and I would talk to people, Tony Fauci, Bart Haynes, Gary Nabel, people that were leaders in the field that I knew. I would tell them about our RNA results, and then they would smile and say, "That's really great data," but they had no interest.

KK: After my presentation, I was asked who's my supervisor.

NN: You had mentioned, Dr. Karikó, that you two had started a company. Was that when you left academia, or did that come later?

KK: We started RNARx in 2006. Our plan was that we would leave academia, or, rather, I was ready to leave many times. RNARx was a virtual company; we still worked at the bench at UPenn. We actually received an small business technology transfer grant from the government by proposing to make pseudouridine-containing messenger RNA coding for erythropoietin to increase the hematocrit value of the animals. To prove that such modified mRNA works *in vivo*.

And we reached a milestone. In total we got $1 million and did a lot of research using that money. We were planning to go to a research incubator on campus and start furthering our company. Unfortunately, we couldn't get the patent for our own company. Our own patent, and we couldn't get it from Penn.

NN: To shift gears a little bit, at BioNTech, what kinds of diseases had the company been working on prior to SARS-CoV-2 emerging? Are there particular diseases that are especially good targets for mRNA therapeutics?

KK: CureVac was the first. They started 20 years ago making mRNA for cancer vaccines, and all of the companies—CureVac, Moderna, BioNTech—all have clinical programs on the cancer vaccine field and of course on many other areas, including protein replacement.

When I went to BioNTech, in 2013, one reason was that AstraZeneca gave so much money to Moderna that I knew that I had to leave academia. If I couldn't succeed with our company, which we established with Drew, then I needed to go to somebody else's company and make sure that things were

going the right way. So that's how I ended up in BioNTech in 2013.

The mRNA-based protein replacement was my number one priority, not vaccines. BioNTech, at that point, didn't have an infectious disease vaccine program. I initiated a collaboration that advanced into the first human trial injecting messenger-RNA-encoding cytokines into the tumors, making the cold tumor hot. This is also protein replacement, not a vaccine, because the mRNA is coding for cytokines and not for an antigen.

And I introduced Uğur Şahin, the BioNTech CEO, to Drew, and now we have a close collaboration with Drew's team.

DW: In 2017, when we published our Zika vaccine, I went to Uğur and spent a lot of time talking to him and showing him the data. Prior to that, the only vaccines they'd worked on were cancer vaccines. The chief focus of their company was on cancer.

I showed them all of our data and how potent the vaccine could be for infectious diseases. He got interested, and he funded my lab to make infectious disease vaccines for him, and we've been doing that ever since. We now have five vaccines going into phase I clinical trials for a variety of infectious diseases.

NN: I want to take a step back and think a little more globally. Equity in vaccine development and access is a persistent global health issue. I'm wondering if you think the success of the COVID-19 mRNA vaccines, and mRNA vaccines more generally, will have any impact on extending the breadth and global reach of vaccines?

DW: This is something that I've been working on my entire career. I had a lab in Thailand 25 years ago and I've had a lab in Botswana and South Africa for the past about 15 years.

Last spring, when COVID was getting big, and Moderna and Pfizer-BioNTech were working on their vaccines, I had been working with labs in Bangkok developing other vaccines and other therapeutics. They came to me with the government, and they said, "We're nervous that if the West develops a vaccine, it will be years and years before we ever get any." And they weren't willing to shut their country down for years.

So the government came up with money to make their own COVID-19 vaccine, and I started working with them. We designed our own immunogen. We designed our own RNA. We got lipid nanoparticles from a different manufacturer. They already started their phase I clinical trial. They're midway through it, with great results so far.

I also helped them set up a good manufacturing practice facility to make RNA in Botswana that can be distributed to Thailand and seven surrounding countries. We're now doing the same in South Africa and Rwanda, setting up GMP facilities that can locally produce vaccine, so if it's locally produced, it would be locally distributed. So vaccine equity has been a big issue for both of us for a long time.

KK: Actually, with Drew, we decided early on not to patent the modified RNA technology, because we wanted everybody to use it. But we were told that nobody will use the technology if they can't secure exclusivity.

But we always envisioned with Drew that the mRNA would be much cheaper and could be generated much faster than the corresponding recombinant protein, which requires so much more work and resources to grow cells, and so on. The affordability of the mRNA medicine was important for us.

NN: One final question for you both. One of the most incredible things I've taken away from your story is your persistent vision of the translatability of your ideas, even when others couldn't really see it because a lot of the basic foundational biology still needed to be worked out. Do the two of you have any practical advice for scientists who feel passionate about pursuing an idea that isn't widely supported?

KK: We persisted, but I have to emphasize that we always, when we were working, could see how the project advanced. It is important to see the progress, and, throughout that, pursuing, refining, and improving is crucial. You have to see advancement, getting closer to your goal, then you can stick to the project and succeed.

DW: I'm always in a difficult situation because I have PhD students, I sit on thesis committees, I have postdocs and other investigators. And every so often I have to tell them, "Your project is not going to work. You need to move on to something else."

And they look at me and they say, "Well, you spent 20 years working on RNA and never gave up. Well, why should I stop working on my project"? And as Kati said, we kept going because we had good results, and we kept generating new findings and new results. But 23 years is still a long time to stick with any project. So it's sometimes hard to tell people, "You need to stop. This isn't going to work."