Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Cell
Leading Edge

CellPress

Conversations

Learning lessons from lipids to make COVID-19 vaccines

A game-changing intervention in the COVID-19 pandemic has been the rapid implementation of highly effective vaccines against SARS-CoV-2. The 2022 Canada Gairdner International Award recognizes Pieter Cullis, Katalin Karikó, and Drew Weissman "for their pioneering work developing nucleoside-modified mRNA and lipid nanoparticle (LNP) drug delivery: the foundational technologies for the highly effective COVID-19 mRNA vaccines." *Cell* editor Cheri Sirois caught up with Pieter to discuss how a long interest in basic and applied questions in lipid biology led to this fortuitous collaboration. Excerpts of the conversation are presented below.

Pieter Cullis
University of British Columbia, Vancouver, British Columbia, Canada

Cheri Sirois: I'd like to start by going back to the beginning and ask how you became interested in lipids. Was that always your research focus?

Pieter Cullis: Not at all. I got my PhD in physics doing magnetic resonance. A long way away from lipids. I was working on semiconductors and exploring the properties of those. When I got to the end of my PhD I realized there were more interesting problems in the life sciences than I could see in physics. In physics, really, the major exciting things were done about 100 years ago. I was an experimental physicist. It meant it was very difficult to discover something new. Because if something didn't fit within an established theory the likelihood was, your experiment was wrong.

Anyway, I applied for an award to go to Oxford as a postdoc. To my amazement I was awarded it, so I ended up in Oxford. I had had a couple of seminars where people tried to explain biological membranes to me but the real catalyst was meeting a postdoc from Holland named Ben de Kruijff, who was a membrane biochemist. I had to build a bit of an NMR [nuclear magnetic resonance] machine when I got to Oxford because they had one that was all in pieces and we had to put it together. So the first thing that we did was apply that to looking at the membrane lipids. That turned out to be a huge area of interest for me. Biological membranes contain literally thousands of different species of lipids. We still don't have a clue why they're all there.

One of the things that fascinated me was why there were so many lipids in a membrane that don't adopt a bilayer structure, which is what provides a permeability layer between the inside and the outside. The other thing that interested me was the fact that lipids in membranes, biological membranes, are distributed asymmetrically. In other words, lipid composition on one side is very different than lipid composition on the other. So I wondered whether or not we could generate lipid asymmetry using ion gradients such as pH gradients. That was really what I focused on: lipid polymorphism, which is the term used for the ability of lipids to adopt different structures, and then lipid asymmetry reflecting the asymmetric transbilayer distributions. Those were things that really were driving me for probably from 1975 or '74 when I started to work in this area.

Subsequently I did a year in Utrecht, and then I got a position back at the University of British Columbia. In order to study these strange lipids in membranes you have to use much simpler model membrane systems, like little vesicles, so you can then make it a pure lipid substance, for example, from a membrane. And then you can get some idea what its properties are and what functional roles it might play.

"[W]e had the great good fortune that we were approached by Drew Weissman and Katalin Karikó who, of course, developed messenger RNA in the hope of developing potent vaccines. They said 'gee, we'd really like to try your system out. Not to express proteins in the liver, but to see whether or not if we go in intramuscularly we can actually see a reasonable immune response.'"

We devised a way of making these lipid vesicles. We showed that these non-bilayer lipids, if we incorporated them, these little model membranes would fuse together. That really pointed that these lipids that don't like to adopt bilayer structures really play a role in membrane fusion, which of course is vital to many biological processes.

In order to look at lipid asymmetry, we established pH gradients across these vesicles and then synthesized the lipid, which we called an ionizable cationic lipid, so it had the property that at low pH, it was protonated and positively charged, whereas at neutral pH it was net neutral. The lipid would tend to associate with the side of the membrane that was facing the most acidic medium, so you could generate lipid asymmetry that way.

It also turned out that with these vesicles, we could encapsulate cancer drugs in a very efficient way in response to pH gradients. Of course that dragged us away a bit from the basic studies and we ended up forming a company, me and four postdocs, to deliver cancer drugs more specifically to tumors using these model membranes, liposomes, as delivery vehicles.

After a few years the CEO came to me and said, "I can't raise money putting old drugs into liposomes; we need to be doing gene therapy." This was in the middle '90s when gene therapy was coming into vogue. So I thought, oh boy, this is difficult. Because it was really a change from what we were doing with the small molecules.

The first thing we had to figure out was how to encapsulate nucleic acids in a lipid nanoparticle in a liposomal type system. At that point there were these cationic lipids around, the positively charged lipids, and they will associate with negatively charged DNA, RNA, polymers to give you a hydrophobic nanoparticles system. The problem with those cationic lipids was that they're really toxic. So that was a huge problem. We thought okay, how can we get around this? How can we use something that's positively charged but not have this toxicity issue? Then we came back to these ionizable cationic lipids that we used for lipid asymmetry which were positively charged at a lower pH, say pH 4, but at neutral pH they're not charged. So at, pH 4 which is about the acidity of a lemon, we found we could encapsulate. We were using DNA at that point, small oligonucleotides, we found we could encapsulate them in these lipid nanoparticles and when we went up to neutral, we could get 80, 90, or 100% trapping efficiency. When we took the pH back up to neutral, the DNA or RNA was retained in the nanoparticle. So that was kind of a big deal because then we had a system that could actually be useful *in vivo* because they're not going to introduce this massive toxicity that we were seeing with the permanently positive charged lipids.

That brought us to the attention of a company in Boston called Alnylam, this was in the early 2000s. They were wanting to deliver their small interfering RNA, siRNA. They'd managed to get some very potent siRNA for gene silencing, whatever target we might want. But they had to get these big molecules which had a molecular weight of 13,000 or something like that. They had to get those into cells *in vivo*. That was not obvious, how they could do that.

So we used these ionizable lipids and nanoparticles that resulted from that, and we found that we could deliver the siRNA to a liver with an IV injection and we would then see whatever gene we wanted to silence being silenced in the liver. This was kind of a big deal. It really entailed about a six- or seven-year collaboration where we found that in addition to being able to encapsulate the siRNA, if you optimized the ionizable lipid you could get really much greater gene silencing. In other words, once nanoparticles were taken up by endocytosis, the ionizable lipid can play a role in getting [cargo] out of the endosome, delivering the nucleic acid to the interior of the cell.

We started another company called Acuitas in 2009. In 2012 we said, maybe we can deliver messenger RNA, which is of course much larger than the siRNA, and have that expressed in the liver. We found that yes, we could see some gene silencing in the liver and with further optimization we could see pretty good gene expression in the liver. We're still in the process of getting better and better systems for gene expression in the liver, but about 2014 we had the great good fortune that we were approached by Drew Weissman and Katalin Karikó who, of course, developed messenger RNA in the hope of developing potent vaccines. They said "gee, we'd really like to try your system out. Not to express proteins in the liver, but to see whether or not if we go in intramuscularly we can actually see a reasonable immune response."

You can see how this is a series of rather serendipitous events. The basic bottom line was [that] they were absolutely fantastic vaccines. It turned out that not only did the ionizable

"The really exciting thing is that the vaccine application is just the tip of the iceberg. First of all there are other vaccines, everything from HIV to influenza, et cetera. But it's really enabling all of gene therapy, which is quite a remarkable statement…"

lipid help in terms of getting more protein expressed once they got taken up into the muscle and into the antigen presenting cells to get a good immune response, but they also acted as a very good adjuvant. What Drew Weissman says is that it really facilitates or encourages a particular type of response that results in very specific antibodies.

He would take a vaccine against Zika virus or influenza, he would package it up in the lipid nanoparticle so it would be a messenger RNA coding for whatever protein was associated with that particular infectious virus and send it off to some of his collaborators. One story he told me was he sent this material to a collaborator and didn't get anything back for about three months so he was wondering what was going on. He called him up and the collaborator said, "I didn't believe the results the first time, neither did I believe them the second time. So I did it a third time. Each time it's the most amazing response for neutralizing antibodies…" et cetera, that he'd ever seen.

Of course that brought us to the attention of BioNtech. Acuitas started working with BioNtech and that was on the flu vaccine. It was about 2018 or 2019. And then of course in early 2020 the pandemic hit. BioNtech was working with Pfizer on a flu vaccine. Everything switched over to the COVID situation in January and February of 2020. It ended up that our lipid nanoparticle got incorporated into the COVID-19 vaccine that Pfizer and BioNtech developed. You cannot predict that this kind of series of events would happen.

Then in November when the results were announced that these vaccines were 95% effective right across all age groups and ethnicities and the tolerability profile was pretty good…the 95% part was particularly amazing. So lipid nanoparticles became really popular. Nobody had ever heard of a lipid nanoparticle before but in the last year and a half they certainly have.

I guess to summarize the whole story it's one where it's really very basic research that facilitated all of this. We designed the ionizable lipids not just according to their pKa or their ability to be neutral at neutral pH, but also in terms of their ability to induce these non-bilayer structures in order to get out of the endosome. So very basic research turned out to be extremely useful in terms of the design, optimizing the design of these lipid nanoparticles to get the best immune response, to get the best protein, the protein production. It's an interesting story that interweaves a lot of basic science together with the applications and then also events like Drew Weissman contacting us saying "I really want to try your system as a vaccine." We weren't going in that direction at all. We had no notion that these might be useful in that kind of application. So it's been quite a ride. As you can imagine.

CS: It sure has! What do you see as the most exciting future prospect for lipid nanoparticle application? Does it remain in vaccines or is there another space where this technology is going to be game changing?

PC: Yeah. The really exciting thing is that the vaccine application is just the tip of the iceberg. First of all there are other vaccines, everything from HIV to influenza, et cetera. But it's really enabling all of gene therapy, which is quite a remarkable statement, but we can now use these kinds of systems to express any protein we want, or silence it if you want to use siRNA, and so a lot of things come into focus.

You can think of everything from fibrosis to atherosclerosis and heart disease. Going after Alzheimer's, neurological diseases, is certainly on the table. You can start to bring the whole armamentation of all we've learned in molecular biology to treat these diseases. Rare diseases.

The thing is you can respond in real time, because once you know whatever protein it is you need to make, then making the mRNA to produce that protein, maybe that's a month or so. Packaging it is a day or so. You have a highly targeted, very personalized medicine that's available in a very short time. This is revolutionizing medicine. We're kind of used to taking 15 years and billions of dollars to develop a small molecule drug. Well this just completely shortened that. It short-circuits that whole approach.

I always refer to it as the third generation of pharmaceuticals. First generation is small molecules; second generation is biologics, proteins, antibodies, et cetera. And then the third generation are these gene therapies which I think are going to revolutionize medicine. They're going to really confound regulatory authorities like the FDA because what do you do with a medicine that you can highly personalize for maybe a very small number of people, such as in rare diseases? It's going to change medicine fundamentally.

CS: Your colleagues have called you a role model for how to translate basic biophysical principles into meaningful applications. What advice would you give to scientists thinking about the challenges of moving from basic science into practical applications?

PC: I was always interested in getting involved in therapeutics, and so when I saw something that we'd done, such as the encapsulation of cancer drugs, my immediate inclination was to say okay, well we've got to move in that direction. But you have to keep the basic stuff going as well. Which is hard to do sometimes.

The other part for me has been, how do I keep a team together? One of the things that you do in academia is you can often get very good teams that are working in a highly

productive manner but they tend to dissipate. They take a postdoc somewhere, or whatever it is. So in the '80s I discovered that starting a company was a very good way of keeping a team together. That's really been a driver for me; you have the company going, you get four or five top people in your lab, you retain that expertise and you focus it. It's quite amazing what happens. That's certainly something I would advise people because you can't do things by yourself. It's the old thing, surround yourself with people that are smarter than you are and maybe you'll have a chance of getting somewhere. That was a real driver. I think starting companies can be used in that manner. It certainly has worked well for me.

DECLARATION OF INTERESTS

Pieter Cullis is the co-founder and a shareholder of Acuitas Pharmaceuticals and is a co-founder, chairman, and shareholder of NanoVation Therapeutics.