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0:00:05.8 Sarah Crespi: This is the science podcast for May 13th, 2022. I'm Sarah Crespi. Each week, we talk about the most interesting news and research in Science and the sister journals.

First up, freelance science journalist, Elie Dolgin, he wrote this week in Science about lipid nanoparticles. These are those little fat bubbles that surround the mRNA in mRNA vaccines. We talked about what these particles were originally designed for, and what can be done now to optimize them for vaccines and therapeutics.

Next, is researcher Luda Diatchenko. We talked about her Science Translational Medicine paper on pain chronification. What takes acute pain and turns it into chronic pain and the surprising role that inflammation and painkillers play in this chronification process.

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The mRNA and vaccines against SARS-CoV-2 is wrapped up in little fatty packets called lipid nanoparticles. These fat bubbles were originally designed for something much different, carrying drugs into cells to silence genes. They were not optimized for vaccine delivery. Science writer Elie Dolgin wrote about efforts to improve lipid nanoparticles as a delivery system for mRNA vaccines and therapeutic treatments. Hi, Elie

0:01:28.1 Elie Dolgin: Hello, thanks for having me.

0:01:29.5 SC: Oh, sure. This is really interesting. There's a deep history here. I think we should go there first. And then we'll kinda go to where we are today, what technologies are being developed. So I said this is something that's been around for a while, what was this technology originally used for?

0:01:44.7 ED: If we wanna go way way back to the history of using lipids as delivery systems, they were originally called liposomes, and they have a rich and long history, but they were never really useful to deliver RNA therapeutics until about 20 years ago when a scientist at the University of British Columbia in my hometown of Vancouver as an aside, [laughter] he developed what we now call lipid nanoparticles.

0:02:14.6 SC: Was the intention there to deliver, what was it, nucleic acids to cells for silencing purposes?

0:02:20.7 ED: Yeah, he had been working on formulating cancer drugs and things like that, and was tinkering with the formulas of his lipid systems, as he moved into new kinds of therapeutics, like gene silencing, also known as RNA interference drugs and antisense oligonucleotides. And he was trying to find a way to get the lipids to both bind and package those types of drugs, but also not be toxic inside the body.

0:02:48.6 SC: Right. You don't want the body to get harmed, also you don't want the body to reject

them. So how exactly do these lipid nanoparticles help anything get into the body and into cells? What is their role exactly?

0:03:01.3 ED: Well, the key original innovation that Pieter Cullis developed from earlier liposomes systems is he came up with this thing called an ionizable lipid. It's positively charged during the manufacturing process, essentially, at low pHs and thus, it can bind to mRNA and other RNA molecules that are negatively charged. But then it turns neutral once it's at the kinds of pHs found in our bloodstream, and therefore doesn't cause the same kind of toxicity problems. But to formulate these things, he also had a few other fats, essentially. There's something called a helper lipid that just kinda helps with the general structure. There's some cholesterol, and there's another ingredient called PEG, polyethylene glycol, which affects also structures stability, particle size, and things like that. So it's this four ingredient cocktail, essentially, that is what we now call lipid nanoparticles. And that's what's used to deliver the mRNA vaccines that millions, billions?

[laughs]

0:04:01.2 SC: Yeah.

0:04:01.9 ED: Lots of people around the world have gotten.

0:04:04.1 SC: Right. Well, how does it get into a cell? Is the pH still important for that transition?

0:04:09.2 ED: This is gonna be kind of a very simplified, cartoony explanation. But it bumps up against the outside of our cells, and the cell kinda pinches off its own little fat bubble that encases these fat bubbles, those are known as endosomes. And then actually, the pH becomes important here again as the lipid nanoparticle tries to break free of those endosomes and release its content, the mRNA in this case, into the inside of the cell so that the mRNA can then go to the sites of protein machinery and be turned into the types of viral proteins that are needed to instigate an immune response.

0:04:45.3 SC: These lipid nanoparticles, they're not taxis, they have a lot of different phases they go through, and they're also interacting with our immune system. This was something that was found with the COVID vaccines, that they do elicit an immune response or it affects the immune response in addition to what's going on with the proteins that are developed off the mRNA.

0:05:06.3 ED: They serve kinda this adjuvant function, the kinda aluminum that people might be more familiar with in traditional vaccines. So it serves both this taxi function and it activates the immune system, both for good and ill, you need that immune activation to get the kind of antibodies and T-cells and all the other good immune responses that help us stay protected against COVID. But then, it also leads to these inflammatory reactions that are what causes fevers, chills, and then sometimes even worse, like the cardiomyopathy that has been linked to some of these vaccines, the heart inflammation. I mean, that could also be tied back to these lipid nanoparticles.

0:05:46.0 SC: But an adjuvant is something that most vaccines have. And it basically gets your immune system just a little bit angry, making it pay more attention to whatever else is carried along

with the adjuvant, is that right?

0:05:57.2 ED: Yeah. The challenge now, basically, is to try to harness that adjuvant potential, which we want, but also to limit some of the adverse immune reactions that we don't. Obviously, they're incredibly successful, we wouldn't have had the mRNA vaccines if it wasn't for them. But there's still so much room for improvement to kind of tip the balance in a better way towards the good immune responses. And part of the reason that that balance seems to be not the best, at least outside of a pandemic scenario, is that these lipid nanoparticles actually were repurposed, essentially, for this use. They weren't ever really designed with mass vaccination in mind.

0:06:37.0 SC: Yeah, they were much more specialized.

0:06:38.8 ED: There's that, there's also the fact that usually when people were thinking about mRNA as a drug, they were wanting to treat disease, and you were gonna administer these things intravenously, through infusions into the bloodstream to try to get the mRNA encoding some disease correcting protein to particular cells in the body. That's essentially how these things were designed and optimized. As we look ahead to other types of vaccination contexts, like if we're gonna use these things for flu vaccine, our annual flu vaccine, or something elsewhere maybe we're in less of a rush, and we're not so desperate for a product. I don't know about you, but I don't wanna be knocked flat for a day or two every time I get the flu shot the way that these COVID vaccines do.

0:07:24.1 SC: Exactly. Another issue with this mRNA vaccines, and is related to lipid nanoparticles, is their cold sensitivity or their temperature sensitivity. Like how cold they need to be kept and how difficult it is to maintain a cold chain. Is that something that people are looking into tweaking?

0:07:42.2 ED: Absolutely. Early on when Pfizer was rolling out its vaccine, there was a lot of concern about the fact that these things had to be kept at ultra low temperatures. I think, with the benefit of time, people have realized, "Okay, it doesn't have to necessarily be in -80 freezers, it can just be a normal freezer or a fridge." But still that's not the best in many parts of the world that don't even have electricity to say. Trying to find nanoparticles that, because of the different lipid chemistries can just sit on the shelf at fairly warm room ambient temperatures, that's a huge goal as people try to expand the utility of these platforms into other vaccine contexts.

0:08:19.6 SC: We're mostly talking about tweaks so far, but other people just saying, "We'll just pick any lipids for this. We don't need to start with these four that initiated this. We're gonna go further a field and recompose this set up entirely."

0:08:33.7 ED: To some extent, it does seem like a lot of the innovation might be tweaks around the edges. Maybe more fit for purpose and less kind of radical next generation. You can think of it I guess as low-hanging fruit, but that's... There's a lot of room for improvements there, but people are starting to think a little bit more outside the box, adding extra lipids, adding extra antibody dongles onto the top of the lipid nanoparticles to get them to bind to particular cells, and trying to find all sorts of new ways to improve delivery. A lot of that actually is being done more with an eye to therapeutics where getting things to cells that are the sites of action where diseases are happening is

really important. Less so for the shots in the arm where you're just trying to tickle the immune system, but for sure, people are starting to innovate with everything both the four core ingredients that we know work, but then also thinking about other things you could throw into the mix.

0:09:29.2 SC: How far along have these tweaks come? Are people getting these shots in their arms at this point or are they still in the lab?

0:09:36.3 ED: Well, a lot of the papers that are coming out and proof of concept studies are being done in mice. But some of the bigger companies that have a lot of proprietary discovery operations are beginning to roll out some of their new formulations into early human trials. So Sanofi is one example, they have a flu vaccine formulated with mRNA that they've been testing in Phase 1 trials. And they actually went into the clinic with the same mRNA, but encoded in two different LNPs, and they noticed some stark differences between those two formulations. One gave kind of more middle-of-the-road immune responses, but also middle-of-the-road reactions. And then the other one gave you both much better antibody responses, but also some of those fevers and chills we don't want. So, it clearly shows there's still room for innovation, neither of them was perfect. But it also shows just how much the lipids do matter and how just tweaking that chemistry can totally change the profile of your vaccine.

0:10:35.9 SC: So do you think this is gonna be a rich vein for a vaccine development in the next decade or so?

0:10:40.7 ED: Yeah. I mean I think delivery is gonna be key, especially if we try to expand the reach of mRNA vaccines outside of a pandemic context, outside of the setting where all those side effects that we got with getting these shots, that's fine we needed something, and we needed something quick. But if we're gonna try to use the power of mRNA in all these other disease settings, I think for sure we wanna get things that are more potent, cause fewer side effects, and ultimately hopefully can be used in the developing world and don't have these cold chain storage requirements.

0:11:11.8 SC: What are the other therapeutic uses that are being considered for this?

0:11:15.4 ED: Oh, you name it. [laughter] Is the short answer. Ultimately, the idea with using mRNA as a drug is that just as with the vaccine where the mRNA encoded the viral protein and your body made its own antigen that elicited the immune system, the same idea, the mRNA here would encode say the correct version of a protein that's defective in some genetic disease and kind of a body heal thyself idea that if you can get those mRNA transcripts to the faulty cells, you can actually have a functional protein. Easier said than done, but where the LNPs come in is actually trying to get the mRNAs to the cells that need them. Right now when you deliver them intravenously, they just tend to traffic to the liver.

0:12:00.7 SC: Yeah, you don't want everything making... You don't want everybody making insulin, you want just [laughter] the specific certain cells making insulin.

0:12:06.1 ED: Well, but people are taking advantage of the first generation technology to try to

repair diseases that trace their routes to the liver and people are encoding disease correcting proteins. But also like CRISPR gene editing, which relies on proteins to help you alter the genome, that can be done in the liver, people are delivering those proteins as mRNA and that's happening right now. So yeah, mRNA has a lot of potential, but if we wanna move outside the liver and really get disease correction in all these other ways, for sure the lipids and delivery systems are gonna be key.

0:12:42.1 SC: Thank you, Elie.

0:12:43.1 ED: Thanks for having me.

0:12:44.4 SC: Elie Dolgin is a science journalist based in Massachusetts. You can find a link to the story we discussed at science.org/podcast.

Up next, we have researcher, Luda Diatchenko. We talk about the transition from acute pain to chronic pain and how some painkillers may actually help chronification.

[music]

0:13:13.5 SC: Have you ever thought about chronic pain, what happens in the body when suddenly you're not injured anymore, no specific thing is broken or disordered, but the pain just never subsides? We don't know why that happens. In this week in Science Translational Medicine, Luda Diatchenko and colleagues were looking into why there is this transition, what's activated, what's not activated. And in this case, it's when acute lower back pain turns to chronic lower back pain. And their team found some surprising links with inflammation and painkillers. Hi, Luda.

0:13:52.5 Luda Diatchenko: Hey. Hi, Sarah.

0:13:54.0 SC: This study focuses on a transition between acute pain and chronic pain. So why does it stick around even when your body is supposedly healed? So can you just tell us how chronic pain and acute pain are defined, how they're different from each other?

0:14:09.5 LD: So acute pain, it's something which we recognize there is an injury, and this is a good pain. We want to remove our hand from the hot stove. We understand much better the mechanism of acute pain. But after injury, majority of the time, we resolve pain and we resolve it quickly, but sometimes we don't. And this transition from acute to chronic, this is what we don't understand very well. So usually definition is more than three months after injury.

0:14:44.3 SC: More than three months?

0:14:45.5 LD: Yeah, it can be after surgery, for example, there is a chronic or surgical pain, or lower back pain.

0:14:52.3 SC: Lower back pain, as you say in the paper, is the most common kind of chronic pain.

0:14:57.0 LD: We injure our back all the time.

0:15:00.0 SC: Yeah.

0:15:00.0 LD: But then we heal. So why some of us don't, in terms of pain?

0:15:07.0 SC: Okay. So one approach you took to looking at this transition is you flagged that three-month period as being important, and you looked at what genes were being transcribed, which one were being read off and used by the body for three months in about 100 people. How were the people who got better, who stopped suffering from lower back pain, how were they different from those who converted to chronic pain?

0:15:33.6 LD: We look at the difference in the genes transcribed between people who after they have acute back pain, and then what's happened to their genes in the blood during this or after these three months. The genes in the people who resolve pain, they have a huge activity. More than half of the genome in the blood change the expression, very substantially. And the people who didn't resolve pain, nothing changed in their blood. So they kind of was frozen in their transcriptional activity.

0:16:11.9 SC: So when you say in the blood, that means you're looking at a subset of cells in the body that are in the blood. Do you know what kind of cells turn out to be important?

0:16:21.8 LD: Yes. We look in the blood means we look at the immune system basically. We realized that many cells participated in this healing process. And from all of them, the most significant contribution we can identify was neutrophils. And this was very surprising to us because neutrophils usually are not associated with pain. It's not the mainstream of the immune contribution to pain states. In our case, we saw its neutrophils who kind of get activated and then get very quickly deactivated.

0:17:00.6 SC: Because neutrophils are all about that early response to injury.

0:17:03.8 LD: But we also saw that neutrophils brought other cells, which they should. And so it's just like a first step in the good events, the healing events.

0:17:15.0 SC: So how often were you taking blood during this three months and checking out what was being transcribed?

0:17:20.5 LD: We had transcriptomics only in two time points. We had it in acute episodes, which we identified as four weeks. So people was having no longer than four weeks back pain, and then they had their transcriptomics three months later.

0:17:35.8 SC: What you saw from the transcriptomics, from the people, was that the ones that continued to have pain never saw this change in what was going on with neutrophils?

0:17:47.0 LD: Correct. And this was very interesting because they never upregulate inflammatory

response. And this was the most confusing, I would even say, in the beginning, because we all know that people with chronic pain have so-called low-grade inflammation. It's not exactly inflammation but this kind of inflammatory response. That's why seeing inflammatory response in the people who resolve pain was very confusing.

0:18:14.5 SC: Yes, it's counter-intuitive.

0:18:16.8 LD: Yeah, counter-intuitive. He was re-checking codes, and he was looking back and right and we were...

0:18:22.0 SC: So you went to a mouse model and you said, "Okay, what's going on here with inflammation, neutrophils, and chronic pain and acute pain?" You want to sort out the mechanism, how these pieces are related to each other. So what did you do with... You took away neutrophils and you tried to see if they were continuing in chronic pain?

0:18:42.0 LD: So I should mention that Jeff Mogil, who's also my colleague and professor at McGill, who is the world known specialist in the mouse pain model. So when I shared with him this result, he first said, "Okay, it cannot be," because we all know that if we inhibit inflammatory response is analgesic. They didn't show pain. And then he said, "Wait a minute. How long do we usually follow our mice? We don't. Maybe we should follow them after they become analgesic." And so the first experiments that we suggest, "Let's take dexamethasone," because dexamethasone inhibits any immune response. So just during the acute stage, we treated them with dexamethasone. And we saw, yes, that mice first became very analgesic. When mice was not treated with dexamethasone, yeah, they showed pain, but they resolved this pain in seven days. But mice who was treated with dexamethasone was... Didn't show pain, but then they developed it. And then this pain was lasting for 150 days, which is like half of life for mice. You can compare seven days if you didn't take dexamethasone, and 150 days.

0:19:55.5 SC: You're suppressing the immune response, you're stopping inflammation. So in the short term, yay for you, but in the long term, you're gonna suffer.

0:20:04.9 LD: Correct.

0:20:05.5 SC: What happened next?

0:20:06.7 LD: They said, "What if when we treat mice with dexamethasone? What if we will add neutrophils? In mice, you can do this. So if we add neutrophils together with dexamethasone that we don't have this long-lasting hyperalgesia at all. Somehow Neutrophils they are important. If they're there, they can resolve pain. So we also did the opposite experiments. What if we will remove neutrophils? Not dexamethasone, forget dexamethasone, just neutrophils. If we will remove neutrophils prior to this inflammatory model in mice, this was with mice develop the same long-term hyperalgesia."

0:20:46.0 SC: Basically, the neutrophils are key. If you have them, you don't end up with a chronic pain. If you don't have them, you end up with a chronic pain.

0:20:55.5 LD: Correct. And we don't know if they're the only one. But they're definitely the first one.

0:21:01.8 SC: We should stop here and bring in the painkillers. There are different kinds of painkillers. Ones called non-steroidal anti-inflammatories, you might have heard of those, like Advil and ibuprofen. And then you have a category that are not those things like Tylenol and aspirin. So if you use these non-steroidal anti-inflammatory drugs like Advil and ibuprofen, you start to see it interfering with this pathway, where instead of going from acute pain to no pain, you end up on the chronic pain train. So can you talk a little bit about that interaction?

0:21:38.1 LD: In the animal model, what we did, instead of treating mice in the acute stage, with dexamethasone, we treat them with Ansaid. And we had very similar, a little bit less strong but basically the same response from mice when they originally they show analgesia, they don't show pain behavior. But then they develop long-term hyperalgesia. In other words, they become very pain-sensitive for a very, very long time. And so we compare this in mice with other analgesics. And we use different groups, different type of strong analgesics, and all of them made mice analgesic at the acute stage, but none of them created this long-term pain.

0:22:28.5 SC: So you could show that the pain was not key to healing and not getting chronic pain, it was rather blocking that inflammatory state that seems to be the problem. So let's take it over to people here. This is where you brought in some correlations that you were able to observe in the UK Biobank. Can you talk about that?

0:22:50.8 LD: The best things to do this will be to do clinical trial. So when people have the same injury, and some of them treat it with Ansaid and some of them not. And this is our next step. But right now, what we could do, is we could look at this big publicly available data set. One of them was UK Biobank, and this is half a million people. So we asked a question, from those who report acute back pain, "Can we see if there is a difference for them still having pain in the second and third visit?" Which is between two and six years, if this is dependent on Ansaid they had taken at the acute stage. And so what we found that those who've taken Ansaid at the acute stage are much more likely to still have back pain at the second Ansaid visit, which is two to six years later, they still have back pain.

0:23:46.8 SC: But because it's a correlation, that's hard. You can't necessarily say this is why, because it could be they just had the worst pain. They just took a lot of different kinds of medications, you just don't know yet.

0:23:58.3 LD: Yes, we do few controls for this. So first of all, we compare them with people who take antidepressant which is often used for pain, and we compare this actually this paracetamol, so neither paracetamol nor antidepressant was having this effect. There was no link with the long term pain. And so we also control this analysis for the number of different pain sites. And this is also strongly linked to pain intensity. So if you, in addition to back pain, you have headache and shoulder pain, and knee pain, then generally your pain intensity is higher. So we control for this, and it didn't affect our association, we still had a very strong association with pain chronification.

0:24:42.7 SC: I think a lot of people think, "I might have just hurt myself back there, I'll look at... I'm gonna ice it. I'm gonna take some anti-inflammatory drugs, and I'm gonna get ahead of this." That's been the thinking for medicating an injury. This is going against that.

0:25:00.0 LD: Yeah, this is going very much against what is so intuitive, but as we know, people thought that the Earth is flat because we see it.

0:25:08.7 SC: Yep, it's true.

0:25:10.4 LD: It's kind of intuitive because we've won a fight inflammatory response. And of course, we should be careful because we shouldn't create sepsis. So it should be all under control. But what we learn is our body needs to initiate correct inflammatory response to then initiate anti-inflammatory response.

0:25:31.3 SC: Really interesting. So that's... You mentioned before that a clinical trial where you compare the different medicines and the long-term impacts would be one thing but you also wanna look deeper into the mechanism and try to better understand the way to support inflammatory response without it hurting people.

0:25:48.3 LD: Right, we need to look at how much inflammatory response is needed. The interesting also is counterintuitive to our usual practice is. It's not like a one gene, which is responsible for this, is half of the genome is participating in this. So, the moment when you inhibit one gene most likely, it still can be key somewhere, but from what I see, I, more expect now that there are so many events that any one stone you will put on this stream.

[laughter]

0:26:21.2 LD: On this stream, it just will be carried away, because there is multiple events. So I think what we need to concentrate is to enhance or help our body with this nature of healing, which our body is capable of.

0:26:39.8 SC: Thanks, Luda.

0:26:42.1 LD: Thanks, Sarah. Thank you very much for talking to me.

0:26:45.7 SC: Sure. Luda Diatchenko is a professor at McGill University in the Medical and Dental School.

[music]

0:26:53.9 SC: And that concludes this edition of the Science Podcast. If you have any comments or suggestions, write to us at sciencepodcast.aaas.org You can listen to the show on the science website at science.org/podcast or search for Science Magazine on any podcasting app. This show was edited produced by Sarah Crespi with production help from Podigy and Meagan Cantwell,

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