Carolyn Coyne: This podcast is for informational and educational purposes only and is not to be considered medical advice for any particular patient. Clinicians must rely on their own informed clinical judgments when making recommendations for their patients. Patients in need of medical advice should consult their personal health care provider.

From UPMC Children's Hospital of Pittsburgh, welcome to That's Pediatrics. I'm Carolyn Coyne. I'm a scientist in the Division of Pediatric Infectious Diseases.

Brian Martin: And I'm Brian Martin. I'm the vice president of medical affairs here at Children's. Today, we'd like to welcome Dr. Liza Konnikova. She's an attending neonatologist and an assistant professor and early stage investigator at our Department of Pediatrics, Developmental Biology, and Immunology at the University of Pittsburgh School of Medicine. Dr. Konnikova's focus is on the development of neonatal immunity at mucosal surfaces, and its role in the pathogenesis of diverse diseases such as sepsis, preterm labor, necrotizing enterocolitis. Welcome, Dr. Konnikova.

Liza Konnikova: Thanks so much for having me.

Carolyn Coyne: Thanks for joining us. So Liza, why don't you tell us a little bit about sort of your background. I always think it's helpful certainly for us, but also maybe for the listeners to tell us a little bit about your previous training, and then ultimately what brought you to research.

Liza Konnikova: Sure. So I pretty much did all my training and most of my life in Boston.

Carolyn Coyne: So you're use to the cold.

Liza Konnikova: I am certainly. I did undergrad at Brandeis, and actually worked as a full time tech at the same time. So I've always wanted to do research. But then decided to apply to medical school instead of doing just a straight PhD. And I did MD PhD at Tufts studying very basic signal transduction. I actually studied that three pathway, which I'm back studying again, but from a very different focus.

And then after my MD PhD I was deciding on kind of what to do. And I've always loved babies. So it was a very easy answer for me that I will do pediatrics and neonatology, and wanted to study something from the baby perspective. So kind of mucosal immunology was also a very simple answer to me. So I did pediatric training at Boston Children's, and then I did neonatology training at Boston Children's, and then I did a postdoc with Dr. Scott Snapper looking at mucosal immunity in much older patients than I currently study, looking at inflammatory bowel disease, and how changes in the immune system can cause or make better inflammatory bowel disease.

Carolyn Coyne: So what brought you to the Children's Hospital of Pittsburgh?

Liza Konnikova: Yeah, so I think after my postdoc I was really ready to start a lab and start my own group, and this seemed like a perfect place for us to come. The translational research and kind of the availability of specimens that I study is just amazing here. And we started a lab about a year ago.

Carolyn Coyne: So is there a specific disorder? We sort of mentioned all the different things that you're studying, and so of course it's quite broad, but is there a specific pathway or disorder that you study? And give us maybe a little bit of the clinical perspective as how that has impacted that kind of area of research in your lab maybe?

Liza Konnikova: Sure. So I think our lab currently is actually focused on studying what the normal development is rather than studying a disorder. The disorder that I've always wanted to study is necrotizing enterocolitis, which is a disorder of very premature infants where they get basically severe inflammation of the GI tract, then perforates, and a large majority of them die from it.

The problem with studying that disorder is that we don't understand what normal development is, and so it's very hard to make any sort of conclusions about what's wrong with these babies. And so, our kind of aim right now is to really well describe what the normal development is, and then start studying diseases like necrotizing enterocolitis, or preterm labor after we really understand what the normal immunological development is.

Brian Martin: Can you tell us a little bit about, do you do any clinical time, first of all, and can you tell us a little bit about how that experience, you've had quite an educational pathway, and how has that sort of informed your life in relationships in your lab?

Liza Konnikova: Absolutely. So I do about 10% clinical, 90% research time. And I do kind of serve as time during the day, and I also take call overnight. And I think kind of both seeing the premature babies being born, and realizing that a lot of them are actually healthy as well as a lot of them do get infectious diseases really started me thinking about we really need to understand what the normal development is. And probably the normal development is actually much more developed than what we have thought.

And also, kind of doing service at the Children's Hospital, we see a lot of babies that have necrotizing enterocolitis, that we've seen around for the past probably a hundred years, and we really have no cure. And so that has really driven me to try to figure out what is really causing this disease and how can we make these kids better.

Carolyn Coyne: So you mentioned that a lot of your focus is on normal development. And of course, with NEC, you're going to be focusing on the guts. Where of course mucosal immunity is very important. But are there other organs or tissue sites that you focus on in terms of normal development through pregnancy, and

maybe some things that could impact preterm labor and sort of your role as a neonatologist having to take care of those preterm infants?

Liza Konnikova: Sure. So we actually study two major organ system, kind of two barrier sites. So, we study the GI tract, as I already talked about, and we also study the placenta.

Carolyn Coyne: Those are my very two favorite barriers sites.

Liza Konnikova: Yes. I know.

Brian Martin: Everybody has a favorite. Carolyn, now I'm happy I know yours.

Carolyn Coyne: Don't ask me to rank them, but I love them equally.

Liza Konnikova: I'm fully taking after you, Carolyn. And so, a lot of is known about the maternal part of the kind of fetal maternal interface, but very little is actually known about the fetal maternal interactions. And so one of the projects in our group is to really figure out throughout kind of gestation of how does the fetus communicate with the mom, or try not to communicate with the mom, and then what goes awry to cause preterm labor?

Brian Martin: Can you tell us a little bit about how technological influences in the neonatal ICU have maybe impacted, during the course of your career, the thinking about early life experiences that neonates might have? Like for example, TPN dependence early in life for a subset of populations, and how these sorts of environmental influences affect immunologic development, and this kind of development of neonatal immunity generally.

Carolyn Coyne: Before you continue, for the non-clinicians such as myself, what is TPN dependence?

Brian Martin: Oh, I'm sorry. Yeah, sorry. The total parental nutrition. So these are babies that are in the neonatal ICU that are essentially receiving their feeds through a line, in other words, not sort of a normal feeding process that hits the gut.

Carolyn Coyne: Okay.

Brian Martin: And when I read about your background, and excited to have you on here today, I was thinking about that just recently.

Carolyn Coyne: Excellent.

Brian Martin: Yeah.

Carolyn Coyne: Okay.

Liza Konnikova: Yeah, absolutely. So I mean I think it's been amazing to see that we can really... babies as early as 23 to 24 weeks really have a chance now and can survive. And I think kind of our next era is figuring out how do they survive well, and I think that gets exactly to your point is we need to look at a lot of things. But one of those is kind of your immunological set up, right?

And so a lot of things that we do to our babies are not normal. They're in an incubator, they're in a hospital, they're in an ICU, they might not necessarily get normal nutrition. Those things are really tricky to study, right? Because you have to obtain blood from those babies. And babies have very little amount of blood.

And so we're actually trying to figure out... one of the fellows in my lab was working on a project where she's taking tiny amounts, so two drops of blood from these babies, and trying to really do deep kind of immunophenotyping. So deep kind of looking at what cells are there on a every kind of week or every two week basis to really figure out how are these environmental things affecting our baby development. And that's really not understood now.

Brian Martin: Interesting.

Liza Konnikova: Some of the work that I've done previously and some of the work that other groups have done have really shown that we really need to feed our babies. So TPN is probably really bad for their guts. And so because NEC is so scary to see, people had previously thought that not feeding babies would probably be the best thing. And really over the past 10 to 15 years, we realized that's probably the worst thing for them, and that we should start feeding them as early as possible. And I think the new kind of question is what do we feed them, and what is the best thing for these babies?

Carolyn Coyne: And I imagine by what route of feeding as well.

Liza Konnikova: Correct. Correct. So none of these babies can eat by mouth because they're too small. So they're all being fed through a tube. But it's clear that being fed through a tube is much better than being fed through the IV because when you're fed through the tube, your gut doesn't atrophy, or those cells that line the gut don't die. But when you feed through the IV, those cells that are lining the gut do die.

Brian Martin: That's interesting.

Carolyn Coyne: And so I guess-

Brian Martin: Microbiome.

Carolyn Coyne: Yes, exactly.

Brian Martin: The microbiome-

Carolyn Coyne: You can't get away from it.

Brian Martin: ... comes up again.

Carolyn Coyne: You cannot get away from it.

Brian Martin: And you can't, yeah, whether you're at a newsstand or in a podcast-

Carolyn Coyne: Exactly.

Brian Martin: The microbiome is-

Carolyn Coyne: It's everywhere.

Brian Martin: ... everywhere.

Carolyn Coyne: So following up, sort of I would say on the heels of Brian's question about technological advances within the NICU, one of the things about your research that I'm always very struck by it, and certainly impressed by, is your use of what I would say is really cutting edge technology from just a research perspective. And so maybe you could tell us a little bit about the technologies that you use in your research, and how I would say that the ever kind of growing improvements in these technologies directly influences your research.

Liza Konnikova: Yeah, absolutely. I mean, so I think as I said earlier, normal development hasn't been described. And one of the reasons is because it's so hard to get that tissue. But I think the other reason has been because it's really hard to study. And the tissue is very valuable. And you get very little of it. And so people have studied one specific cell type at a time. Whatever their favorite cell has been. T cells, B cells, macrophages.

But the advances recently in the technology have really allowed us to look at all the cells at the same time. And so, the technology, and there's a whole number of technologies, but the technology I use is called CyTOF, or mass cytometry. Which is basically, what I call it, fax on drugs basically. You are able to use cytometry, but because the antibodies are linked to heavy metals and not fluorophores, you can combine a whole lot of them.

And so, we routinely combine 40 antibodies at one time, which allows you to look at 40 plus different cell types at the same time. And so you can really start describing what cells are there, and how they're interacting with one another. Instead of focusing on one particular cell, focusing on the whole tissue at one time. And I think that really has driven the advancement in the field of... we now see there's so much more diversity than we had ever appreciated within all the different populations. And that the different population can interact with one another.

Brian Martin: So it sounds like it's an ecosystem.

Liza Konnikova: It is definitely-

Brian Martin: You're able to examine-

Liza Konnikova: Exactly.

Brian Martin: ... this as an ecosystem versus-

Liza Konnikova: Absolutely.

Brian Martin: ... a standalone kind of process.

Liza Konnikova: Correct.

Brian Martin: As the clinician in the room, I was really struggling there for a second. I was like...

Liza Konnikova: Exactly.

Carolyn Coyne: Don't be embarrassed.

Brian Martin: I'm going to make it through this podcast.

Carolyn Coyne: So you mentioned, I actually really like the ecosystem-

Liza Konnikova: I do too.

Carolyn Coyne: ... analogy.

Liza Konnikova: I think I might use this.

Carolyn Coyne: I have to say, that's quite good.

Brian Martin: Well, I was an ecology major in college-

Carolyn Coyne: It's very good.

Brian Martin: ... which baffled my accountant father.

Carolyn Coyne: Yes.

Brian Martin: He was like, "You're studying stream ecology? Like basically an excuse to go fishing, right?" But yeah, I think those kind of things, like some of your early experiences in education kind of color your perspective, but when you were speaking, I was like, wow, this sounds similar, far removed, but similar to stream

ecology in that you have complex interdependent relationships. That it's ideal to be able to take a look at them at a high level as a whole.

Liza Konnikova: Yeah.

Carolyn Coyne: And how does that... I mean you were talking again as sort of I like this idea of what's normal development, what's abnormal development. And sort of do you know anything yet about how that ecosystem changes during that? So during the process of NEC, and maybe again this gets at lack of tissue, but how will you address those questions? Is that your ultimate goal basically to compare the ecosystems of a normal and abnormal state?

Liza Konnikova: Sure, absolutely. So we used to think that probably kind of T cells and B cells don't play a huge role in that. Because, at least in mice, the adoptive immune system is really not developed in pups. But our recent research has shown that both T cells and B cells are actually quite developed, and really occupy a large proportion of your gut even in preterm infants as early as 16 weeks gestation.

And when we have looked at our samples from patients that have developed NEC, they're fully lacking the adaptive immune system to go along with the previous hypothesis that maybe the immune system wasn't developed. But now we know that it was developed. And so we think that there's a large contribution of the adoptive immune system that plays a role in developing NEC. And so I think that that's kind of how we're going to try to study it.

Carolyn Coyne: And how much of that develops in utero? So what is the influence then of... one of the things that I find very fascinating just about pregnancy is sort of that interface that exists, and how much really communication exists between certainly the fetal and the maternal side, but also vice versa. So how does that process kind of ultimately either predispose, protect, does it, if a baby is born either prematurely, or even normally, the eventual predisposition maybe to disease?

Liza Konnikova: Right. So we only really looked at the GI tract for now to very significant detail. And what we've shown is that as early as 16 weeks gestation, which is very early on in your second trimester, all of your major immune populations that are supposed to be there are already there. And not only are they there, they're functional. So they're able to produce the cytokines, that they should be producing an adult tissue. They're able to kind of react to stimulation in similar ways to the adult tissue. And they are also specific cells that have retained some memory. So there's classical markers that people use to show that these are memory cells, and those markers are expressed on the cells in the fetus as early as 16 weeks.

So, I think the majority of the pre-setup of what the immune system should be like actually happens in utero. And then once the fetuses are born and are much more exposed to the microbiome, then there's changes in that environment.

But the initial environment, or ecosystem as you were saying, between the immune cells, the epithelial cells, and all the other cells that are there is actually quite pre-setup. How that happens, I don't know.

And I think that's kind of the next question that we're trying to answer. My thought is that because fetuses swallow amniotic fluid continuously starting at 14 weeks gestation, right, they are getting antigens from mom that are crossing the placenta, and then into the amniotic fluid, and they're swallowing those. And we have showed that your development of the immune system is actually much earlier in the small intestine than the large intestine. Consistent with that, that as they're swallowing, they're first going to get to the small intestine and that system is going to develop. Then it will get to the large intestine, and then that system is going to develop.

Carolyn Coyne: Well in the context of congenital disease, of course there's a very few number of pathogens that'll actually induce congenital disease, but some of those of course then infect the fetus through the oral route, Toxoplasma gondii for example is one. And so, I think understanding the basics of what immune cells are there, will actually I think give us really important insights into congenital disease, microbes, what does or does not protect him a baby from in utero infection, which I think is really fascinating.

Brian Martin: This makes me want to go wash my hands. But saying that, I have a question, and this might be coming from a little bit out of left field, but are the cell lines and the tissue samples that you're studying, being whether or not it's an animal model, are they well controlled for, or has there been any attention paid to control for some of the other areas that we know affect health?

And where I'm going with this is like social determinants of health, right? Where know that there are certain aspects that have a positive predictive value on whether or not you may have a premature baby in the first place. And health systems sort of I think are struggling to really engage pregnant women with appropriate prenatal care and to do everything we can to avoid ideally having a 23 or 24 week baby. And any ideas or thoughts about the sort of epigenetics circumstance with all of this.

And I'm sorry if that question comes from out of left field, but when you're talking about this, and particularly at these ages of development, I'm like, well what's happening to mom clearly is what's happening to the child. Right? And at these critical stages of immune system development, is that part of the conversation?

Liza Konnikova: That's a great question. It's very tricky to study unfortunately. And I think because we're just starting to study at this point, we just kind of are describing what's normal. And I think once that's well-described then we can start getting at your questions. The maternal part of this is much trickier to study ethically because these are kind of fetal tissue, and so we don't have any information on the mom whatsoever. But I think as we can show how important this normal

development is, and how critical it is to understand it to study all sorts of diseases downstream, as Carolyn was saying, then I think we should be able to start answering more of the questions that you're answering. But for now, we have no information on the moms.

Brian Martin: Thank you.

Carolyn Coyne: Thank you for joining us, Liza. That was really fascinating.

Brian Martin: Yeah, absolutely, Liza. Thanks for very much for your time.

Liza Konnikova: You're so welcome. This was really fun.

Carolyn Coyne: You can find other episodes of That's Pediatrics on iTunes, Google Play Music, and YouTube. Be sure to subscribe to keep up with new content. Also feel free to leave us a review and tell us what other topics you'd like our experts to cover. Thanks again for listening.