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Carolyn Coyne: From UPMC Children's Hospital of Pittsburgh, welcome to That's Pediatrics. I am 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. We're here today to welcome Dr. Timothy Hand. He's a scientist who specializes in gastrointestinal immunology, and his work is focusing on the development and regulation of immune responses against microbiota, and how gastrointestinal infection may unleash responses against bacteria, and how the immune system is controlled. We look forward to talking to him a little bit about his work, what brought him to Children's, and kind of where we are and where we're going.

Carolyn Coyne: And so for, Tim, for you, one of the aspects of your research that I find most engaging and exciting is really the focus that you have on neonatal health, and sort of the impact that can happen in a premature infant, in terms of how infections in the intestine can lead to complications. Could you tell us a little bit about the work that you do in that area? And perhaps even more generally about some of the things that you've been looking at?

Dr. Hand: Yeah, thanks for that question and thanks for inviting me. We've kind of shifted our research focus since coming to Pittsburgh. That was sort of one of the reasons why we chose to come here. My, sorry, should say we, my wife and I, when we moved here from the NIH, one of the aspects of our work that we have shifted is that we're interested in how the microbiome interacts with the host, and coming to a children's hospital, it just became clear to us that one of the most important moments of this relationship between you and the bacteria that are in your intestine or on any other surface is that moment of first colonization.

Dr. Hand: What we have really shifted most of our lab to start working on is how is this moment, this handshake, this first introduction between yourself and your immune system and the bacteria that are going to live inside of you for the rest of your life, how is that negotiated? One of the things that has been really, you know, shifted enormously by coming here to Children's has been able to access some of the samples from the neonatal intensive care unit at Magee, and being able to look at how, in fact, the mother's breast milk and how antibodies in the breast milk may actually be shaping that relationship, actually modifying those bacteria so that they are less invasive when they first interact with the host.

Carolyn Coyne: And so, is that the kind-of first handshake? So is the first handshake of an infant gut sort of through feeding, you know, through breast milk, or formula in some

cases? And does that then have a direct impact on the health of the intestine or how the microbiome is formed and shaped?

Dr. Hand: Yeah, I mean, yeah, that's a great question. Unfortunately it's a bit more disgusting than that, you know?

Brian Martin: Go for it.

Dr. Hand: Yeah.

Carolyn Coyne: We're all friends here.

Dr. Hand: So what we're-

Brian Martin: Disgust us.

Dr. Hand: We're particularly interested in premature infants. Many of these infants are born by C-section, so of course they won't gain access to the, you know, the vaginal and the fecal microbiota, by virtue of the vaginal birth. Then, unfortunately, because many of the infants we work on are severely premature, these infants will spend most of their first few weeks of life in incubators, and not really experiencing the evolutionary interaction that an infant typically would go through. So the organisms we find actually, unfortunately, in these kids are much more random and almost like passengers not by design. We see things such as E. coli and Staph. aureus and Staph. epidermidis, things that would be on the skin of the nurses and of the mothers that are handling the kids, not necessarily what was in their intestines, which is what you really want to start getting into the kids.

Carolyn Coyne: Well, and what I would say is, you know, we hear a lot about this, in the news media and certainly at scientific meetings, for those of us that are basic scientists, about the microbiome and how the microbiome controls your life and controls your mood and whatnot. Could you maybe just talk a little bit about the microbiome? You know, is it different? You mentioned sort of differences in bacteria from skin surface versus the vaginal canal, and maybe talk a little bit about those differences just more generally in the sense of are there big differences in the kinds of bacteria at all of our different kind of contact sites and mucosal surfaces?

Dr. Hand: Sure, that's a great question, and actually it's sort of a fundamental question, because, and people sort of gloss over this, but sort of a seminal finding in the field was that the bacteria at each different site, you know, your skin, your lung, your nose, your intestine, they're all unique and they're actually more similar between two different people than any two body sites are on that same person. What that means to us is that these are not random assortments of organisms. These are actually being shaped by the environment, which implies that it's an evolutionary relationship. These organisms are interacting with the host in

important ways. It's really been in the last decade that we've even started to unravel some of these ways that they interact.

Dr. Hand: The example I always use, and this is probably the most important example that we have is, people always tell you to eat your fiber, right? Fiber's good for you. Fiber is a way to maintain a healthy lifestyle, healthy body.

Brian Martin: I can hear my wife is saying that.

Dr. Hand: Yeah, yeah. But I mean everyone needs to eat more fiber and more leafy greens. Without your intestinal microbiome, you would not digest any of that. You do not have any of the enzymes in your intestine to digest any fiber. It's all provided by a set of very fastidious anaerobic bacteria that essentially ferment all of your fiber into things that you can then digest.

Carolyn Coyne: Brian's going to be discussing that fact tomorrow morning at breakfast.

Brian Martin: Oh yeah, absolutely, this is going to be a hot topic tomorrow and the weekend with my kids, no doubt. No, and I'd like to thank my intestinal microbiome for its robust performance over the years and all of the horrible things I've thrown at it.

Brian Martin: What brought you to Pittsburgh specifically? Said you were at the NIH before Pittsburgh. We talked a little bit about, so far, about your work with the microbiome, and I would love to ... I could talk to you forever about the ecology of these different microbiomes and body sites. I'd love to hear more about that, but what brought you here as a basic scientist after you came out of the NIH with your wife?

Dr. Hand: Well, I mean, one of the first things I will mention is that we were extremely comfortable with the research environment here. We knew a lot of the people who were already working here, both at Children's and also over in the other campus at Oakland. So specifically, you know, much of the immunology department at Yale, where I did my PhD, has in fact moved to the Yale West campus here at the University of Pittsburgh.

Carolyn Coyne: Or perhaps they're the Pittsburgh East campus.

Dr. Hand: ... Pittsburgh East campus, yeah, exactly. That's a better way of putting it. These people were instrumental in bringing here. We're very comfortable with them as researchers and as being people who were going to be supportive of us in terms of the immunological community. As well, there was just a feeling around Pittsburgh, you know, not just the Children's Hospital but the entire Pittsburgh research community, of being a place that's sort of ready to burst. I mean there was just so much going on and there was so much interest in things that were in and around the topics that I was interested in that it just seemed like it was going to be an environment that was really going to be both beneficial to my

research, but also that I would be able to step in and really, I think, transform other people's research and work in a collaborative way, and really start to do, you know, what we started to call like our soup-to-nuts research projects, where we are taking clinical observations that we learn about from our collaborations with the medical community here at Children's, identifying really unique aspects of that clinical cohort, and then modeling it in some of the animal models of the microbiome that we're so famous for ... not so famous, within very small circles.

Brian Martin: Oh, we think you're famous.

Carolyn Coyne: You're famous.

Brian Martin: We hear you're very big in Germany.

Carolyn Coyne: You're on our podcast, Tim, that makes you famous.

Dr. Hand: Right, yeah, you know modeling those, in the systems that we are renowned for, at least in the smaller immunological circles, and that really is what's happened. We have some wonderful collaborations here with some of the researchers who work on these premature infants, and it has just been an amazing experience. We had opportunities to go to some great places and we are not even the slightest bit ... we have no second thoughts about Pittsburgh. It's been really, really wonderful in multiple ways.

Carolyn Coyne: So what have you found? You mentioned before that you were really looking sort of at tissues and samples at the women's hospital, on premature infants and babies in the NICU. What have you found in terms of the microbiome and how that could impact the health of these infants?

Dr. Hand: Yeah. If you remember, I mentioned that your microbiota is necessary for doing things like digesting fiber. What's unique about premature infants is that their microbiome is completely different from the healthy adult microbiome. It really is a collection of whatever organisms are in their environment. So remember I told you that we know that these organisms are evolutionarily shaped by the body. In these kids it's the exact opposite. We just seem to almost see random assortments of organisms. There are certain organisms we see more, certain organisms we see less. This wouldn't be that much of a problem except that we didn't evolve to be born at, you know, 25, 26 weeks of gestational age, and the intestine's not prepared for these organisms. It can end up with a series of different, really serious complications, of which the one we're most interested in is necrotizing enterocolitis.

Dr. Hand: Sort of the going hypothesis about how this disease works is that, as a result of prematurity, you get damage to your intestine, and we're still not quite clear on how that happens, but then the bacteria that are present there, they invade. That invasion of the intestine causes a massive immune response. You end up

actually having sort of an immunopathological response against the microbiota of the intestine, which can be-

Carolyn Coyne: How common is necrotizing enterocolitis?

Dr. Hand: ... So it's quite uncommon, but that's because prematurity isn't common. Amongst premature infants, we see it in somewhere between seven to 15% of all premature infants. It's extremely important because of that 15% of very low birth weight infants, up to a quarter of those children will unfortunately succumb to the disease. Many of them we have to go in and remove large stretches of their intestine, which can have huge long-term consequences.

Dr. Hand: So what we would like to do is prevent disease entirely. The only therapy that has worked here at Children's and across the country is by feeding kids breast milk. What our project was was to determine what it is in the breast milk that is important. We think that we have some new data, which we're just about to submit and hopefully have published, where we think that actually maternal IgA is the important component.

Dr. Hand: So what can happen is that we think that it's important what the specificity of those ... want to say IgA, I mean immunoglobulin A. This is the antibody that gets secreted into your intestine and also, not coincidentally, but on purpose, it also gets secreted into the breast milk of mothers who are lactating. We think that this IgA is actually capable of shaping those first communities that go into an infant's gut and really preventing the invasion of those organisms in the intestine. We think that perhaps in the future, by targeting the most effective breast milk samples, that is the samples with the antibodies that are specific to the right set of organisms, or in this case actually the wrong set of organisms, you know, we might be actually able to prevent more nec than we have in the past.

Carolyn Coyne: So do you think that that IgA prevents the bacteria from infiltrating sort of into the intestine? Or do you think it's more of the effect of that IgA on other sort of more immunological kind of pathways and processes?

Dr. Hand: That's a great question, and actually we don't know yet. That is the next step of our experiments. We think it's an important enough finding just to have identified what is the active component of breast milk. You know, just sort of to start talking about it. But you know, as far as the mechanism goes, we don't know. Actually, it's been a huge mystery in immunology, what immunoglobulin A does.

Carolyn Coyne: In terms of it being in the breast milk?

Dr. Hand: In terms of what it does anywhere. I mean this is the most secreted molecule that you have, particularly your mouth is secreting grams of it every week, into

your salivary gland. Your small intestine is secreting a gram of it every day. I mean a gram is a lot of protein, I don't know if-

Carolyn Coyne: It is.

Dr. Hand: ... if anyone's had to make protein-

Brian Martin: When I'm lifting a lot, it's-

Carolyn Coyne: Brian can get at least two grams.

Dr. Hand: ... Right.

Brian Martin: ... Absolutely.

Dr. Hand: But we have a huge effort to make this molecule, but we don't know what it does. So part of our efforts as a basic science lab is to determine, you know, can we use this particular system where we've seen huge effects of this molecule to sort of parse out what it's doing? It would be easier for us if someone had told us what it was doing already, but it's been a big mystery.

Carolyn Coyne: Never works that way.

Dr. Hand: Yeah.

Carolyn Coyne: Basic science.

Brian Martin: Tim, do we know about normalization of this patient population? You sort of spoke about the ex-25-week preemie and an abnormal microbiome, secondary to the environment that you spoke about. Do we know about the evolution of the microbiome as the infant grows and changes? Do we see a refreshment from kind of like exogenous sources as a child's exposed to different environments and a normalization process that occurs? Or do we know how-

Dr. Hand: Yeah, sure. I mean, yeah, that's a great question. I mean, our work definitely applies to full-term infants as well. I mean, of course, the stakes are maybe not as high for those infants, because they generally, except for very rare cases, never develop anything like necrotizing enterocolitis. But yeah, there's basically a standard pathway the kids go through, and we do think that the maternal antibodies are important for that as well.

Dr. Hand: So what we think, and this is sort of, you know, we're not well understood, but just as a phenomenon, when we as a field have been looking at infants that were fed formula versus infants that were breastfed throughout their life, essentially what seems to happen is if you're getting formula, the maturity of your microbiota seems to lag a few months. So there's some effect. And again, because we don't know what IgA does, we don't know how this works, but there

is a phenomenon where getting breast milk IgA actually makes it so that you get the beneficial organisms first.

Dr. Hand: This will be a big effort in the field to determine how this works, and not just in terms of immunology but especially in terms of microbiology, because a lot of these organisms have been very difficult to grow and to work with. They're all very fastidiously anaerobic, meaning that even a whiff of oxygen will kill them. It has made doing genetic manipulations of these organisms to test mechanisms very, very difficult. So the tools are just coming online now, and I think there will be, soon, answers, but we sort of don't know enough yet.

Carolyn Coyne: So I noticed that you're a scholar in the Mellon Institute. So what is the Mellon Institute at Children's and how has that impacted you?

Dr. Hand: The Mellon Institute has been great, sort of putting a foundation down for us as we moved to Pittsburgh. Essentially what it is, it's an incubator for pediatric research here at Children's Hospital to give us a little bit of a buffer, a monetary buffer, to be honest, in terms of being able to do some groundbreaking research that we wouldn't be able to immediately fund via sort of the traditional funding mechanisms. This money, or sorry, this gift from the Mellon Foundation has been transformative to my lab.

Dr. Hand: We would ... the NIH, or sorry, the necrotizing enterocolitis work I've been telling to you is not funded by the NIH yet, so we've been doing all of that work with funds from the Mellon Institute. This I hope is the exact kind of project they had in mind when they put the money together. This is the type of project that's happening in all four of the Mellon Institute labs right now. You know, some of our most risky, but also most potentially transformative and groundbreaking, work is funded this way because, you know, the NIH by nature is a conservative group. They're government, so they want to make sure that things are going to work. Whereas having this kind of incubator and having this kind of sort of startup funds is sort of allowing for more risky, for lack of better word, more risky stuff.

Carolyn Coyne: High-risk, high-reward type stuff, right?

Dr. Hand: High-risk, high-reward yes, exactly, yes.

Brian Martin: Sounds like a great environment in which to be able to take some chances that could make a profound impact.

Carolyn Coyne: Absolutely.

Brian Martin: That's what we hope, yeah.

Carolyn Coyne: Okay, well thank you for joining us today, Tim. I learned a lot.

Brian Martin: Yes, thank you very much.

Dr. Hand: Thanks.