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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: I'm Brian Martin. I'm a vice president of medical affairs here at Children's. It's our pleasure today to introduce our guest, Amanda Poholek. She's an assistant professor in the Department of Pediatrics and the Department of Immunology at the University of Pittsburgh School of Medicine. Her research interests include transcription factors, chromatin regulation, T cell differentiation and effector function, and next-generation sequencing.

Welcome, Amanda.

Carolyn Coyne: Welcome, Amanda.

Amanda Poholek: Thanks so much for having me.

Carolyn Coyne: So that was a lengthy, very scientific description I would say of your research.

Amanda Poholek: It was. It was. Yeah.

Carolyn Coyne: Brian sounded like a real scientist there.

Brian Martin: I tried.

Carolyn Coyne: Why don't you tell us a little bit about what you do?

Amanda Poholek: Yeah. So I definitely would say I'm a basic scientist who's interested in the immune system and in the cellular differentiation of that process. And so let me give you a little bit of background of why I think those things are interesting. So the cells in your body all have the exact same DNA, right? That's the blueprint for everything, and yet your cells all do different jobs. And that's really fascinating to me. How does that work? You have one blueprint but yet you get to do different things based on that cell type. So even in the immune system that's true. The immune system is a makeup of lots of different cell types, and they all have different jobs to do. But they still have to work not only in concert together as a system but they're constantly interfacing with other cells in your body because they have the ability to go everywhere. Your brain cells stay in your brain, but your immune system cells, they can go to every part of your body. So they also have to interact with all of those other cells. And yet every single one of those cells has a basic blueprint to do the same jobs.

So how that works is that you have this process called epigenetics, and you have this process called transcription factors that basically go in there and they tell the DNA exactly which parts to turn on and turn off and function. So that way all of those different systems in your body have the ability to work together in just the right way. The immune system to me is a really interesting system because when something goes wrong, you end up with a really severe disease, and that can be quite pervasive and have all different variety types of diseases including Crohn's disease, inflammatory diseases, responses to infectious disease and how you respond to a virus or bacterial infection, or sort of where I got my start, which was thinking about autoimmunity and how things can go wrong in the sense of actually driving an autoimmune disease like Lupus or rheumatoid arthritis. And these are diseases that also effect children in large part.

So there's lots of really interesting ways to think about how the cells in your body are getting different signals to do different jobs and how they interact with other cells in your body and then those can result in diseases when things go wrong.

Brian Martin: Tell us a little bit about how you got started sort of from the undergraduate to graduate school transition. Like what lit your fire specifically-

Amanda Poholek: Yeah. So I'm glad-

Brian Martin: ... of dysfunctions.

Amanda Poholek: No, I'm glad you asked that question. So my family background has a lot of autoimmunity in it, which is why I brought that up earlier. My maternal grandmother died really young, way before I was born when my mom was only 13 years old of multiple sclerosis. This was a time for when autoimmunity wasn't well understood. So they actually thought her disease was largely neurological. So most of the treatments that she received, which at the time were cutting edge, were not addressing the immune system whatsoever because they just didn't understand what it was.

Brian Martin: Mm-hmm (affirmative).

Amanda Poholek: So that has sort of pervaded through my family. My sister and I have a cousin who have sort of similar symptoms of types of autoimmune diseases. Not exactly MS, but it became very clear to me that this was something that was being passed through our family in a hereditary way. But yet it was having its effect in slightly different ways because it wasn't the exact same disease every time. When I started graduate school, I knew I was really interested in sort of how cells functioned and how that when something went wrong, that led to diseases. But I was really open to what I wanted to work on. And so I started working in a lab that was focused on Lupus because that was an autoimmune disease, a systemic autoimmune disease. That was my first foray into the immune system, and I just became totally fascinated. I couldn't walk away.

So my graduate work was in the Department of Rheumatology at Yale University, and there we studied sort of the genetics of autoimmunity and Lupus and T cells in Lupus and how they specifically contributed. T cells are one of the really important parts of the immune system that can be considered kind of like the generals. They sort of have the ability to tell the other cells what to do and where to go and how to do their jobs. So we studied how T cells in Lupus contributed to that disease and how they particularly would sort of respond when they shouldn't and act as if they were seeing an infection when they weren't seeing an infection and fight self instead of fighting non-self or fighting an infection. And that's really the result of a systemic autoimmune disease like Lupus.

From there, I became really interested in specifically how do T cells do their jobs. What is it that they need to see? And then once they see those things, what are they actually go on to do? And that really led me into the world of transcription factors, which is a really fascinating group of proteins that can specifically bind to the DNA, make changes, turn genes off and on, and really get at that idea of how the DNA in your body can be the same in every cell but not necessarily act the same way in every cell.

So I've been continuing to study how transcription factors change T cells and their function in different contexts, and during my post-doc, I started to learn at the NIH, the National Institute of Health, how those transcriptions factors can have really important jobs. And we started to really examine them using a process called next-generation sequencing. And that allowed us to actually really look where those transcription factors were binding to the DNA and how they specifically were doing those jobs and specific cell types, specific ways. And that's really been sort of the beginning of everything that I now study in my own lab. Looking at T cells in different diseases and transcription factors and how they can participate in that process often using next-generation sequencing as our technology.

Carolyn Coyne: So I guess sort of on the heels of that, next-gen sequencing, this has been I'd say a revolution certainly in science and our ability to look at expression levels. Can you define a little bit what that means? Sort of how that's different. And I would say just on a technical level scientifically what that allows you to do that maybe you couldn't do in the past.

Amanda Poholek: Right. So in the past when we wanted to know where something was happening in the DNA, we kind of had to know where to look to start. We'd have to sort of have a basic idea and very carefully walk along the chromosome and try to find where things were, and it was very labor intensive, it was very slow, it was very expensive. What next-generation sequencing has done is given us the ability to kind of look everywhere all at once, and then see where the interesting places are. So it's a technology that allows you to start with nucleotides and then basically map them back to the genome and say where the interesting bits of what I started with. So you can look at what we call the whole transcriptome, which is sort of the part of your cell that's generating the proteins. So you kind

of know the functional part of your cells, and that allows you to look at what's actually being made and what's going on in that cell at the functional level. Or you can look at the actual DNA itself and look for potential variations or mutations that could potentially lead to changes in the function or have some sort of deleterious effect, depending on that cell and its context and function.

Brian Martin: What sort of technological evolution was required for us to get to this sort of next-gen sequencing place where we are now, and what sort of challenges does that technology pose to you and your lab in terms of funding, in terms of like how you get to do what you do?

Amanda Poholek: Right. So the technology was obviously not something that my lab worked on. This was something that lots of really smart bio engineers were focused on doing. It basically was the concept it massively parallel sequencing. So you basically took what used to be a one at a time reaction and you were able to sort of lay it down flat on a chip if you can imagine and do it in parallel multiple times, much, much faster than you used to be able to do. So it was physically the creation of a machine that had the technology to do that.

The technical challenges by and large, which are now actually being resolved again due to more amazing technology that people are coming up with every day, was the ability to look in smaller and smaller cell numbers. So when we started this technology, it still required a fair amount of starting material. So if you're looking at a specific cell, you'd want a lot of them. And sometimes that was easy to do and sometimes that was really hard. I think that's really a place where next-gen sequencing at UPMC Children's Hospital of Pittsburgh is really important because when you're dealing with patient samples from children, you're often talking about not a lot of material to start with. So it's really critical to take this technology to a place where we can look in smaller and smaller cell numbers.

So now when we look at sort of the transcriptome that I referred to before, we can now do that very easily in single cells. And that allows us to do some really interesting things. It allows us to address the potential of heterogeneity in a cell population. So even though, for example, my cell types T cells, they're all T cells, but they may be doing different things. So each individual T cell might be important to look at a in a different way. So we can do that now.

Carolyn Coyne: I remember pouring sequencing gels as a graduate student with a lot of radioactivity, and it was a labor intensive and not pleasant experience.

Amanda Poholek: Exactly. Exactly.

Carolyn Coyne: So you mentioned before the ability to sort of take patient samples, and is this either a technology or something that you're interested in doing in terms of looking at the transcriptional profile let's say in patients with autoimmunity? And do you see this as sort of what one might refer to as kind of personalized

medicine? The ability to look on a person specific level at expression levels and how they might be different.

Amanda Poholek: I'm glad you brought up personalized medicine because that's something I'm personally really interested in, and I think is really important. I think is probably the way that a lot of things are going to go, particularly when you're talking about complex diseases like autoimmunity. I mean, the reality is these symptoms might be similar, but the underlying causes can be due to a number of different things. So even if you talk about Lupus, there probably is more than one 'type' of Lupus. We just haven't been able to really capitalize on that because they're all a collection of symptoms. But if you could start by looking at the DNA or the RNA in those cells that are driving those disease processes, you may find that, "Oh, okay. We can actually take this group of patients and say their underlying cause for these symptoms are X, Y, and Z. And these group of patients are A, B, and C." So we really need to use specific treatment plans for each of those patients that we can understand just by knowing exactly which cells are involved, what those cells are doing or what they're not doing when they should be doing something else, and how we can sort of redirect them into the right way.

So I really feel that next-generation sequencing is going to be a critical component of personalized medicine because it's one of the only ways that we can really look at the patient level and say, "What's going on in this individual person?" By having more and more and more of those patients together, we can actually start looking at the broader scope and saying, "Okay. What's normal?" Because sometimes that's even hard to do unless you're looking at control patients or regular people. So if we know first of all in a large group of people what's normal because normal is still going to have a huge range among different races and ethnicities and ages and backgrounds, and then we can really say, "Okay. What's not normal? And which of those not normal situations are driving symptoms in one direction or another direction?" Then we might be able to really start to understand how we can treat people in a specific way.

And this technology is growing so rapidly that it's really getting to a place that we can take people into the hospital or even have them send their samples in the mail sometimes, run them through the sequencer, and within a week or two weeks, have some data or information that would really help us with their treatment plan specifically.

Brian Martin: You just took my question, which was what's the turnaround time on this? I mean, this sort of technology is amazing to me as an non-scientist, the concept that we would be able to garner such information. And so this is really starting to come to fruition within a two week or so turnaround time that we could have actionable information in the hands of front line clinicians with the assistance of this type of technology.

Amanda Poholek: I mean, I think not right now today.

Brian Martin: Not right now, but that's where this is going.

Amanda Poholek: I mean, you can see it, and you can see it happening in the next five to ten years where it really would be that way. I mean, there's certainly the main problems of course with these types of situations are infrastructure. And the bottleneck in a lot of this is sort of the analysis process. There's just not enough people who know how to do what needs to be done and then translate that back to either scientists or more importantly clinicians. What is that information mean and how can I display it to them in a way that they can really quickly make a decision?

Brian Martin: Let's talk about that for a moment because that's interesting because I'm a systems, non-scientist systems person on the hospital side. So tell me what does that workforce look like and how are we here at Children's Hospital and UPMC dealing with a future state, which sounds like it's going to have incredible demand for this skill set?

Amanda Poholek: I think you really need to have a system where you have medical records that are easily accessible to everyone within the system. And you need to have a system where you have sort of a group of individuals who can sort of be sitting there crunching through pipelines or at least sitting up pipelines that automatically run through those data sets so that the data comes in from the machine and then it goes straight into the computer system. And it can either be filtered very easily through automatic pipelines with reasonable levels of computing power that can then be sort of translated directly back to the clinician. I mean, a lot of this is all sort of in a computing system. So having those computing systems really pull together in a way that made sense and having the right individuals being able to sort of push that information along in the right way.

The machines themselves, they exist as well, and they're relatively easy to use. A lot of that has been put together in very clinically certified mechanisms so that you have everything behind a wall of privacy, and everything is robotics and sort of treated... Standardization obviously is a key thing here. So everything is treated in a very standardized way. And you have individual ways of dealing with each part of that pipeline. But UPMC has invested in a new genome center that certainly has the machines that are capable of doing these types of things, and then it just really comes down to connected computing infrastructure.

Carolyn Coyne: And so bringing it back to sort of the basic science that you do, so the ideas that you're going to apply the next-gen sequencing to look at let's say immune cell populations and perhaps why my immune cells will or won't do what they're supposed to. And I guess that's sort of what I'm wondering. You mentioned before that obviously all of our cells have the same starting DNA, but maybe you could talk a little bit about what regulates that DNA maybe differentiated between different kinds of cell types. A T cell versus a B cell.

Amanda Poholek: Right. So I mean, I think this again comes back to the transcription factor conversation. There are specific populations of proteins that actually go to the DNA and sort of turn on or off regions of it, and what's really interesting to me is that the chromatin actually acts not in this linear fashion that we'd like to think about, right? But actually is this giant three dimensional map, and some of the folks how have done some sort of chromatin restructuring and assembly. When you see it, there's like individual balls that kind of... It's almost like a ball within a ball, and you can see all these individual regions sort of interacting together. So the cells have come up with some really clever ways to sort of take those proteins and use them in different sections of the DNA together. So you can really have efficient processing.

Something like 5% of your genome is actually the coding portion that drives those proteins. And the rest of it, which we used to call junk DNA, we now know as really not junk. It's the sort of directions. It's the non-coding region or the directions that tell all of those proteins that function on that chromatin to say, "This one on. This one off. Bind here. Don't bind here. Bring in this person. Don't bring in this person." And all that really leads to that kind of context dependent and cell type specificity that really drives systems.

So we have one transcription factor in our lab that we look at as a model transcription factor, and one of the reasons that we really like this one transcription factor is that it's not just expressed in the immune system. It's actually expressed in more than just the immune system. It's earliest role is actually in placental development. Something I know that you're really interested in, Carolyn. So if you don't have this particular transcription factor, the placenta forms, but it can't attach. And so that lead to embryonic lethality. It's really interesting, right? It's also really important for primordial germ cells specification, which is basically the cells that go onto drive the sperms and the eggs. So you can get sterility if it's not expressed in that cell type.

Brian Martin: One important protein.

Carolyn Coyne: Yes.

Amanda Poholek: Yes. Exactly.

Carolyn Coyne: It is.

Amanda Poholek: It was originally identified in B cells actually, which is another immune cell type, the one that makes all your antibodies. And it's considered the master regulator of antibody production. So if you don't have this one transcription factor in your B cells, then you can't make any antibody, and antibodies are critical component for all kinds of responses to infection. But it can also drive autoimmunity if expressed inappropriately.

In T cells, if you get rid of it just in T cells, we found that it tends to lead to multi-organ inflammation that's sort of systemic. And it's sort of considered to be sort of helping to control effector responses. But what we found really recently looking in an asthma model that's been really interesting to us is that it's actually responsible in T cells to actually promote asthma.

Carolyn Coyne: And this is Blimp-1, right?

Amanda Poholek: Yeah. The name of it is Blimp-1. I didn't want to get too-

Carolyn Coyne: That's fine.

Brian Martin: That's okay. We're happy to call it by name.

Carolyn Coyne: Okay. We can call it by name.

Amanda Poholek: So if you don't have Blimp-1 in T cells, it can actually promote asthma. So some new data in our lab that's still not published has really led us to realize that even in the T cell, when and where Blimp-1 is expressed can have different effects. Whether it's expressed early can maybe promote a type of cell that actually will drive a disease like asthma, but when it tends to be expressed more late in the T cell differentiation process, it actually drives cell death. And if you don't have it, you have too much effector responses, and you drive autoimmunity like colitis. And so now you're just talking about this one protein, Blimp-1, just in one cell type, T cells, that can still have context dependent functions depending on when and where and how it gets expressed, not to mention whether it's expressed in different cell types. So that's sort of our model transcription factor for understanding just globally how one protein can have such really interesting effects on different cells, cellular differentiation of function, and how inappropriate expression can really lead to disease.

Brian Martin: Impressive. Interconnected.

Carolyn Coyne: Well, this has been very educational. Thank you, Amanda.

Amanda Poholek: No-

Carolyn Coyne: Thank you for joining us.

Amanda Poholek: Absolutely. Glad to be here.