Marie: Hello and welcome to *The Parkinson's Research Podcast: New Discoveries in Neuroscience*. I'm your host, Dr. Marie McNeely, and I've partnered with The Michael J. Fox Foundation for Parkinson's Research to bring you to the forefront of the field of neuroscience to discuss the latest advances and discoveries with leading experts.

The Michael J. Fox Foundation created this podcast for researchers, clinicians, and industry professionals with the hope that these conversations and the resources that we share will advance your efforts and partnerships to improve brain health. We are welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

Today we are excited to welcome our guest, Dr. Malú Gámez Tansey. Listeners, Malú is the Norman and Susan Fixel Chair in Parkinson's Disease, Professor of Neuroscience and Neurology, and Director of the Parkinson's Foundation Research Center of Excellence at the University of Florida College of Medicine. She's also editor-in-chief of Nature Partner Journal *Parkinson's Disease*. Malú is working at the intersection of immunology, neuroscience, and genetics to better understand how inflammation and immune system responses impact brain health and may contribute to neurodegenerative diseases, including Parkinson's disease. So, we look forward to talking more about her research today. Malú, we are thrilled to have you here. Welcome to the show. How are you?

Malú: I'm wonderful. Thank you, Marie, for having me today.

Marie: Well, we are so excited to have you on the show and to learn more about you and the wonderful work that you're doing. So, Malú, can you start by telling us a little bit more about your background and perhaps how you ended up working in this particular area of research?

Malú: Sure. I grew up on the border in West Texas, went to elementary school in Mexico and then high school in El Paso, then decided I wanted to study biology after all the wonderful science fairs that I did in high school, thanks to an amazing biology teacher, Margaret Jackson, who is still with us. And she encouraged me to develop my love for science. So I went to Stanford, graduated with a bachelor's and a master's in biology and initially was thinking I wanted to go to med school.

But life takes interesting turns. And I ended up, after doing a year of technician work at UT Southwestern, I ended up applying to grad school. From there, I went to a postdoc at Wash U in Jean Johnson's lab. And it was an amazing time to be finding dopaminergic neuron trophic factors that could help the survival of these vulnerable populations. And that's how I got interested in Parkinson's and in neurological disorders, age-related neurodegeneration to be more specific.

Marie: Oh, very cool. And to provide our listeners with a little bit of background here, what have been some of the key pieces of evidence that you've seen in the field really supporting a link between inflammation or immune responses and neurodegeneration?

Malú: We knew for quite some time that at postmortem, in autopsy tissue, there were signs of inflammation in brains from individuals who had had Parkinson's. And at the time, we didn't know, couldn't tell, how early that inflammation and activation of immune cells had started.

And so, people assumed that it would be after the neurons had died. But in fact, what has happened in the last decade or so is that we've been able to image individuals with Parkinson's through positron emission tomography or PET using specific ligands that pick up inflammation. We now know that this inflammation starts early in the disease and it may not increase as a disease advances, but it stays at a sustained level. And that alone may be sufficient to drive progression. And finally, the genetics, several genes related to inflammation, such as LRRK2, progranulin, and HLA-DR variants have been associated with increased risk for Parkinson's. And that tells us that immune cells and non-neuronal cells may have a contribution to play in terms of your genetic predisposition for late-onset Parkinson's.

Marie: Absolutely. And perhaps to bring in another piece of the puzzle here, you don't only look at cells in the brain and inflammation in the brain, you're also looking at the gut. And I think this connection has been drawn in the literature previously about problems in the gut potentially being linked to problems in the brain. So, can you talk about the evidence in this area?

Malú: So, we are very interested in the connection between the brain and peripheral organs. One of the things that we recognize is that your immune system, in all tissues, is in charge of keeping the house clean and tidy, removing toxic proteins and debris. So, one of the ideas is that perhaps knowing that Parkinson's is a systemic disease, it doesn't just involve the brain, but that there are manifestations outside the brain, including gastrointestinal dysfunction.

One theory is that, for some people, the disease may start outside the brain, perhaps with aggregation and propagation of the culprit from Parkinson's, which is alpha-synuclein. And if that alpha-synuclein in the gut increases with triggers like inflammation and other bacterial exposures or pesticides, anything that will give inflammation, has been shown to increase the synuclein levels. That that could be a trigger for the misfolding and aggregation, which could then go from the gut into the nervous system, either through the anatomical connections with the vagus or simply through the circulation and across the blood-brain barrier. So,

the peripheral organs are definitely in communication with the brain, and it is still a testable hypothesis that it may begin outside the brain for some individuals.

Certainly the preclinical models in rodents and non-human primates support this idea, but it's still being tested. And the other piece of that is that because Parkinson's is a systemic disease, we feel that the immune system will respond to neurons in trouble. And because there's a communication between the brain and the outside, the peripheral immune systems in your blood, those cells are able to receive signals from the brain that come out into the circulation. And sometimes the cells that go into the brain can sense those signals. And our idea is that by focusing on immune responses in your blood cells, we might be able to pick out early clues about risk for individuals that have certain immune dysfunction, and that could lead to more inflammation, which would then increase the risk for neurodegeneration.

So, by looking at the peripheral immune cells in the blood, we hope to get clues about risk and our ability to stratify patients who have underlying inflammation, who may be the best candidates and more responsive to any intervention that could quench that inflammation, and therefore reduce their risk or progression for Parkinson's.

Marie: Oh, Malú, this is fascinating. And I'd love to dive into some of the details of the research that you're working on right now in the lab. So, can you tell us, I know there's a variety of projects that you're working on, but what are some of these specific questions that your lab is currently investigating at the edge or the frontier of this field?

Malú: One of the things that we recognize is that if the propagation of any misfolded protein from the peripheral organs, like the gut into the brain, if that does in fact occur, it's going to be very difficult to test that hypothesis by just looking at humans because we expect that that propagation will take decades. If you look at the data, the individuals with gastrointestinal dysfunction that have Parkinson's disease, they report that those GI disturbances started 10 to 20 years before they had their motor symptoms.

So, we suspect that, if indeed this is what happens, that the synuclein propagates from the gut into the brain, that it takes a long, long time in humans. So, what are the alternatives so that we can study the potential mechanisms that contribute to that? So that we can target them and perhaps develop more effective therapies? Well, one option is to look at non-human primates. That also takes a fair amount of time and is rather expensive. We could look at rodents and we do. And so some of these models, we use rodents, which have a shorter lifespan, and therefore the process takes a little bit shorter time.

Now, we've also chosen to look at flies. And *Drosophila* is a model organism where their lifespan is much shorter. So, it's 20-some days. And that way, we can still study the potential propagation of synuclein, or tau, or any other misfolded protein from the gut into the brain. And in this case, we also have been able to pick up some really interesting behaviors like disruption of the sleep wake cycle, which we know also happens in Parkinson's.

So, by combining a whole different kind of approach and different models, we hope to be able to not just cross-validate, but perhaps go a little faster by using models that have, you know, shorter lifespans, but where the anatomy is highly conserved and basically relevant to humans. We have some projects that specifically ask, what are the mechanisms by which the brain immune cells communicate with the blood immune cells? Does that communication break down as we get older?

And we have some evidence that it does. Other people in other research groups have shown that the microglia are likely sending signals to the peripheral immune cells. There are Th cells in the brain that are very vulnerable (the dopaminergic neurons) to inflammation. And so, one possibility is that as we become older and more inflamed, peripherally, that there's more inflammation sensed by the brain, and that compromises vulnerable populations like the dopamine neurons.

But the other thing that people have shown is that there are T cells, which are part of your adaptive immune system, in the gut that are really important under certain circumstances of producing substances like interleukins that act far away, not in the gut, but act on cells in the brain that have receptors for these proteins. And so, we're very interested in understanding how the brain communicates with the gut and other immune cells in the blood so that we can understand how that conversation or crosstalk breaks down as we're aging, as we become exposed to different things in our environment, perhaps lifestyle choices like diet, and repeated chronic infections, which tend to produce something called chronic antigenic load, which basically contributes to the accelerated aging of your immune system.

So, age is the largest risk factor for Parkinson's, as well as Alzheimer's. And the aging of the immune system is something that hasn't been really explored in terms of how it may contribute to these age-related diseases. And I think that's a missed opportunity. So, we are very interested in that.

And we have some evidence from a LRRK2 project looking at immune function of the monocytes and the peritoneal macrophages that are able to be induced or stimulated in the dish. And we're getting clues about how some of these mutations associated with increased risk for Parkinson's appear to change the immune system in a way that when the mouse is young, these mutations, which

have persisted in the population in humans for a long time, they give you an immunological advantage, almost as if you had a really good immune system when you're young.

But then when the mouse is old, it actually completely flips, and the immune cells become exhausted. And “immune exhaustion” is a term that has been used within the context of chronic infections. And T-cell exhaustion is something that's been studied in terms of being able to leverage that to combat infections and in cancer. But the immune exhaustion with regards to the innate immune system or the monocytes in myeloid cells hasn't really been studied that extensively and not within the context of neurodegeneration.

So, we're very excited that some of this new evidence relating to immune exhaustion in the innate immune system may be a really important process that happens during aging and also may be associated with some of these mutations in genes that are associated with increased risk for Parkinson's. If that turns out to be correct, then we might be in a position to leverage some of the drugs in the space where people are using it to reverse or mitigate immune exhaustion and be able to slow down the process that's mediated by that.

Marie: That's really interesting. And I know, Malú, you mentioned, in general, neurodegenerative diseases. You mentioned Parkinson's, of course, and Alzheimer's also. Are you seeing similarities between the processes that are occurring in different neurodegenerative diseases? Or are there specific differences maybe that you're seeing in Parkinson's that make it unique in this regard?

Malú: A lot of times we get asked, why do you study both? Well, we do believe that immune function and inflammation is one of the things that may be a unifying theme in the age-related neurodegenerative diseases and even in others that are not so late-onset like frontotemporal dementia, ALS. And we feel that the immune system, because the immune cells are sentinels of the body, that they should be able to give us clues about neurons in trouble, before they die. And the problem is that we've not been looking to the immune system for any clues. We've been looking for signs of neurons dying, like neurofilament light chain and degeneration peptides.

But I think that's too late. So, instead of looking for that, we should be looking for the immune response that's sounding the alarms, with the immune system being the first responders to neurons that are vulnerable and in trouble. So, what we find in these diseases when we look for potential immune biomarkers, they do appear to have some things in common. But I do think that the triggers that send you down one path versus the other are different enough that, while you do have some people that develop both PD and Alzheimer's pathology, the exposures

and the environmental piece, as well as the genetics for these, are different. So for instance, in Alzheimer's, you have other genetic risk factors like ApoE4, TREM2, CD33, and these are immune molecules that are associated with increased risk for Alzheimer's.

Now, interestingly enough, there's some crossover. Right? So, ApoE4, which is the greatest genetic risk factor for Alzheimer's, makes people more inflamed. So, if you're more inflamed, you are going to have a dysregulated immune system, and you'll be able to pick that out.

Now, why something like APOE4, which is in immune cells and astrocytes, would give you a risk for Alzheimer's versus Parkinson's is the $64 million question. But I do think that it's a combination of gene by environment interplay. So, you're born with a set of genetic risk factors, what I call the genetic dance card, and you then accumulate additional exposures as you go through life, including environmental exposures, pesticides, infections, you make choices about your lifestyle, exercise, etc.

And this interplay of your genetics with those environmental factors is what determines your overall lifetime risk for those diseases. So, you're not going to have a lot of crossover because the triggers are different and the genes that make microglia functional, or other immune cells functional, are not really shared that much. There's one gene progranulin that has actually been associated with frontotemporal dementia. There are some variants in progranulin associated with Alzheimer's and some variants associated with PD, but they're all different variants. So, how those selectively confer risk for one disease versus the other is something that we're very interested in. So, we're working on that piece of the puzzle.

When we run a study for biomarkers of inflammation, a lot of them are shared between these diseases, but others don't appear to be. So, some non-specific things like neurofilament light chain and synuclein, we know that those are going to be sometimes high also, or changed, in more than just parking sense. So, it's okay, you just can't use them for diagnostic purposes because they're overlapping, but you could still use them for progression markers across time and in studies.

Marie: That makes sense, Malú. And you touched on this a little bit, but what methods or approaches are you using to start to answer some of these questions?

Malú: So, we are not a lab that is known for a single technique. We really pride ourselves in using whatever method and approach is the most appropriate to answer the question. And to be honest, I've been fortunate enough to mentor

incredibly smart people who get interested in a technique or a question. And they go in that direction, and they dive in, and then they bring it to the lab.

And so, we use immunological approaches like flow cytometry, we use immunohistology, molecular biology, delivery of certain genes through AAV (adeno-associated virus) or lentivirus. We use cell culture, we use rodents, we use a lot of clinical samples and biofluids because it's important to really understand the human disease first. And to understand the biology of what's going on there, so that you can more appropriately model those features in any other non-human system that you want like the mouse, or the monkey, or the fly. And so, using cell culture and organotypic slices, as well as mice, and flies, and obviously human samples, we are very interested in the heterogeneity of the system. For instance, not all microglia are the same, they vary between different regions.

We care about the gut-brain access, as you mentioned. We want to know, what part of the gut is it associated with, inflammation, synuclein aggregation. And so, we've gotten into a lot of single-cell techniques these last few years, especially those that give you the resolution in terms of spatial geography in that tissue. So, single cell spatial proteomics and transcriptomics, I think are going to be really important for the field so that we can understand what cell types need to be targeted so that you don't have collateral damage by targeting an entire set of cells that you shouldn't be touching. And that's an important concept because, just like the cancer field progressed from radiating an entire body or chemotherapy for things that you're going to damage more than just the cancer cells, we're going to have to do the same thing for neurological disorders, especially in terms of immunity and inflammation. Because you don't want to do anything that's going to compromise the function of the immune system. You don't want to immunosuppress the individuals, especially those that are older.

Marie: Absolutely. And I think this work that you're doing is tremendously important. So, can you talk about the potential impacts of your research?

Malú: I think the impact that I hope our research will have, and it's been a long road of 20 years when initially there was very little interest in inflammation and immunity in Parkinson's or really any neurodegenerative disease. The field has moved from being very neuron-centric to recognizing, as one of my colleagues likes to say, that the neighborhood matters. That the glia and the immune cells in and around neurons are so critical for support of the vulnerable neuronal populations.

And I hope that our work will do two things. One is, give us clues about how that aging or dysfunction of the immune system, who I consider the caretakers, the parents, if you will, of the dependent children, the neurons, how they change with age, how they change with environmental exposures and lifestyle choices. And

eventually, they need to be rehabbed, or rebooted, or rejuvenated in some way to expand the amount of time that they're able to actually take care of those neurons. Because I suspect that neurological disorders and neurodegeneration in specific, especially that related to aging, is a combination of neurons getting older and becoming dysfunctional, but the caretakers also becoming older and dysfunctional. So, it may be easier to replace and rejuvenate the caretakers than to replace the neurons, because those have formed circuits, right? They were not going to be able to reestablish once they become damaged.

So, I hope that our research will give us clues about how the aging and dysfunction of the immune system increases risk. I hope that our work will have the impact to be able to look to the immune system for clues that neurons are in trouble. And this includes any peripheral immune cells that are in communication with the brain immune cells and cells that are coming in and out of the brain, carrying signals and manifesting a dysfunction in the blood. And in terms of therapeutics, I would hope that once we understand the process by which the immune cells are giving us clues, that we'll be able to target those pathways and be able to come up with better, more efficient immunomodulatory therapies to reduce risk and progression and either delay or prevent these diseases.

I think that it will be hopefully within my lifetime, because we're very, very close. And we are paying very close attention to the cancer field and the autoimmune disease field, because they really have broken the code for how to leverage the awesome power of the immune system to be able to fight disease.

Marie: Definitely. And I know you've had some really cool papers come out recently. So, Malú, could you share some of just the latest findings that your lab has, or perhaps results that you're just the most excited about at the moment?

Malú: Yeah, we've had some really excellent work within the space of communication between the central and peripheral immune compartments using single cell transcriptomics in very elegant, but simple experiments that some of my team members have done. We have come to appreciate something that was suspected a while back, which is that if you give a peripheral inflammatory stimulus through something like endotoxin or like a polysaccharide, that the brain is going to sense that even though that endotoxin does not cross into the brain.

And part of that is signal that is mediated through circulating inflammatory factors called cytokines and chemokines. And for a long time, people did not think that there was any real communication, that cells from the peripheral blood could enter the brain. The brain was considered immune privileged, right? Well, we believe the brain is not immune privileged, it's immune specialized, which means that there is communication across the blood brain barrier, and some cells can come in, but they need a password.

As that blood brain barrier breaks down, there might be more leakiness. And the same happens in the gut, we believe, and we're actively pursuing that line of research. And so, what we did is we asked the question, can you look at single cell gene expression in the brain and in the blood in different parts of the brain? Specifically looking at, say, the microglial activation in the microglial gene expression, and do the same for cells in the blood, like the monocytes that are circulating, and answer the question as to whether the microglia are communicating with those peripheral cells.

And the way you can do this is because if someone is expressing a ligand, a protein that's going to be made by one cell and then act wherever its receptor is, you can look at ligand receptor pairs and infer cell-to-cell communication. So, what was fascinating about some of these studies is that when you have no peripheral inflammation in the system, the microglia appear to be communicating with each other a lot. So you find ligand and receptors in microglial populations in the brain. But as soon as you introduce peripheral inflammation or endotoxin, now the receptor expression in microglia and in the periphery changes such that it appears that microglia are now sending more signals to cells that are outside the brain.

So, that communication between brain cells themselves and brain versus outside brain is changing when you have inflammation on board. And we think the implications of this are really important because we know that a short duration, acute inflammatory signal is probably not going to be bad for your brain because it resolves. But if you have constant peripheral inflammation, such as some of the comorbidities that we have like obesity and metabolic syndrome and a lot of other autoimmune conditions which increase inflammation in your body, then that chronic inflammation is going to dysregulate this conversation between the brain and the periphery such that the cells themselves become sick.

And then that message, that crosstalk, becomes really garbled. And that's, I think, an opportunity for pathology. So, that's one study that we're very excited about because it gives us direct evidence that the gene expression changes at the single cell level to now microglia sending signals outside the brain. What they're saying, we're not exactly sure, but they could either be calling cells in, or they could be sending signals that the outside immune cells decide, okay, microglia need help, here we come. That's an exciting finding.

Some of the other things that we've been involved with is the idea that colitis or gut inflammation may affect the brain, and we want to know how that happens. So, we're doing some studies that develop a model of colitis in the mouse and testing under what circumstances, and under what genetic predisposition

conditions, does a colitis stimulus affect the brain in terms of nigrostriatal dopaminergic neuron function or inflammation gene networks.

And what we find, which is really interesting, is that when you have chronic gut inflammation, you end up dysregulating gene networks in the brain that have to do with immune function, oxidative stress, protein misfolding, and myelination. And the link to myelination through inflammation hasn't really been that well-studied. But if you remember, the pathways that degenerate most in Parkinson's are those that are poorly myelinated. So, if gut inflammation is disproportionately affecting myelination, then that could set up an association between inflammation and the demise of dopaminergic nigrostriatal pathway, which tends to be poorly myelinated.

Marie: That's really interesting. And Malú, how do you, I guess, see these findings then moving the field forward for Parkinson's disease research?

Malú: The way we see them moving forward, and we are very actively working to get this other paper out, is to try to understand how some of the associations between, say, risk for inflammatory bowel disease and Parkinson's, whether there's anything to them, right? What is the connection between people with IBD having increased risk for Parkinson's and some of the genes in LRRK2 and variants in those genes give you increased risk or decreased risk for both of those diseases?

We are very interested in understanding, is there anything in the molecular signature, if you will, in the colonic biopsies or gut biopsies of people with IBD that look like those in Parkinson's? And so, do we see any patterns that are emerging in people with IBD that are still represented or are also represented in people with Parkinson's (always relative to control)? And the interesting thing to me is that we think that that connection between IBD and Parkinson's has to do with the timing issue, right?

So, few studies have connected IBD with increased risk for Parkinson's. And so, people are like, oh, well, that makes sense. Gut inflammation, synuclein, and propagation. It could or could not be synuclein-mediated. But one thing that is interesting is that if you think about people with IBD, they get that early in life. This is not an age-associated condition. And what ends up happening is that a lot of them are treated with anti-inflammatory drugs, anti-TNF drugs, Enbrel, Remicade, to get the inflammation under control. Well, it turns out that in another set of patients with IBD who have been treated with anti-inflammatory, specifically anti-TNF drugs, have now a 78% decrease in incidence of Parkinson's.

So that's kind of huge, right? And it suggests that it's the inflammation early in life that lasts a long time. And that may be what's giving you the increased risk for

Parkinson's. But if you quench it, if you target it, because you have another disease, then you're helping out your brain.

The interesting thing about that is that those drugs have very little brain penetrance. So, how is an anti-inflammatory that has no brain penetrance protecting you against Parkinson's disease? Well, number one, we now know that Parkinson's is not just a brain disease, it's a systemic disease. And these drugs may be acting peripherally to lower inflammation there and keep that conversation between the periphery and the brain more functional.

The other thing that we know is that people with these diseases, because they have inflammation, they may have a leakier gut barrier and a leakier blood brain barrier. And if that's true, then their own disease is helping those drugs that they're taking for that disease get into the brain. And that may be another possibility as to how these drugs, that are mostly supposed to be acting peripherally, could potentially be protecting the brain.

Marie: Well, I think this is all very fascinating work. And I guess, you hinted that there might be some surprises or some unexpected results that you're seeing in the work that you're doing. What have been some of the biggest surprises for you, Malú?

Malú: Well, some of the biggest surprises have been that the mutations that appear to be associated with an increase for familial Parkinson's through LRRK2, for instance, depending on the immunological or environmental stress that you expose the mice to, they don't always seem to be at a disadvantage, right?

Especially the young mice. That may explain why these LRRK2, D219S, and 1441 mutations have persisted in the human population, rather than been selected out. Because we're not supposed to live this long, but when you're young, they give you an immunological advantage. Now, if your cells become immune exhausted as a result of having this incredibly good young immune system, that may be one reason why you now have increased risk for Parkinson's as an adult.

What's really exciting though is that those mutations, D219S, don't have full penetrance, which means if you have that mutation, it doesn't necessarily mean you're going to get Parkinson's disease, which is the good news. Because it says that the environmental, gene by environment piece, the environmental piece, is entirely up to you. It could be modified by diet. It could be modified by exercise. It could be modified by fewer exposures. So, it's not really a Parkinson's sentence if the penetrance of the mutations can be modified by environment. And so, that has been a surprising thing to me that we expected any organism with these mutations to be disadvantaged from the get-go. And that did not happen. It's

actually that you're advantaged immunologically early, and then things switch when you're older.

The other surprise perhaps shouldn't be that surprise, but has been the sex differences that we see between males and females in terms of inflammatory responses, in terms of how you resolve inflammation, in terms of how much inflammation you can withstand. And that may give us clues about why Parkinson's seems to perhaps affect more males than females, and why Alzheimer's affects more females than males. Perhaps it's not inflammation, but we do now know that it's very important to look at both sexes when you are doing animal studies. And when you're looking at humans, you need to make sure that you sex-match and age-match as much as possible. And to first assume there are no sex differences, but then to analyze your data looking at potential sex differences, because it makes evolutionary sense to me that males and females are wired differently for dealing with stress and dealing with inflammatory and immunological insults.

Marie: Absolutely. And I think this just perfectly illustrates the multiple layers for Parkinson's disease and Parkinson's disease research. I mean, we talked about how you're looking at kind of the immune system side of things, of course, the brain side of things. And now thinking or perhaps starting to think about the hormonal or endocrinology or some of these sex differences that might also be contributing as well.

Malú: Yeah, absolutely. There have been some really fascinating studies by other groups working in spinal cord injury and pain, showing that microglia in males versus females are wired very differently in terms of how their purinergic, or ATP-sensing, signaling goes. And that's fascinating to me because it really suggests that in order to manage pain better, perhaps because of childbirth and other things, the females have to be wired a little differently. That's fascinating. And we don't really know how that necessarily predisposes you to age-dependent neurological disorders, but I'm pretty sure it's not irrelevant.

Marie: Absolutely. And I guess thinking forward towards the future, Malú, what are some of the next steps for this line of work for you?

Malú: The next steps are really to continue to understand the biology of the gut-brain-blood axis, to understand the functional relationship between these organs, the metabolites that are being made by the brain cells, the blood cells. And then, of course, in the gut, the bacteria, the virus, the fungi that are there

Because of this active axis of communication, if we understand the functional relationships between them, who's making what and for what purpose, we will then be able to understand how things in this axis decay or go awry with aging or

with exposures, too many birthdays, that kind of thing. But also, because once we do that, then I'm absolutely confident that we will be able to find new targets for intervention, for rejuvenation of the system to delay or extend people's health span. Because nobody wants a long lifespan. You want a long health span. And so that's, I think, the goal to make sure that we understand how it's changing and how to be able to target those changes to be able to have better quality of life.

Marie: Oh, Malú, that is so exciting. And I think a perfect segue for my next question here, which is taking a step back and looking at the big picture. How is your work bringing us closer to that ultimate goal of finding a cure for Parkinson's or contributing to improving the therapies for people who are living with Parkinson's today?

Malú: I'd like to say that what we believe is important is to be able to update and revise your thinking as new data emerges. It's important to be able to learn from the discoveries that we have. And learning really is adapting to change. And what's been really good in the last 10 years or so is that the field of neurodegeneration has finally come to appreciate that the immune system and inflammation is not just a byproduct of dead neurons, because the way you think of a disease is what gives you ideas on how to prevent it or treat it.

So, if everybody's just focused on the neuron, we're never going to be able to take advantage of what else could we help in the system to prevent these diseases. And so it's been huge that people have now come to appreciate 20 years after we started and really paid attention and revised their thinking. Because I think that's been very important for both fields of Parkinson's and Alzheimer's, especially as we look beyond the misfolded protein problem. Right?

If you're going to try to dissolve synuclein aggregates or amyloid plaques, you have to think about how that comes about. And the way that comes about is through activation of the immune system with immunotherapies. So, even that, the only thing we have today that could potentially slow down progression, modestly, but clearly, right? The immunotherapy depends on the immune system.

And so, it's time to think about how else can we boost and rejuvenate and reboot that immune system once you are at a point where time has passed. I think a lot can be done when you're young. So, the message is good diet, good exercise, lots of sleep. And if you can start to do that in your 20s, then it's great because the inflammaging process really accelerates after age 30. So, if you can be proactive, that's great.

But for those who are beyond that, I think we need to find other ways to slow down and intervene. It's a very exciting time because finally, neuroimmunology, it's sort of like the next frontier, I think.

Marie: Oh, certainly. And Malú, I love that your work is really helping or encouraging perhaps the field to develop this comprehensive understanding and really take into account this whole complicated biological context and not kind of get tunnel vision and just focused on the neurons because I think it is really easy to do in science when you're trying to tease apart just one specific question. So, can you talk a little bit more about how your work has perhaps benefited from some of the tools, resources, collaborations, or even prior work that's out there, particularly anything funded by The Michael J. Fox Foundation?

Malú: I mentioned that we've been at this for 20-some years now. And to be honest, when you asked me about the trajectory, I left out the part about me being in biotech after my postdoc. And when I was in biotech is when I learned about inflammation. And it was kind of a surprise to me because I wasn't that interested in immunology when I was a graduate student, despite the fact that I had lots of friends in immunology.

But I ended up with this project in biotech that has to do with the next generation TNF inhibitors. And of course, now I'm forced to learn about the system, and I became fascinated with it. And as we were finishing our studies, and we were fortunate enough to be able to publish them in *Science* in 2003, I recognized that at the time, this was 2002, that there was a lot of literature coming out about inflammation and neurodegenerative disease.

And of course, the idea was neurons died, you get inflammation because the phagocytes, the microglia show up to clean up the mess. But I had a different thought about that. And I thought that maybe it wasn't that simple. So, when I made the decision to go back to academia, after being in the biotech sector for a couple of years, I thought that it would be a really good opportunity to take these research tools at the time, which were really meant to study peripheral inflammation, to put them in the brain.

And of course, the company that I was with thought I was a little crazy and said, you want to do what? Put them in the brain? Why? Right. And so, I thought, well, you know, there's a lot of evidence coming out. The McGeers had done some work, and it was clear that something was going on in the brain in terms of inflammation. And I thought, we could be wrong, but at least it's a testable hypothesis.

So, they allowed me to take these research tools with a material transfer agreement. And I started working on the role of inflammation in Parkinson's when I set up my lab, and nobody wanted to fund any of that. Like the NIH was like, what are you talking about? And The Michael J. Fox [Foundation], kind of out of nowhere, came up with this RFA, or request for application, called the role of

inflammation in Parkinson's disease. And I thought, wow, isn't that just perfect timing?

So, we submitted our first grant and got our first grant 20 years ago, exactly, from The Michael J. Fox Foundation to study how inflammation, driven by soluble TNF, played a role in the death of dopaminergic neurons in two different preclinical models of Parkinson's-like degeneration. And that's sort of the beginning of the whole story.

Without that support, we honestly would not be here because they were innovative enough and willing to take a risk on what seemed like a crazy idea at the time. But now, I think it's clear that it's an important process that may be leveraged to reduce risk, to intervene, and immunomodulate. So, we have incredible gratitude to The Michael J. Fox Foundation and all the reviewers who saw potential in this line of work. From there, we were able to get other grants. I think we've received a lot of support from The Michael J. Fox [Foundation] throughout the years.

But we were able to then de-risk the question and be able to get NIH funding and of course funding from other foundations like Parkinson's Foundation. And then we've merged into Alzheimer's, and then FTD, and we did a little bit of depression, and ALS because inflammation appears to be at the center of a lot of things that go bad.

Marie: Absolutely. And Malú, I love this story. I think it really emphasizes the fact that The Michael J. Fox Foundation is really dedicated to considering all good ideas and all good approaches to really tackle this problem from a lot of different directions. And I think your work specifically is a tremendously perfect fit for that RFA. So, well-timed there.

Malú: It was. I can't tell you how important it has been, The Michael J. Fox Foundation, for a lot of early-stage investigators, for people who have innovative ideas, but again, are either nobody in the field, or they're just too early and they don't have enough of a track record.

So, without the support of The Michael J. Fox Foundation, we would not have as many brilliant minds as we have now. And a lot of them working on inflammation and immune system. So, they really blazed the trail for cutting edge, high-risk, high-reward. And I think it's absolutely paying off.

Marie: That's wonderful. And I guess looking broadly across the field, are there any advances in neuroscience or perhaps other fields that you think need to happen in order to really accelerate your research or the field as a whole?

Malú: I do think that the multidisciplinary and diverse interactions of team science are very important for the next stage. And I think The Michael J. Fox Foundation, along with the Aligning Science Across Parkinson's, or ASAP, have been really at the forefront of this, which means partnerships between academics, private sector, the government, NIH. It will take a huge village to get to where we need to be. And I think The Michael J. Fox Foundation and other foundations that recognize the incredible importance and value of talking to people outside of your immediate surrounding. That's how we're going to get to solving these problems.

And we need more team science. We need more open science. We don't need people to hold on to their data for years. We need access to the data sets so that you can basically start a revolution where people can see things in your data that maybe you haven't. And that's how we're going to get there with a lot more diverse teams and more open science.

Marie: Absolutely. I think the future is bright for Parkinson's disease research. And like you said, it's an exciting time to be working in the field. But what do you see, Malú, as one of the most important unanswered questions to be worked on in Parkinson's neuroscience today?

Malú: I think we have several important unanswered questions. And one of them is if this disease starts outside the brain, how can we think about developing biomarkers of early detection, instead of just looking where we know the disease is going to end up? Right? So, we need to look outside the brain in peripheral organs, in skin, in gut, quite possibly in olfactory epithelium, because I do think that we need to look not just where the light is.

And it's fine that everybody started studying just the dopaminergic neurons because that's an easy thing to model. And it's a very obvious pathological finding. But it's almost like looking through the ashes of a fire to decide how did the fire start? And so, I think we need to take a step back and look for early biomarkers that can help us diagnose risk and delay or halt progression.

So that's, I think, one of the most critical things for me and challenges in the field now. I also think that there is some controversy as to whether synuclein pathology is absolutely necessary for the degeneration of the vulnerable populations in the brain. And I think that the data related to LRRK2 Parkinson’s suggests that maybe it's not required. A lot of those individuals, when you look in their brains, do not have synuclein pathology. Some do.

So, I think a major challenge for us is to establish, if it's not synuclein, then what else is it? And of course, my cognitive bias is that LRRK2, as a regulator of inflammation and immune responses, it may have to do more with the direct effects of inflammation on those populations, like dopaminergic neurons, which

are extremely sensitive to things like soluble TNF when they're oxidatively stressed. You don't have to have over expression of synuclein or too much synuclein in those neurons to kill them if they're oxidatively stressed and there's a little bit of inflammation around. So, I suspect that understanding the difference between synuclein requirement or not, is it really the only culprit, is going to be a second major challenge for us?

Marie: Definitely. Well, Malú, you've given us a lot to think about today, and we truly appreciate you sharing your expertise and your insights. So, thank you so much for joining us on the show today.

Malú: I really appreciate your time, and I encourage everybody to become more knowledgeable of how you can help the field find an answer. One of the most important things is enrolling in a clinical trial, wherever you are.

We need a lot of healthy controls, and if your family or friends are afflicted with any of these diseases, especially Parkinson's, you can be part of the solution if you contribute your time. So, thank you.

Marie: Absolutely. I couldn't agree more. Well, Malu, thank you again so much for joining us and listeners, it's been great to have you here with us as well. If you want to know how The Michael J. Fox Foundation can help your research, please visit michaeljfox.org/researchresources.

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