What is Tolerization?

You can listen to the audio of this podcast at: youtu.be/PtYzqrNi6pg

[00:00:02] **Intro:** "ABCs of NMOSD" is an education podcast series to share knowledge about neuromyelitis optica spectrum disorder or NMOSD. A rare, relapsing autoimmune disorder that preferentially causes inflammation in the optic nerves and spinal cord. "ABCs of NMOSD" podcast series is hosted by SRNA, the Siegel Rare Neuroimmune Association, and in collaboration with the Sumaira Foundation for NMO and Guthy-Jackson Charitable Foundation. This education series is made possible through a patient education grant from Horizon Therapeutics.

[00:00:59] **Krissy Dilger:** Hello everyone and welcome to the "ABCs of NMOSD" podcast series. Today's podcast is titled "What is Tolerization?" "ABCs of NMOSD" is an education podcast series to share knowledge about neuromyelitis optica spectrum disorder. My name is Krissy Dilger, and I will be moderating this podcast. This podcast series is hosted by the Siegel Rare Neuroimmune Association, in collaboration with The Sumaira Foundation for NMO and The Guthy-Jackson Charitable Foundation. This podcast is being recorded and will be made available on the SRNA website and for download.

[00:01:39] "ABCs of NMOSD" is made possible through a patient education grant from Horizon Therapeutics. Horizon is focused on the discovery, development, and commercialization of medicines that address critical needs for people impacted by rare, autoimmune, and severe inflammatory diseases. They apply scientific expertise and courage to bring clinically meaningful therapies to patients. Horizon believes science and compassion must work together to transform lives.

[00:02:10] For today's podcast we are pleased to be joined by Drs. Terry Smith and Michael Yeaman. Dr. Terry J. Smith, the Frederick G.L. Huetwell Professor Emeritus in Ophthalmology and Visual Sciences at the University of Michigan, is an internationally known endocrinologist who has studied Graves' disease, its eye manifestations, and related autoimmune disease for over 20 years.

[00:02:35] Dr. Smith received his medical degree from the University of Missouri School of Medicine and completed his residency at the University of Illinois in Chicago and Sinai Hospital in Baltimore. He has completed fellowships in biophysics at the University of California School of Medicine, San Francisco, in molecular biochemistry at Columbia University in New York, and clinical endocrinology at the Pritzker School of Medicine, University of Chicago.

[00:03:04] Dr. Smith is the author of over 275 articles and book chapters and has been awarded five patents for his research discoveries. He has been elected to the Orbit Society, is chief scientific officer for the National Graves' Foundation, and serves as reviewer for numerous scientific journals. Dr. Smith has been funded continuously by the National Institutes of Health and the Veterans Administration since 1983.

[00:03:31] Dr. Michael Yeaman is Professor of Medicine, David Geffen School of Medicine at UCLA, Chief Division of Molecular Medicine, and Vice Chair, Department of Medicine, Harbor-UCLA Medical Center. He

is also the Director of the Institute for Infection & Immunity, Lundquist Institute for Biomedical Innovation. He joined the faculty of the UCLA School of Medicine in 1992, after graduating from the University of New Mexico School of Medicine, and completing NIH and AHA Fellowships. Dr. Yeaman serves as Chair Medical Advisor of the Guthy-Jackson Charitable Foundation.

[00:04:10] Along with his research on NMOSD and related autoimmune diseases, he has pioneered design and development of novel anti-infective agents and vaccines for antibiotic-resistant infections. Dr. Yeaman has published over 250 papers and serves on the editorial boards of premier journals. He holds more than 50 issued U.S. patents and has received notable honors including the NIH Innovation Award, the Weitzman Memorial Research Award, the Alexander Research Award, and the National Research Service Award.

[00:04:44] On a parallel note, Michael is a composer and performer with original works in films and documentaries. His music has been acclaimed by venues including the Los Angeles Times. He has a special interest in applying music and art to medicine, focusing on therapeutic neuroplasticity. His music is available on iTunes®, Spotify®, Amazon® and other streaming services, and expressed in the Pandora® Music Genome.

[00:05:11] Thank you both for joining us. If you want to just get us started by introducing yourselves.

[00:05:18] **Dr. Michael Yeaman:** Hi Krissy. My name is Dr. Michael Yeaman. I'm a Professor of Medicine at the UCLA School of Medicine in Los Angeles.

[00:05:28] **Dr. Terry Smith:** I'm Terry Smith, Endocrinologist at the University of Michigan.

[00:05:38] **Dr. Michael Yeaman:** Well, we really appreciate SRNA's invitation to talk about tolerization. We've called this discussion "Tolerization 101" because the immune system is a very complex and, in some ways, mysterious aspect of the body. But just as sometimes the immune system can make mistakes, we also think that those mistakes can be corrected and that is called tolerization or re-tolerization and hopefully those kinds of corrections can be applied to cures for NMOSD and MOGAD and that's what we'll be talking about today. Why don't we move into this? The first thing of course, we always like to make sure people know about our disclosures. I do receive funding from the National Institutes of Health, the Department of Defense, and I am an advisor for the Guthy-Jackson Charitable Foundation.

[00:06:40] **Dr. Terry Smith:** I as well have been an NIH funded investigator for multiple decades and also serve as advisor to the Guthy-Jackson Charitable Foundation.

[00:06:58] **Dr. Michael Yeaman:** We should also point out that we have both been either speakers or consultant advisers to Alexian, Genentech, and Horizon. With that as our background, we wanted to really focus in on four specific objectives today and we want to try to do this in a way that's as relatable as possible. First a quick refresher on the basics of immune detection and protection and then we'll move right into how is immune tolerance established. What is immune tolerance and how does it come to exist? Then we'll move on to how is immune tolerance lost? Because loss of immune tolerance is the basic problem with autoimmune disease. Then most excitingly we'll focus on how can immune tolerance be restored. We want to make sure everybody understands that while there is a lot of information that will convey today, we can't possibly cover everything and a lot of the complexities of the immune system we'll just have to summarize for today's discussion, so please bear with us in that respect.

[00:08:20] **Dr. Terry Smith:** I would just add that this quest for understanding how to restore immune tolerance is aspirational. This is really shared by not only patients and their providers with NMO, but all auto immune diseases.

[00:08:50] **Dr. Michael Yeaman:** It's a great point Terry in some ways if we can solve the problem of immune tolerance in NMO and MOGAD it could open doors to solving auto immunity in many different conditions. Let's move on and we'll now begin to dig into the basics of immune detection and protection in a way that really starts to talk a little bit about what the fundamentals are of the immune system and how they might be understood. Let me just start with a quick reminder that there are two basic dimensions of the immune system that protect us against illness and cancer and other issues. One of the dimensions is called innate immunity and this is the immune capability that can act very quickly and without having to learn from experience. The other dimension is adaptive immunity, and this is the immune system that learns from experience and remembers what it learns. Both are involved in immune tolerance, but much of what we'll be talking about today has to do with adaptive immunity.

[00:10:08] **Dr. Terry Smith:** Many of you are probably asking why did nature create two distinct arms of immune response? The answer is that these two arms were actually evolved at very different times. Therefore, once you think of the immune system as not a static entity, but one that continues to evolve as the human race evolves.

[00:10:48] **Dr. Michael Yeaman:** Theoretic I would even take that one step further and just remind everyone that the immune system learns throughout our lives. So, what are immune system knows today is not identical to what it will see and know and remember tomorrow and all of this is comes back to immune tolerance. But let's just think now about what are the main cellular drivers of the immune response. We want to focus on those that people might recognize from their names and also really get into some of their functions as they relate to immune tolerance.

[00:11:24] The three cells that we're going to talk mostly about today are the cells that drive immune response. Sometimes people call these the regulators or the governors of immune response. They include antigen presenting cells, these are often abbreviated as APC, T Cells or T Lymphocytes and B Cells or B Lymphocytes. With those three cells in mind, we wanted to just go quickly through a refresher of how they talk to one another and work in ways that protect us from threats.

[00:12:05] There's really three steps in this regard, the first is what we call antigen presentation. In this step the antigen presenting cells which are constantly looking throughout the body for possible threats or abnormal cells, or even infectious microbes will detect an antigen, they'll process it and then display it in a way that puts pieces of this antigen on their surface. This activates the energy in presenting cell so that it can then talk to the naive T cell or B cell in a way that requires two signals. One is the signal from the antigen presenting cell to the T cell or B cell and the other is the counter response from the T cell or B cell to the antigen presenting cell and we call this the molecular handshake.

[00:13:06] **Dr. Terry Smith:** One should glean from this slide and those that follow the notion that there is a dynamic interplay between very different cells, but cells that are interdependent and one cannot function properly without the rest of the gang.

[00:13:33] **Dr. Michael Yeaman:** Well said buddy. The step two in this regard after T cell activation is B cell activation. Here the activated T cell that has been told by the antigen presenting cell about an antigen interacts with a B cell in a very similar way that requires at least two signals, and we call this the molecular handshake between the T cell and B cell. But it also involves cytokines that are generated by the T cell that further tell the B cell how to react, not just whether to react.

[00:14:16] **Dr. Terry Smith:** What are cytokines anyway? Michael. I think I can offer at least a brief description. These are usually proteins, they're relatively small and they're produced by all of the cells that have been

mentioned thus far and they get released and they stir up the neighborhood. Some of the effects of these cytokines are very specific and others are quite broad stroke actions and like everything else in nature, they are associated with both good and bad consequences.

[00:15:11] **Dr. Michael Yeaman:** The basic name for cytokines derives actually from Greek origins cyto meaning cell and kine meaning to take action. These are small proteins that cause cells to take action. Now, once a B cell is conditioned by signaling through the T cell interaction and by cytokines it undergoes activation so that it matures and does something called class switching where it changes its subclass type from IGM which is the default mode to other types of antibodies like IgG or IgE or IgA for example.

[00:15:54] **Dr. Terry Smith:** This is not junior high when we switch classes at the belt. Right, Michael?

[00:16:02] **Dr. Michael Yeaman:** That's right. We're talking about changing types of antibodies, because different kinds of antibody can have different effects and we'll talk more about that as we go. The third step really that's involved in immune response to an antigen is referred to generally as the process of inflammation. The cells that are involved in the inflammatory response are several. They include what are called granulocytes and those include neutrophils and eosinophils. One thing that's interesting about NMO and MOGAD is that granulocytes can accumulate within the central nervous system at sites of damage that we see in patients who are having onset disease or relapses. There are other cells that contribute to inflammation that include macrophages and natural killer cells as well as a special type of T cell called a CD8-positive T cell.

[00:17:03] **Dr. Terry Smith:** All of these cells are present at all times, both under states of health when we feel good and in states where something's going wrong. It's the number of these cells and whether they're turned on or turned off and the context, the gang they're hanging out with that really determines whether these cells are our friends or foes.

[00:17:43] **Dr. Michael Yeaman:** That's where we're going next, because the main point about immune tolerance is whether all these cells can tell the difference between normal healthy tissues and cells and molecules in our bodies or whether they make mistakes and misrecognize these types of cells and tissues. Let's move right into that.

[00:18:08] Let's talk now about how is immune tolerance established? This is one of the most mysterious and as I call it, miraculous aspects of the immune system that really, I think deserves a few minutes to think through with you. First, what is immune tolerance? In a nutshell immune tolerance is how your immune system protects you against internal or external threats such as cancer or infection without harming any of the healthy molecule cells or tissues that make up the body. Put another way immune tolerance is the system of checks and balances that prevents the immune system from targeting healthy cells and tissues.

[00:18:58] **Dr. Terry Smith:** Bottom line, the immune system walks a perpetual tightrope and it's like the Goldilocks paradigm, a little too hot a little too cold, just right.

[00:19:16] **Dr. Michael Yeaman:** We want it to be just right. Exactly. Not just right in terms of amount of response, but also to which targets it is responding and that's where we really get into the key point of tolerance. Tolerance requires the immune system to tell the difference between self and non-self. In terms of self the immune system wants to tolerate healthy molecules, healthy cells, healthy tissues, normal beneficial wound healing, normal growth, and development, for example. On the other hand, it does not want to tolerate pathogenic microbes, pre-cancerous cells, cancers, tumors, abnormal wound healing or abnormal growth and development. We want the immune system to leave healthy things alone but go after things that are not healthy and that's the basic self, versus nonself concept.

[00:20:23] **Dr. Terry Smith:** As I'm sure all of the audience are thinking, well aren't there a lot of mistakes made? This is really complicated. I don't know about you, but when I enter a large room with many people sometimes there's some mistaken identity and this can lead to embarrassment, but thankfully the immune system has a number of checks and balances that make sure that the embarrassment doesn't lead to a fiasco.

[00:21:08] **Dr. Michael Yeaman:** Or to harm. That's really what we're going to talk about next. The key cells for toleration are antigen presenting cells, T cells and B cells and we'll talk about that again as we go through this. What we want to talk about next is how the T cells and B cells really come to know the difference between self and non-self. I like to think of the finest as the T cell university. What is really one of the more interesting and fascinating aspects of the immune system is that most of the immune system cells that we are talking about today are born in the bone marrow. Your immune system comes from your bone marrow and that includes T cells and B cells, white cells, granulocytes, and the other cells that we've been talking about. Well in ways that are both mysterious and amazing, T cells find their way to a tissue that is called the thymus and it's wrapped around your esophagus just above your heart and it contains several different compartments. Inside of the thymus is where T cells go, and they are tested in three ways that we'll talk about in just a moment.

[00:22:36] Likewise, B cells are made in the bone marrow, and they find their way largely to the spleen, so we think of the spleen as the B cell University. Both get their elementary school education in the bone marrow, but T cells go to the thymus, B cells go to the spleen and like the thymus the spleen has several compartments wherein B cells undergo very specific tests to see if they are tolerant. Let's just talk a little bit more about that process now.

[00:23:15] T cells and B cells must pass three exams. T cells in the thymus, B cells in the spleen if they are to be allowed to play a role in the immune system. The first test is can they recognize an antigen presenting cell. If yes, they move on, if no they are deleted. The second test is can they do the molecular handshake with the antigen presenting cell that is presenting to them the antigen of interest. If yes that's called positive selection, they move on if no they are deleted. We don't want T cells or B cells that can't do this specific molecular handshake.

[00:24:08] Finally, can they distinguish between self and non-self, that is can they tell the difference between something that's good and healthy, or something that is potentially a threat and needs to be removed? If the answer is yes, they move on. If the answer is no, negative selection, they are deleted. I just want to point out one other point buddy before you comment. Only 1% of all T cells and B cells successfully passed these three exams.

[00:24:45] **Dr. Terry Smith:** You're probably thinking to yourselves this is really an inefficient process. Here we go to the trouble that is our immune systems go to the trouble of making all of these cells and we only wind up with about one or two percent of those cells actually passing muster and doing something that's potentially productive. This I think will become apparent to you as we go through the next few slides just how this inefficiency allows for diverse functionality so that an enormous number of antigens can be dealt with and just speaks to the enormous repertoire of the immune system and the cast of characters that the immune system is called on to pass judgment over.

[00:26:07] **Dr. Michael Yeaman:** Sometimes I like to think about this as what the immune system can do versus what it does do. This immense capability, but only allowing cells that pass these exams is really key to tolerance. Well then what happens to the few graduate cells that actually pass these exams and make it through the test grounds? Well, they go to very specific places in the body. One of the most important places is the lymph node.

[00:26:43] Our body has lymph nodes throughout tissues and in various places and it's really a secondary circulatory system for lymphatic fluid and for cells such as those we're talking about today. T cells and B cells go to the lymph nodes some of them stay in the spleen and the whole point for their going there is that this is where antigen presenting cells that have been roaming throughout the body to look for things that might be threats like infection or abnormal like cancer cells detect what they see and bring it back to the lymph node where they can show these antigens to T cells and B cells there

[00:27:30] **Dr. Terry Smith:** Virtually all of you have experienced for instance infections or it states where you're running a fever and you're not feeling well, and you can feel these lumps and bumps in your neck or maybe in your groin or under your arms and those are lymph nodes. That's the situation where this coffee house chatting results in enlargement of the lymph nodes as cells are recruited in and join in the cabal to actually then leave and to protect us from whatever the problem in our health state happens to be.

[00:28:31] **Dr. Michael Yeaman:** We think of the lymph node as the social club where antigen presenting cells and T and B cells come together to talk about what's happening in the body and if needed to respond. If needed T cells and B cells that are shown an antigen and educated that they think there is a threat will then leave the lymph node in response to go track down the potential threat.

[00:29:00] That is how immune tolerance is established. It's a system of checks and balances where immune cells emerge from the bone marrow are educated and tested in the thymus or the spleen and then move to lymph nodes now where they can tell the difference between self and non-self and are prepared to react if antigen presenting cells show them something that's a potential threat. Well then, an important aspect of autoimmune disease is then how is immune tolerance lost? This is what we'll talk about next. I think Dr. Smith mentioned earlier that autoimmune disease and loss of immune tolerance is basically a molecular and cellular case of mistaken identity and Terry maybe you want to talk a little bit more about that here.

[00:29:56] **Dr. Terry Smith:** As Dr. Yeaman has described there's this bi-directional functionality that information is flowing both to and from each of the component's cells shown on the slide. This is important, think of it as a combination lock if you will and all of the tumblers have to align in a certain way in order for positive identification to be made. As we've alluded to previously there are mistakes that can occur and these can occur either with regard to the antigen presenting cell, the T cell the B cell or maybe it's a self-antigen that's looking a little funky to the immune system. Maybe that has to do with some of the bad company that the antigen is hanging out with on that evening. But for whatever reason there are multiple steps where cells and molecules of the immune system can misbehave and misidentify self as for.

[00:31:42] **Dr. Michael Yeaman:** That's really the key to autoimmune disease is when the immune system thinks that a normal or healthy cell or molecule or tissue is not normal and it will attack it, it will think it is foreign or a threat. There's a lot of different places where immune tolerance can be lost a lot of different steps.

[00:32:05] What causes loss of immune tolerance? What causes this mistaken identity? Well, there are several possible steps in this regard. First, sometimes self-proteins are expressed out of place or with structures that are not normal. They're misfolded, they are decorated with different types of chemicals that should not be and oftentimes this comes down to a problem with either DNA or RNA. It can also be after the protein is made and how it's processed.

[00:32:46] Also, there can be dysfunctions in how antigen presenting cells work and the complexes that are generated to do that. Those are called the MHC complex or the major histocompatibility complex. This is the set of proteins that actually does the molecular handshake and that can be a problem. Sometimes the receptors on T cells or B cells can be problematic. There's a system called the recombination activated

genes or ragged jeans that shuffle the deck of T cell and B cell receptors to generate diversity and sometimes mistakes occur there, and the T cell receptors or B cell receptors might miss recognize normal proteins or cells as being abnormal. Of course, infection, pre-cancer or neoplasia and cancer can all lead to loss of immune tolerance including through processes such as molecular mimicry or antigenic misrecognition, reversal of antigenic ignorance and a lot of different possibilities. Finally, pregnancy or transplantation where a person receives an organ or a tissue from another person can also lead to loss of immune tolerance. There's a lot of different ways where tolerance can be lost.

[00:34:17] **Dr. Terry Smith:** Bottom line here is that sometimes loss of immune tolerance is a good thing, cancer infection, sometimes it's a bad thing and that can lead to some of the problems caused by immune system dysfunction. Among those autoimmune diseases are really the top of our list today.

[00:34:56] **Dr. Michael Yeaman:** The consequences along those lines Terry, of loss of immune tolerance include autoimmune disease, but they can also manifest as pregnancy miscarriages and transplant rejection, poor wound healing, impaired growth and development, and even other issues. But we're talking about autoimmune disease today and how we can possibly restore immune tolerance. We're coming to that pretty much as our next topic, which again really gets us into the exciting and potentially very hopeful area of restoring immune tolerance for cures.

[00:35:38] Then how can we restore immune tolerance in these conditions? Long story short, we've talked about how immune tolerance is lost by mistakes in identity and a number of different interactions with the self-antigen and between the cells that do the recognizing and responding antigen presenting cells. T cells and B cells.

[00:36:06] Basically, if we are going to correct immune tolerance that is to restore immune tolerance, we've got to correct these problems in ways that overcome whatever mistakes were made. That sounds like a big job and a complicated job given that we're talking about many possible steps, but one of the really promising ways that we and many people are now focusing on restoring immune tolerance in NMOSD and MOGAD is to reverse this combination lock as Terry mentioned. The beautiful thing about a combination lock is you don't have to change all the numbers. All you have to do is change one of them and that lock will not work anymore. If we're trying to reverse an autoimmune disease that involves a bunch of different tumblers, if we might just be able to change one of them the others might follow. That's a concept that we're going to talk about now.

[00:37:12] What might be the actual strategies to restore immune tolerance, we've listed a few of them in our minds and if you're able to see the slide show you can see the list here. But for issues that have to do with DNA or RNA problems there is a new technology that probably many of you have heard about called CRISPR DNA editing. That can potentially correct problems in DNA that lead to loss of immune tolerance. We know that there may need to be ways to correct the production the location or the structure of an antigenic protein and there are ways to do that now.

[00:38:01] The immune system can be taught how to reprioritize antigen presenting cell T cell and B cell responses to certain antigens over others and that's called immune deviation. The two areas that we'll focus on today have to do with either deleting auto reactive antigen presenting cells, T cells or B cells, that is actually having the immune system kill the cells that misrecognize normal self-tissues and that's called censoring or deletion. Finally, to reeducate the immune cells that are responsible for tolerance, the energy in presenting cells T cells or B cells, and this is called reprogramming or retraining.

[00:38:49] **Dr. Terry Smith:** As we get more and more sophisticated in our understanding of diseases like NMOSD and MOGAD we will be able to pair specific technology for retolerization to the problem. The NIH

has a huge drive now to enhance what we call personalized medicine where individuals rather than groups of patients are the target of therapy development. I think what Dr. Yeaman is really leading into has to do with the multiplicity of potential therapeutics and how each of them may provide a separate non equal pathway to solving diseases like NMO and MOGAD.

[00:40:14] **Dr. Michael Yeaman:** Great points Terry. Tolerization cures is really going to be the combination of personalized medicine meets precision medicine and that's what we wanted to talk about in the last part of the discussion. That is what are some examples specific ways that colorization might be restored in patients who have NMOSD or MOGAD. We just wanted to give a few examples here.

[00:40:47] The first involves something called CAR-T or CAAR-T cell therapy. We won't go into a lot of detail here, but the CA of CAR-T or CAAR-T refers to something called chimeric antigen. What that means is it's a receptor that can recognize certain kinds of antigens because the receptor is made of a mixture of receptors, so that's the chimeric part. Here what happens is healthy T cells are drawn from the patient, you've heard about PBMCs, that's where we would get healthy T cells. They're engineered or modified in the laboratory so that they now have these chimeric antigen receptors.

[00:41:38] The other thing in addition to expressing the chimeric antigen receptors is these cells are now programmed to be killer cells. Once they are able to recognize cells that mistakenly identify auto antigens, they can also kill them. What happens is these CAR-T or CAAR-T cells are then returned to the patient where they can seek and destroy auto reactive T cells and auto reactive B cells in the patient. That's one way we delete auto reactive T and B cells.

[00:42:20] **Dr. Terry Smith:** I think that the CAR-T technology is a great example of how huge leaps forward into the future derived from sharing of technology between different sorts of human disease. In this case cancer and auto immunity and the impact of this technology is mind boggling, because it really reduces a disease or a group of diseases to their fundamental molecular components. That for those of us who are biologists is just really exciting.

[00:43:22] **Dr. Michael Yeaman:** In many ways autoimmune disease and cancer are molecular opposites when it comes to tolerance, cancer too much tolerance, autoimmune disease too little tolerance. It's a great example of how different diseases can help solve one another. Another example of ways to tolerize in NMOSD and MOGAD has actually gone pretty far into the clinic already and it's called dendritic cell or antigen presenting cell vaccines. Here dendritic cells which are antigen presenting cells are taken from the patient they're exposed to the auto antigen in the laboratory, but under conditions which make the antigen presenting cell tolerant and it will then educate T and B cells that it interacts with to be tolerant to that energy. The tolerizing dendritic cell then is now educated in the laboratory, its return to the patient where it will teach T cells and B cells to be tolerant to that same antigen.

[00:44:32] **Dr. Terry Smith:** It's like turning a weapon into a walking stick. It really, I think sabotages bad intention and turns it into good.

[00:44:57] **Dr. Michael Yeaman:** It's turning a weapon into a peace sign. I agree buddy, it's a nice way to think about it. Another example of ways that are already being used experimentally to tolerize in diseases that are related to or even within NMOSD and MOGAD landscape are inverse RNA or DNA vaccines. Most of the time we think about vaccines for infectious disease as being able to turn up the immune system to a specific target. For example, the spike protein of the SARS-CoV-2 virus that causes COVID-19. Inverse vaccines turn the immune system down to a specific antigen like aquaporin-4 or MOG. Here the nucleic acid either the RNA or DNA is often encoded in some type of a capsule like a nanoparticle where it then either encodes for

the production of the autoantigen or its variant that will lead to tolerance. Typically, this is not just regular DNA or RNA, but it's special kinds of DNA or RNA that will teach the immune system, in particular, teach the tolerizing dendritic cell or antigen presenting cell that rather than responding in an inflammatory way to this antigen it should respond in a non-inflammatory way to it. Again, once the antigen presenting cell is educated to be tolerant there will be no second signal to the T cell or B cell and when there's no second signal to these cells, they are either energic which means they will not respond or they are deleted, either way we're protecting against autoreactive T and B cells.

[00:47:01] Let me move on. We have other examples here one of which is engineering of autoantigen epitope. As you know most antigens are proteins and autoantigens are self-proteins to which the immune system responds and causes autoimmune disease. Well, we know through a lot of different experiments done by some very important groups around the world that certain parts of antigens can be engineered, so that they act as decoys and instead of causing the immune system to be inflammatory toward them it can turn off the immune system response to those antigens.

[00:47:49] Here for example a dendritic cell might be exposed to an engineered autoantigen epitope for example imotopes or epitopes are two instances under conditions where the dendritic cell is tolerizing and then those return to the patient where they tolerize the T cell or the B cell. Lastly one of the more interesting recent approaches is to actually leverage a very natural tolerogenic system in the body and that is the red blood cell MHC matching process that occurs.

[00:48:31] We all know that if someone is to be transfused with red blood cells from a donor, they have to be carefully matched otherwise there can be problems and that is due to very specific matching and tolerance of red cells within a patient. Well, if that's true, some pharmaceutical and biotech companies have said why not use that as a way to tolerizing against an autoantigen. The strategy is to take a piece of the autoantigen and either insert it into or express it on red blood cells and then simply let the tolerance mechanisms that clear red blood cells do the rest of the work.

[00:49:17] Some of these red blood cells that are now decorated with autoantigen go to special antigen presenting cells in the liver which then become tolerant to that antigen and then go down to present a tolerizing message to T cells and B cells so that they become tolerant to that autoantigen. Again, these are just a few examples. There are several others that we'd be happy to talk more about. But again, a lot of exciting work going on now in industry that we're very hopeful will be curative.

[00:49:51] When we think about cures, we wanted to just mention in response to sometimes we hear well, is tolerization a treatment or a cure? Well, it's a little bit of both actually. It will be a treatment if the therapy induces temporary tolerance to the autoantigen. There may need to be occasional redosing, but the important point is that there would be no need for immune suppression.

[00:50:22] Tolerization therapy would be a cure, if it induces permanent immune tolerance and that's our goal ultimately. We'll probably not get there immediately, will probably need to find some redosing strategies and that thing. But that would be fine, I think patients would be very happy if we could induce immune tolerance even if it meant you had to get four infusions a year or two infusions a year, especially if you were able to get off of immune suppressing therapy. That's really the important idea here.

[00:51:00] Those are some of the specifics about how tolerization might be applied to NMOSD and MOGAD and we do like to always remember that there is a road map here. The first thing is to identify the autoantigen, then to figure out what targets and effects which sells then to figure out a tolerizing strategy, what's the best technology to deal with this particular loss of tolerance? Then to put that to the test and optimize it in

the laboratory, then into clinical trials, which of course first and foremost focus on safety and effectiveness. Then of course it goes to the regulatory agencies for review and approval.

[00:51:44] One very hopeful aspect of NMOSD and MOGAD, is that many of these steps have already been achieved and now we're starting to see some of the fruits of our efforts over the last 8-10 years that the foundation has been working on this that are now moving into the clinic. By comparison, unfortunately diseases that are more common like multiple sclerosis or type 1 diabetes, which are also autoimmune, we don't know that much about which autoantigens are most important and we don't know really a lot of the processes that lead to these autoimmune diseases. Again, NMOSD and MOGAD, these might be the little diseases that could open the door to solving all autoimmune disease.

[00:52:39] We wanted to leave you with the thought about, hopefully this has been helpful, but if you want to learn more about tolerization, there's a couple of places you might think about. The Guthy-Jackson Charitable Foundation website now contains a microsite that is under the breakthroughs banner tab, the top of the website and it will take you to a subsite that's called pioneering curative therapies. In there you can learn a lot about tolerization and also, you'll be able to see more than a dozen or two dozen companies that are working in this space.

[00:53:21] I think with that we just really wanted to thank the Siegel Rare Neuroimmune Association and all of its staff and leadership and really being a partner with both the Guthy-Jackson Charitable Foundation, but with patients in really trying to do everything we can to save and improve lives. We really appreciate the opportunity to talk about tolerization today and look forward to hopefully doing so again in the future.

[00:53:55] **Krissy Dilger:** Thank you both so much. We really appreciate your time and this incredible presentation. I learned a lot about tolerization that I didn't know and I'm sure everyone who listens to this presentation as well will learn a lot of it too. Thank you both for your time and thank you for joining us. I hope to see you all again so we can continue this conversation.