Immunology of Rare Neuroimmune Disorders

Part 1

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Dr. GG deFiebre: [00:00] Hi, everyone and welcome to the SRNA's "Ask the Expert" podcast series. This podcast is titled "Immunology of Rare Neuroimmune Disorders Part One." My name is GG deFiebre and I moderated this podcast. SRNA is a non-profit focused on support, education, and research of rare neuroimmune disorders. You can learn more about us on our website at wearesrna.org. Our "Ask the Expert" podcast series is sponsored in part by Horizon Therapeutics, Alexion, AstraZeneca Rare Disease, and Genentech. 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. Alexion, AstraZeneca Rare Disease is a global biopharmaceutical company focused on serving patients with severe and rare disorders through the innovation, development, and commercialization of life-transforming therapeutic products. Their goal is to deliver medical breakthroughs where none currently exist, and they are committed to ensuring that patient perspectives and community engagement is always at the forefront of their work.

Dr. GG deFiebre: [01:15] Founded more than 40 years ago, Genentech is a leading biotechnology company that discovers, develops, manufactures, and commercializes medicines to treat patients with serious and life-threatening medical conditions. The company, a member of the Roche group has headquarters in South San Francisco, California. For additional information about the company, please visit gene.com. For this podcast, we're joined by Dr. Anastasia Vishnevetsky and Dr. Phil Bilodeau. Dr. Vishnevetsky is a Neuroimmunology fellow at the Brigham Mass General MS and Neuroimmunology Fellowship program. She graduated from the Mass General Brigham Neurology residency and completed her internship at Brigham and Women's Hospital. She has recently been awarded the Sylvia Lawry Clinical Research Fellowship as well as the NeuroNEXT Clinical Trials Fellowship and is currently focusing on clinical trials addressing symptomatic aspects of demyelinating diseases such as fatigue, pain, and spasticity. Dr. Bilodeau completed a medical degree at McGill University in Montreal and then moved to Boston to train in neurology at Harvard Medical School, Massachusetts General Hospital, and Brigham and Women's Hospital and is currently a senior resident and incoming neuroimmunology fellow. Dr. Bilodeau studies the interactions between the immune system and the nervous system with particular interest in novel therapeutics to induce immune tolerance and multi-omics phenotyping of neuroinflammatory disorders.

Dr. GG deFiebre: [02:36] Welcome and thank you both so much for joining us today. To start, Dr. Bilodeau, do you mind just giving an overview of what our immune system is and what the difference between innate and acquired immunity is?

Dr. Philippe-Antoine Bilodeau: [02:52] I think fundamentally, the goal of the immune system is to fight what is foreign and foreign can mean different things. Classically, people think of things like infections, but it can also mean things like cancer cells. Traditionally, we've divided the immune response into two different arms.

The first arm is called innate immunity and the second arm is called acquired immunity. And you can think of innate immunity as essentially infantry. So, it's trained to recognize patterns that could represent a threat and to respond rapidly to that threat. Innate immune cells actually do this through things called pattern recognition receptors and those receptors are expressed on pathogens and cancer cells. And there's a couple different subtypes of immune cells in the innate immunity arm. Some of them are called macrophages, some others are called neutrophils and there's also dendritic cells. And just like infantry, the innate immune response is not specific. In other words, it's going to respond to any threat the same way regardless of whether it encountered that threat before. It is still a critical first line defense against pathogens and the innate immune system is the first to encounter a threat. If infantry comes across a threat generally what they're going to do is they're going to hold it off and they're going to relay the information back to their commanding officers so they can send specialized units to eliminate the threat.

Dr. Philippe-Antoine Bilodeau: [04:26] And those specialized units, that's essentially the adaptive immune system. The adaptive immune system includes things like B cells and T cells. B cells generate antibodies and T cells are sort of the commanders that regulate the immune response and make sure that it's coordinated. Another big difference between the innate and the adaptive immune system is that the adaptive immune system has a memory. If you fought off an infection 10 years ago, there are still some B cells and T cells in your blood that remember that infection. So, if you get infected again, they'll be able to generate a response much more rapidly. That's actually one of the things that we rely on when we make vaccines. So, the COVID vaccine for example, what it does is it induces memory of an infection without the need to actually get the infection. And that's how it's effective in making sure that the immune response is much more rapid and much more efficacious if and when you do get infected.

Dr. Philippe-Antoine Bilodeau: [05:27] And while the innate immune response responds to patterns, the adaptive immune response is actually a lot more specific. It responds in fact to specific proteins expressed by things like infections and cancer cells. And that's why it's so effective is because it is very, very specific. And one of the ways that the adaptive and innate immune system communicate together is through small molecules called cytokines. These molecules act as essentially a signal to trigger or dampen the immune response. In fact, one of the new medications for neuromyelitis optica called tocilizumab targets one particular cytokine and in doing so prevents relapses. So, I think that shows just how important those molecules are because if you get rid of even one of them, you can turn down the inflammation quite a bit. Another strategy is to get rid of the immune cells that are doing the damage. So, some of our medications for NMO and multiple sclerosis do that by eliminating B cells. Medications like inebilizumab or eculizumab are B cell depleting agents and they act by getting rid of the adaptive immune system or at least part of the adaptive immune system.

Dr. GG deFiebre: [06:45] Great. Thank you so much for that overview. I think it's really clear how different aspects of the immune system and the differences between the innate versus acquired immunity. And so, Dr. Vishnevetsky, Dr. Bilodeau talked a little bit about it, but do you mind just talking about the different immune cells and how they function?

Dr. Anastasia Vishnevetsky: [07:09] Yeah. Absolutely. I think Phil covered-- touched on a couple of the different types of immune cells. But big picture, I'm going to talk about different categories of white blood cells and kind of the first three subdivisions of white blood cells are granulocytes, monocytes, and lymphocytes. Within the category that we call granulocytes, there's three actually subcategories, which are more involved in the innate immune system and those are neutrophils, eosinophils, and basophils. Lymphocytes are probably the category that's most important for neuroinflammatory disorders and so I'll focus in on that. But for the granulocytes I just mentioned, so neutrophils are really focused on targeting bacteria. Eosinophils are involved in our allergic reactions and allergic responses, and they also target parasites or parasitic infections. And

then basophils are also involved in allergic responses and release histamine amongst a few other functions. There's also, I said there was granulocytes, which included those three types of cells that I just talked about.

Dr. Anastasia Vishnevetsky: [08:19] The next category is monocytes, which the kind of two subcategories for monocytes are macrophages and then also dendritic cells. Dendritic cells are cells that actually present antigens or little specific molecules or foreign bodies that then need to be destroyed by lymphocytes in that last category. So that brings me to lymphocytes, which have three subcategories. One is B cells which a lot of you might have heard of already and are really important for neuromyelitis optica. There's also T cells and then there's also natural killer cells. So, B cells and T cells as well as the complement system, which I'll talk about in the end, are all components of the adaptive immunity system or the acquired immune system that Phil was talking about. So, B cells are a type of white blood cell. They're also a type of lymphocyte and they produce antibodies. These antibodies are a component of immunologic memory. So, when we talk about for example, in the last few years, you've been around on this planet, you've probably been hearing about having an antibody response after a vaccine. So, when we talk about that, we're really talking about essentially a vaccine is given and the vaccine is often presenting a specific molecule or antigen that both the vaccine and the COVID virus have in common.

Dr. Anastasia Vishnevetsky: [10:03] And then B cells, our B cell receptors essentially bind to this antigen or to this molecule and that causes them to be activated and to essentially become more mature and when they mature, they are able to create specific antibodies against that molecule or against that antigen. Some of the B cells will turn into plasma cells, which are actually the cell that's kind of the final pathway of a B cell that's actually generating the antibodies. And other B cells will turn into memory B cells and essentially circulate around in the bloodstream and contribute to our immunologic memory. They're going to be those B cells that stick around so that if you encounter the infection again in six months, they have a kind of fast track to generating those antibiotics. They don't have to go through that whole maturation process all over again. Thinking more about MOG antibody disease or aquaporin-4, when we talk about aquaporin-4 or MOG antibodies, we're actually talking about the products of B cells that have an abnormal response to a self-antigen. So normally, there is an aquaporin-4 channel, water channel. It's not an aquaporin-4 antibody, it's just a water channel. And when we say that someone has the aquaporin-4 antibody, it's an abnormal antibody that should not be there that targets that normal water channel.

Dr. Anastasia Vishnevetsky: [11:36] Similarly for MOG, myelin oligodendrocyte glycoprotein, is a protein that's normal and present in all of us on myelin, which helps to insulate our nerve connections and also on oligodendrocytes, which make myelin. And so, these are kind of abnormal antibodies. So, B cells are really at the root of creating antibodies, both the ones that we want for vaccines and also the ones that we don't want in autoimmune disorders and neuroinflammatory disorders. Another thing, just to note, you might hear the term humoral immunity thrown around. So humoral immunity versus cell-mediated immunity are two terms, humoral immunity is really kind of the antibody-based immunity, so B cell-based immunity, whereas cell-mediated immunity or cellular immunity is more focused on T cells. All T cells are not the same, are not created equally. There's a bunch of subdivisions of T cells. There are helper T cells that are actually more involved in helping B cells be activated and also help release other cytokines that help activate additional white blood cells. So, they can be part of both the cellular or cell-mediated immunity and have some role in human immunity. There are also cytotoxic T cells, which are also called natural killer T cells and those release molecules that kill viruses and other antigens.

Dr. Anastasia Vishnevetsky: [13:18] And then there's memory T cells, which can help-- which essentially can-- similarly to memory B cells, are there after your body has fought off an infection and they helped you deal more easily with any infections that you might get in the future. And finally, there are regulatory T

cells, which actually help to tamp down the immune system. They help to keep the immune system more in control. So those are a few different types of T cells that are important to think about. And then finally, if you think about NMOSD in particular, the complement system is particularly important. So, the complement system is essentially-- it consists of a bunch of small proteins, small molecules that are made by the liver and they circulate in our bloodstream. They're usually inactive. But when a trigger stimulates the beginning of this complement cascade, there's a bunch of divisions [or] cutting of these proteins that then activates the proteins. And there's a few different things that can occur when the complement cascade is triggered. One of them and one of the most important is that there's something called the membrane attack complex that can form, and it can then essentially cause destruction of whatever it is that-- the membrane of whatever it is it's attached to. Sometimes that's bacteria but it can also be, in the case of NMOSD, an astrocyte, which is a normal healthy cell that we need in our nervous system. Complement activation can also just trigger phagocytosis, which means essentially enveloping or kind of eating of the cell and then also just inflammation in general by attracting other types of immune cells that we've already talked about, like neutrophils or macrophages. And so, blocking the complement cascade can essentially have quite a few different types of effects at the same time.

Dr. GG deFiebre: [15:36] Great. Thanks so much for that in-depth explanation of the immune system and you tied it a bit to some of the rare neuroimmune disorders. I know you mentioned NMOSD or neuromyelitis optica spectrum disorder and then MOG antibody disease. But what makes-- so at SRNA we talk about rare neuroimmune disorders like those two as well as ADEM or acute disseminated encephalomyelitis, AFM, acute flaccid myelitis, optic neuritis, and then transverse myelitis. So, what makes these neuroimmune disorders, that term? And then what happens in certain cases with the blood-brain barrier in these disorders, Dr. Vishnevetsky?

Dr. Anastasia Vishnevetsky: [16:20] Yeah, absolutely. So, I think a lot of understanding the inflammatory and neurobiological disorders comes down to understanding that a fundamental role of the immune system is to distinguish between self and not self. In particular, the immune system needs to distinguish between self and danger. And all of these conditions are fundamentally based on the immune system getting confused and mistaking self for danger and thus attacking. That's why these are called autoimmune conditions and why that's very different from what you want when your immune system is fighting an infection, when you want your immune system to be more activated. All of the conditions you mentioned differ in terms of specifically what type of mistake they're making and where in the immune system the mistake is occurring. So, is it predominantly in the B cells? Is it in the T cells? What's the target? But many of the conditions that you mentioned are actually really syndromes rather than distinct diseases. And what I mean by that is that there might be really a lot of underlying immunologic causes for a collection of imaging findings and symptoms that we, as neurologists would call for example, ADEM, acute disseminated encephalomyelitis or optic neuritis or transverse myelitis.

Dr. Anastasia Vishnevetsky: [17:42] And to really illustrate what I mean by saying that this is a syndrome rather than a disease, if you think about MOG antibody disease and neuromyelitis optica spectrum disorder and multiple sclerosis, we know that all three of these can cause optic neuritis. Often that's the first question when someone has optic neuritis is, is it one of these things? We also know all three of these disorders can cause transverse myelitis. But we also know that drugs that work for multiple sclerosis don't necessarily work for neuromyelitis optica and you need to understand the underlying immunologic mechanism to really get at these different disorders even if they initially might look the same. There are also cases of optic neuritis or transverse myelitis that are unrelated to any of those diseases. Some of them are caused by other rarer systemic diseases or conditions like sarcoidosis or lupus or Sjogren's disease. Some of them can be related to an infection in rare cases.

Dr. Anastasia Vishnevetsky: [18:41] So I think it's tough to say what is the underlying cause, neuroimmunologic cause of something like transverse myelitis or optic neuritis because we just know that there's so many different ones and a lot of what basic science research is focused on today is still trying to further break down the transverse myelitis that we haven't explained yet, that we don't know is part of MOG antibody disease, we don't know is part of multiple sclerosis and separating it out and saying, well, what is it and how do we target it? MOG antibody disease and neuromyelitis optica spectrum disorder are two entities, especially the aquaporin-4 positives subgroup of NMOSD, are two entities that really have a much more specific mechanistic kind of-- we have a more specific mechanistic explanation for, and we know where in the nervous system the abnormal target is. So, for aquaporin-4 we know that that's a water channel most often present on astrocytes, and we know that there is antibody-mediated destruction around those water channels and on those astrocytes, whereas MOG antibody disease is more focused on oligodendrocytes and myelin.

Dr. Anastasia Vishnevetsky: [20:00] So those are kind of-- all of these, however, regardless of which neuroimmunologic disorder it is, is essentially just the immune system mistakenly targeting self and causing a variety of different symptoms. In all of these cases, you need some degree of blood-brain barrier breakdown. And so, what is the blood-brain barrier? It's really a highly selective border between the brain and the rest of our body, or really between the nervous system and the rest of our body, that allows for only very specific molecules to cross into the brain. This is formed by cells of the capillary wall. The capillaries are the smallest blood vessels that we have. It's also formed, I mentioned astrocytes, which are important for NMOSD, but the astrocytes end feet are a very important part of the blood-brain barrier. They essentially surround the capillary and create this barrier.

Dr. Anastasia Vishnevetsky: [21:09] And these other cells called pericytes as well. And there's a lot of different ways that the blood-brain barrier can break down and we don't always know what the first impetus for that is or what the first trigger for that is. There are metabolic changes and difficulties that can cause breakdown of the blood-brain barrier. There's physical injuries and trauma that can cause blood-brain barrier breakdown. The reason that's important is because that allows for white blood cells that otherwise wouldn't be able to get into the brain to get in there and cause damage. It's also an important concept because we want to often deliver drugs that are effective in the brain and the blood-brain barrier can be a really important barrier for that and for drug development. So, it's an important consideration for pharmaceutical companies when they're developing a drug. They need to think about, are we targeting the immune system in the periphery or in the brain? And if we're targeting it in the periphery, is there still some activity left in the brain that we're not targeting? And things like that.

Dr. GG deFiebre: [22:19] Great, thank you. And then, Dr. Bilodeau, to add to that, what role do we think molecular mimicry plays in these disorders?

Dr. Philippe-Antoine Bilodeau: [22:29] Anastasia put it pretty well. I think fundamentally autoimmunity represents a failure of tolerance in recognizing self as foreign. One of the ways that that can happen is through something called molecular mimicry. Molecular mimicry concept is that someone's immune system encounters a threat whether that's a pathogen or a cancer cell. But in addition to reacting to that threat, it also starts reacting against proteins in someone's own body that resemble that of the pathogen or the cancer cells. And in doing so, it's actually one of the mechanisms that can lead to autoimmunity. A good example of this is multiple sclerosis because recently there was a big study that gained a lot of recognition in the media, and it linked the Epstein-Barr virus to multiple sclerosis. And one of the mechanisms that could be at play here is molecular mimicry. So, we know that most people are infected with EBV at some point in their life, it's probably one of the most ubiquitous viruses that's circulating. But we know that in some people the immune system mistakenly thinks that the proteins in the brain are EBV proteins and in doing so starts

attacking oligodendrocytes, the myelin-producing cells. That's a hypothesis that hasn't been proven yet, but that's one of the ways that a virus can cause an infection.

Dr. Philippe-Antoine Bilodeau: [23:58] We also know that things like transverse myelitis, MOG antibody disease and ADEM, these diseases can happen after an infection. It's been recognized for decades and decades and one of the ways whereby an infection can cause a neuroinflammatory disorder is molecular mimicry. And often the symptoms of the actual infection can be mild, it can be just a cold, but then a few weeks later, people can develop neurologic symptoms. And what we think happens in the background is that the immune cells, the immune system appropriately reacts against the virus, but in doing so, it also generates immune cells that recognize proteins in the brain and in the spinal cord and those proteins, the reason why it generates those is because they look like the virus. So, the immune system is tricked into attacking the nervous system while it's trying to fight off the virus. And the reason why it's tricked is because those proteins can actually be surprisingly similar. Another somewhat related concept is that when the immune system attacks a pathogen, so an infection, it can cause a lot of bystander damage. So, if you have a lung infection, for example, there could be quite a bit of inflammation around the area of the lung that's infected and that damage can release some proteins that the immune system is not used to.

Dr. Philippe-Antoine Bilodeau: [25:22] So these proteins now suddenly look foreign to the immune system, even though it's the body's own protein, the immune system thinks that it's danger. It thinks that it's not self and it starts attacking those proteins. So that's another mechanism that's somewhat related to molecular mimicry that can cause autoimmunity. And so far, we've been talking about infections, but the same can actually be true for cancer cells. In fact, paraneoplastic neurologic disorders are inflammatory diseases that are caused by an aberrant immune response against cancer cells. So, for example, some proteins can be expressed in an ovarian cancer, and they may be similar to proteins that are expressed in the cerebellum, the back of the brain that controls balance. Through molecular mimicry, patients can start attacking their own cerebellum instead of just attacking the cancer cells. And so all of these mechanisms, molecular mimicry, bystander damage, combine to create autoimmunity. But it doesn't happen in everyone though. Most people who get a viral infection or a cancer don't develop autoimmune diseases and that's because we have mechanisms of tolerance, which is a very important concept that we'll talk about in a couple of minutes.

Dr. GG deFiebre: [26:40] Great. Thanks so much. And then Dr. Vishnevetsky, what is the relationship, you talked a bit about this but between B cells and MOG and aquaporin-4 or AQP-4?

Dr. Anastasia Vishnevetsky: [26:52] Yeah, so B cells are really kind of the troublemakers of the immune system for MOG and aquaporin-4 related diseases. So, they're white blood cells that make antibodies and when we say that someone has MOG antibody disease or aquaporin-4 positive NMOSD, we mean that we have found antibodies against MOG or aquaporin-4 that should not be there. So, the B cells made a mistake. So, MOG and aquaporin-4, as I said, are naturally present in the body. And when antibodies target one of these antibodies by accident, they can cause damage. So, for MOG, they can cause damage to myelin. Interestingly, MOG antibody was used to essentially make a model of multiple sclerosis for a long time because multiple sclerosis is also caused by damage to myelin. It turned out later though that there's a whole kind of additional disease MOG antibody disease that's more specifically related to this particular glycoprotein that's found both on myelin and on some oligodendrocytes. With aquaporin-4, this is a water channel, it's present in astrocytes, as I said that it's a very important part of the blood-brain barrier.

Dr. Anastasia Vishnevetsky: [28:16] And so that actually explains some of the findings we see when we take a picture when we get an MRI of people with NMOSD and aquaporin-4. We see a lot of damage around areas close to where cerebrospinal fluid flows, around areas where water is flowing essentially because that's where we see these water channels most commonly. In both cases it's, again, the B cells that are making

these antibodies that are causing the damage. But I want to give one caveat to what I said about the B cells making antibodies that cause the damage. So, in aquaporin-4 related disease, we feel very comfortable with the fact that the antibody itself is a large part, not the only part but a large part of what causes damage to the nervous system. Essentially get rid of the antibody or get rid of the cells that are making the antibody, the B cells, and you can help get rid of the disease.

Dr. Anastasia Vishnevetsky: [29:14] That's the idea of drugs like rituximab, which targets a B cell marker or inebilizumab, which targets a different B cell marker. Both of these get rid of B cells and that way you get rid of essentially making these antibodies that are causing trouble. However, there's also an idea of something called a nonpathogenic antibody. Essentially that would mean that the antibody is a marker of a disease process that's going on, but it is in and of itself not necessarily a key part of how the nervous system is damaged. Sometimes when there's damage to the nervous system and destruction of parts of the nervous system, those destroyed parts become recognized by the nervous system and antibodies can be generated. So, for MOG antibody disease there's actually pretty active debate about the degree to which the MOG antibody itself is causing the damage versus whether it's in small part causing the damage and there's a lot of other processes going on. And so that's still an area of active research. Might also be part of the explanation for why B cell therapy is more impressively effective in aquaporin-4 related NMOSD than it is in MOG antibody disease.

Dr. GG deFiebre: [30:30] Great, thank you. And so, we've talked a bit about damage being done via the immune system to parts of the central nervous system. A question we get a lot is that, is there a natural repair of the immune system after injury? So, after someone is diagnosed with one of these conditions, is there a natural repair process that occurs of the immune system after this damage is done? Dr. Bilodeau, do you mind answering that question?

Dr. Philippe-Antoine Bilodeau: [31:00] Sure. So, the immune system can easily regenerate itself and the immune cells are very good at replicating rapidly, that's essential to their function. I think a related question is whether there is a natural repair of the nervous system after injury and that one is a little bit harder. So, the quick answer is no. And the long answer is a bit more complicated. Classically, we know that the peripheral nervous system can regenerate itself. The peripheral nervous system refers to peripheral nerves and nerve roots, essentially things that are outside the brain and the spinal cord. So, we know that if you cut off a nerve, over time it will grow back. It will be long, but it eventually will grow back. Historically, we've said that the central nervous system cannot regenerate itself, that it cannot repair itself. The reality is a little bit more complicated than that. Of course, if a neuron dies, it's true that it cannot grow back. There is no way of making new neurons in the brain. There's actually quite a bit of exciting research right now trying to understand why that is, why is it that a neuron in the brain can't regenerate itself but peripheral nerves can. And a lot of groups are trying to change that, trying to replicate the conditions of the peripheral nervous system in the brain and see if they can stimulate repair. But it is still true fundamentally that if you kill a neuron, the neuron will not grow back.

Dr. Philippe-Antoine Bilodeau: [32:27] And similarly, if you kill off oligodendrocytes, the cells that make myelin, which helps speed up the signals between neurons, there will be a scar tissue that will develop. But I think it's a little reductionist to think of central nervous system injury as just being about neurons and oligodendrocytes dying. There's quite a bit more going on. First of all, there's a lot of other cells in the brain. Anastasia talked about things like astrocytes, which provide a scaffold in the brain and form part of the blood-brain barrier. There's also cells called microglia which are specialized immune cells in the brain whose function is to clear up debris. And all of these cells can be affected to a certain extent when there's inflammation in the brain and some of that is reversible. We know for example that oligodendrocytes can eventually grow back if they're not permanently injured. We also know that things like swelling in the brain

and in the spinal cord, which can cause a lot of symptoms and things like ADEM and NMO and MOG and actually most neuroinflammatory disorders.

Dr. Philippe-Antoine Bilodeau: [33:31] We know that that swelling for the most part is reversible. In fact, steroids, which are used essentially for all of these disorders are particularly good at getting rid of swelling and that's why people can feel better fairly quickly after we start steroids. Another important concept is plasticity. So even if there is irreversible injury to neurons and oligodendrocytes, the brain is actually pretty good at rearranging neural networks and trying to find ways of bypassing the injury. That's one of the reasons why rehabilitation works so well actually after neurologic injury. They've done studies and they've shown that there's structural changes in the brain after patients go through rehab and we can image that using advanced MRI techniques. These adaptations are one of the reasons why patients with neuroinflammatory disorders can have worsening of their symptoms when they're sick. In other words, their brain adapted to the injury but if you add the stress of an infection, it's no longer able to compensate and some of the old symptoms can come back. That's something that we call in neurology recrudescence and it's different from a relapse which is caused by new inflammation in the brain or in the spinal cord. The question of how to promote repair in the central nervous system is probably one of the most active areas of research and one of the biggest unmet needs in neuroimmunology.

Dr. Anastasia Vishnevetsky: [34:58] To add a little bit to what Phil, Dr. Bilodeau, was saying, recrudescence is something that we really see quite often in neuroimmunology and the neurobiology clinic. But it's also something that we can see with other types of injuries, the strokes, but for example, with our MS or NMOSD patients, we also often see that when someone has the flu or someone has a UTI, they might have symptoms that other people would never really have. They have worsening numbness or tingling. They have really, really profound fatigue or they have worsening weakness. Some people actually experienced, after the vaccine, after the COVID vaccine, they would experience some of those symptoms and get really nervous about a new relapse. What we saw was that people weren't actually experiencing new relapses but just the stress of essentially having an immune response, that's really the goal of the vaccine, was enough to kind of overburden the compensatory pathways that Dr. Bilodeau was talking about. So, it's a really helpful way to understand what's happening to the body and why, even though some of those parallel pathways might make it seem like the body's fully repaired itself under stress, we see how that isn't fully the case.

Dr. GG deFiebre: [36:17] Thank you for that addition. Dr. Bilodeau talked about steroids, for example. If someone has a recent onset or experiences a true relapse, so not just a worsening of symptoms but an actual new inflammatory attack, they might be given acute treatments like steroids. And so how do these work immunologically, Dr. Vishnevetsky?

Dr. Anastasia Vishnevetsky: [36:40] Yeah. So, you would think that as someone who prescribes these acute treatments all the time, I have a really quick and easy answer for this. And when I say acute treatments, the ones I really think of as being the most important for neuroimmunology are glucocorticoids or steroids and IVIG and plasma exchange or also called therapeutic plasmapheresis. And although I do feel like I have a really great tip of the tongue answer for how a lot of our chronic immunotherapies work, the truth is that for our acute therapies, they are so multifactorial, and they are so messy in the way that they work that there is still ongoing debate. Even though most of these drugs are actually-- or most of these therapies are actually really, really old and predate any of our newer therapies, there's still debate about how they work and how they work for different diseases. They probably are doing slightly different things whether we're treating them for MS-- using them for MS or NMOSD or something like rheumatoid arthritis or lupus. But I'll give it my best shot. So, steroids, the type of steroid that we really use, there's different categories -there's

mineralocorticoids, glucocorticoids - we really use glucocorticoids in neuroimmunology and the drugs that we're giving are really imitations of glucocorticoid hormones that our body produces naturally.

Dr. Anastasia Vishnevetsky: [38:14] For decades these have been some of the most commonly prescribed drugs that are immunomodulatory, so modulate or change the immune system, not-- partially immunosuppressive as well. And the way that glucocorticoids typically work is that the glucocorticoid hormone binds to glucocorticoids receptor. So, without the glucocorticoid hormone, the glucocorticoid receptor is totally inactive. But the glucocorticoid receptor signals information when it's activated through a number of pathways to actually change gene transcription. So, it changes which genes in an individual cell are being transcribed and made into proteins. It does that-- steroids essentially regulate transcription of genes in a ton of-- pretty much all of our different cells and they regulate it differently in many different cells. But the overall effect of glucocorticoid activation is that it interferes with immune system activation. So, it interferes with the differentiation or maturation of different types of T cells. We talked about T cells, which are a type of lymphocyte type of white blood cells.

Dr. Anastasia Vishnevetsky: [39:46] So it interferes with the subdivision into those different types. It also suppresses activation of proinflammatory cytokines. We talked about cytokines, IL-6, things like that. It kind of tamps down the cytokine activation as well. It also can lead to destruction, auto-destruction, or apoptosis of different types of white blood cells and different types of immune cells. So, there's really a wide variety of different ways in which glucocorticoids can mediate their effect. They're very, very dirty drugs. They have a lot of off-target effects, which is why we don't like to use them long-term. But they're also really good for the fact that they're so dirty because when we don't know what's going on yet, when we haven't made a diagnosis for example, when we just want to kind of hit the immune system and tamp it down regardless of what's causing it, regardless of whether we understand the underlying immune mechanism, glucocorticoids are really great and can often work for all of the-- many of the different conditions that we talk about.

Dr. Anastasia Vishnevetsky: [40:59] So that's glucocorticoids or steroids. Another pretty complicated acute therapy is IVIG, which is a bit newer, also a bit pricier, which can be an issue for it. But IVIG stands for intravenous immunoglobulin. So, immunoglobulins are really another name for antibodies. There are many different types of immunoglobulins that have different letters - IGA, IGM, IGE, IGG. And just one thing to note, IVIG means it's IV, you also can actually get immunoglobulin subcutaneously. Then it is actually called SCIG. And so IVIG is a pooled preparation of normal human immunoglobulins obtained from several thousand healthy donors. And IVIG, like glucocorticoids, has a lot of different effects. One of the things it does is it interacts with the complement cascade, and it prevents formation of the membrane attack complex and that way it prevents complement-mediated cell death and tissue damage. That's one of the ways it works. IVIG also ends up having what are called anti-idiotypic antibodies, which are really a type of antibody that targets and binds and neutralizes autoantibodies. So IVIG, the antibodies that we're giving to you might go out and bind antibodies like anti-MOG antibodies or anti-aquaporin-4 antibodies or other antibodies that we don't even know are there but are causing damage.

Dr. Anastasia Vishnevetsky: [42:44] So it can neutralize those antibodies, that's another way that it can work. It also can just neutralize additional cytokines and complement-related proteins and so decrease inflammation that way. So, in addition, it can inhibit activation of additional cells like monocytes and macrophages, and it can be also potentially toxic to neutrophils or eosinophils. So just generally kind of pushes you away from a proinflammatory environment to an anti-inflammatory environment. I think IVIG is pretty nonintuitive because we also give IVIG to people who have immunodeficiencies, who need more of an immune system, and they need more immune protection. So, I think that just really underscores how complicated the mechanism of

action of IVIG is. There are quite a few drugs that are being developed to try to mimic certain aspects of IVIG's mechanism of action to see if it can decrease some of the off-target effects, make it more specific. So that's kind of an active area of research.

Dr. Anastasia Vishnevetsky: [44:01] And then finally, the last acute therapy I wanted to talk about is plasma exchange. Plasma exchange is a little bit more straightforward. Essentially, a patient's blood plasma is removed, and you give additional blood products in replacement. The removed plasma is essentially thrown out, you get rid of it. That plasma probably had lots of autoantibodies and you replace it with donor plasma. One of the benefits of plasma exchange is just allows for really, really rapid removal of disease-causing autoantibodies from the circulation. But it's important to know that plasma exchange in and of itself won't fix really any underlying neuroimmunologic condition because the production of the autoantibodies by B cells will continue. You're just removing the autoantibodies; you're not removing all of your B cells along with it. So, you have to kind of use something else to target the B cells and stop the production of new antibodies. But in the meantime, plasma exchange can really quickly get rid of those antibodies. It's a little bit more difficult to arrange and it has some other side effects. You have to have a large catheter to administer it. So, it's less commonly used than things like steroids.

Dr. GG deFiebre: [45:27] Great. Thank you. And so, in addition to those acute treatments, as you said, steroids, IVIG, and plasma exchange, can you just talk a bit about the approved therapies or therapies in general for NMOSD and how they work immunologically?

Dr. Anastasia Vishnevetsky: [45:43] Yeah, absolutely. This is kind of the easy one you're setting me up. All of the new therapies for NMOSD are based on some really elegant immunology and kind of basic science that paved the way for the development of these drugs and we know exactly what they're doing and how. I'll start with the B-cell depleting therapies. So inebilizumab, Uplizna is a new monoclonal antibody. Essentially what that means is it's an antibody that's developed-- just an aside in terms of how these monoclonal antibodies are even made by a company, essentially you can have cells that are given instructions to create these types of antibodies. And you have these massive reactors full of cells that are making these antibodies and monoclonal just means they're from one type of clonal cells. So, you have these antibodies that you would receive as an infusion and they target CD19, which is a cell surface marker on B cells. I didn't talk about the different stages of maturation of B cells but there's many different stages and inebilizumab is one of the ones that targets B cells at almost all the stages of maturity. Really importantly, it targets plasma cells, those kind of final B cells that make antibodies and so it targets them and destroys them.

Dr. Anastasia Vishnevetsky: [47:24] And so when somebody has been treated with inebilizumab, they have something that we call B cell suppression, or we hope that they are B cell suppressed because that's really how the drug works it's by leading someone to be B cell suppressed. The rest of your immune system, the entire innate immune system still works. So, a lot of, you're targeting of bacteria, parasites, a lot of these different things still works. Your T cells still work but your B cells are really reduced. Rituximab is really similar. It targets a CD20, the CD20 marker also on B cells. It's on slightly different B cells than inebilizumab, that's why they're a little bit different but really very, very similar idea. These are both drugs that are suppressing B cells, causing B cells to be depleted and that's how they work. Ocrevus or ocrelizumab used for MS, also ofatumumab or Kesimpta used for MS, are similar in that they target B cells for destruction. Moving on, eculizumab or Soliris is a drug that targets the complement system.

Dr. Anastasia Vishnevetsky: [48:43] The first complement inhibitor, I believe, that was used in-- that came to market at all, it was not developed for NMOSD initially. It was developed for paroxysmal nocturnal hemoglobinuria and was then repurposed for both NMOSD and also myasthenia gravis, another rare neuroimmune disorder. So going back to one of the earlier questions, I had said that complement proteins, these little proteins that

are made by the liver and just kind of circulate not active in the bloodstream, they go through a series of divisions or cleavages that lead them to kind of be active. So eculizumab blocks the division of one of the complement proteins, C5 five into-- and usually when it divides, it divides into C5a and C5b. So C5a is just a more generally proinflammatory anaphylatoxin. And C5b goes on to form the membrane attack complex. So, when eculizumab blocks C5 from dividing or from being divided by binding it, it really stops the complement system there. Ravulizumab is also under development for NMOSD and is a longer acting C5 blocker.

Dr. Anastasia Vishnevetsky: [50:10] So very, very similar, eculizumab and soon to be likely on the market for NMOSD ravulizumab. And then the final kind of big drug that's been approved for NMOSD is a little bit trickier to describe how it-- exactly where in NMOSD it targets. But that's satralizumab, which Dr. Bilodeau mentioned as well. So, it's a monoclonal antibody as well like the others and it targets a cytokine receptor. So, interleukin-6 is a cytokine and there's an interleukin-6 receptor and that's the target of satralizumab. And IL-6 is a really powerful proinflammatory cytokine. We actually find that it's elevated in the cerebrospinal fluid of patients with an NMOSD, and it stimulates immune activation via both B and T cell mechanisms. So, both humoral immunity and cell-mediated immunity. And so, by binding the interleukin-6 receptor, it really shuts down the activity of IL-6. Tocilizumab, which is related essentially is a similar drug, also blocks binding between the IL-6 receptor and IL-6 and prevents activation of the complex. But we tend to use satralizumab more frequently because there's a kind of dedicated trial for an NMOSD, but tocilizumab has been looked at as well.

Dr. GG deFiebre: [51:39] Okay, thanks so much. And then Dr. Bilodeau, you talked previously about tolerance. Do you mind describing what tolerance is?

Dr. Philippe-Antoine Bilodeau: [51:51] Sure, yes. Tolerance is probably one of my favorite topics. So yeah, I think I alluded to it a little bit earlier, but autoimmunity has essentially two fundamental requirements. The first one is that you need to generate autoreactive T cells or B cells, really autoreactive immune cells. And the second one is you need to have failure of tolerance. In the example that I mentioned earlier, it's not sufficient to simply generate immune cells that react to both BBB and the central nervous system. If it was, essentially everyone who gets BBB would potentially develop multiple sclerosis, which obviously isn't the case. There also needs to be failure of tolerance. And tolerance means that your immune system does not react against your own body. It does not react against self. The first mechanism of tolerance happens very early in life in the thymus and bone marrow at the stage where immune cells are first generated.

Dr. Philippe-Antoine Bilodeau: [52:47] So B and T cells are randomly generated and by chance some of them will react against self. There are mechanisms in place in the thymus to eliminate those immune cells that react against self. Essentially what happens is you've got these scavenger cells that present all these self-antigens, so these self-proteins and tells the immune system, you're not supposed to react to that. And if a cell happens to react as one of those cell antigens, it dies. And there is actually a disease called autoimmune polyendocrinopathy syndrome, type one that's caused by failure of the central tolerance. And patients get this very dramatic widespread autoimmunity very early in life. But we know that central tolerance is not perfect, and some autoreactive immune cells are going to escape. In fact, we know that about 30% of the cells that escape the thymus have the potential to react against someone's own body. And that's what peripheral tolerance is for. Peripheral tolerance happens after B and T cells enter the circulation. And it's dependent on a type of cell that we've talked about earlier briefly called regulatory T cells. Regulatory T cells essentially directly interact with autoreactive immune cells and shut them down and they do that through a bunch of different mechanisms.

Dr. Philippe-Antoine Bilodeau: [54:05] But the balance between elimination and tolerance is very delicate. So too little tolerance can lead to autoimmunity, but too much tolerance can lead to things like cancer or

ineffective response to infection. And a lot of the immunotherapy that we use for cancer and in fact, even for autoimmune diseases tries to shift that balance away from tolerance or in the case of cancer, away from tolerance and towards elimination and in the case of autoimmunity, away from elimination and towards tolerance. One of the consequences of that is that for example, cancer patients who are on immunotherapy can get all sorts of autoimmune complications and that's a really good example of how important tolerance is. One of the newer therapeutic approaches in neuroimmunology is to work on inducing tolerance. In other words, instead of trying to get rid of the immune cells that are doing the damage, we can try to teach the immune system to stop attacking the nervous system and to become tolerant again. In other words, to restore that balance between elimination and tolerance.

Dr. GG deFiebre: [55:13] Great. Thank you so much. And thank you both so much for that really comprehensive overview and I think easy to understand overview too of the immune system and how it relates to these disorders. Before we end, I just wanted to see if you had anything else you wanted to add about the topic or upcoming research or anything like that that you think is important to mention. Dr. Vishnevetsky.

Dr. Anastasia Vishnevetsky: [55:37] I think jumping off of what Dr. Bilodeau was saying, the immune system is really all about balance and a lot of different-- a lot of interrelatedness and a lot of different interactions between different aspects of the immune system. I think any time we try to teach or learn about the immune system, we have to make a ton of simplifications because otherwise you end up with one of those diagrams with arrows pointing in every which direction. But I think that it's important to keep in mind that even when you have a concept of humoral immunity as separate from cell-mediated immunity and things like that, that there's a lot of interactions between all of these different systems and also a lot of redundancy, which leads to some of the less intuitive impacts of immunotherapy and a lot of this. So, for me, for example, it was really surprising to think about that you could essentially suppress somebody's B cells and that they weren't going to get incredibly sick.

Dr. Anastasia Vishnevetsky: [56:47] And I think for a long time, we were a lot less likely to prescribe B cell therapy because we thought about it as just something that's really dangerous and going to put people at risk for really significant infections. And although there are certain infections that you're a little bit more at risk for with B cells, the degree of infection that we might have expected hasn't panned out and that just really underlines how many different fail-safe methods we have in the immune system and it's really just about maintaining that balance. And I wouldn't be surprised if a lot of our conceptualization about some of these neuroimmunologic diseases changes in the future or if we have new diseases that come out and that we're talking about in the next five or 10 years that we're all debating the nomenclature of because we just discover that they exist. So, it's definitely a new-- a very rapidly evolving field, which I think is good for patients. There's a lot of opportunities to get involved in research and there's also a significant chance that research is going to directly impact you. And so, I'll leave it at that.

Dr. GG deFiebre: [57:57] Great. Thank you. And Dr. Bilodeau, anything else to add?

Dr. Philippe-Antoine Bilodeau: [58:01] Yeah, just going off what Dr. Vishnevetsky was talking about earlier and just now I think it's worth highlighting just how revolutionary the discovery of aquaporin-4 was. Prior to that, a lot of these patients were labeled as having multiple sclerosis because we didn't know any better. They had a disease that looked like MS but somehow, they just didn't respond to MS treatment. And in the span of really essentially only 10 years, which in the research world is pretty short, so in the span of 10 years between when aquaporin-4 was discovered in 2004 at the Mayo Clinic and now, we've really worked out exactly what happens from the generation of the antibody to the cell death in the brain. And we've developed three treatments that directly affect that. And I think it's an unusually successful and an example of I think what neuroimmunology research should aim to do in the future. And I think that a lot of our efforts in the future

should be aimed at trying to find more specific biomarkers, trying to find more autoreactive-- or autoantibodies because once we do that, we can better characterize the disease and only through better characterization of the disease can we get specific treatments and ultimately help patients.

Dr. GG deFiebre: [59:26] Great. Well, thank you both so much. I really appreciate you taking the time to chat with me today about this important topic, so thank you.