Ruth Adewuya, MD (host):

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Ruth Adewuya, MD (host):

Dr. Lawrence Steinman is a professor of Neurology and Neurological Sciences, Pediatrics, and Genetics. Dr. Steinman's research focuses on what provokes relapses and remissions in multiples sclerosis. He has developed two antigen specific therapies, using DNA vaccines for MS and type 1 diabetes. He was the senior author on the seminal 1992 Nature article that reported the key role of a particular integrin in brain inflammation.

Ruth Adewuya, MD (host):

Dr. Steinman has received numerous honors and awards, including the John M. Dystel Prize from the American Academy of Neurology and the National MS Society for his research on MS, and the Charcot Prize for Lifetime Achievement in MS research. He has twice been awarded the Senator Jacob Javits Neuroscience Investigator Award by the National Institution of Neurological Diseases and Stroke, and is a member of the National Academy of Sciences and the National Academy of Medicine, which was formally called the Institute of Medicine. Can you start by talking about how long has EBV been thought to be a possible trigger for MS?

Lawrence Steinman, MD (guest speaker):

Well, for at least 10 to 20 years, EBV has been on everyone's radar scope. As I was studying neurology at Stanford as a resident in 1977, there were other viruses that people were paying attention to. The measles virus was one, some of the other herpes viruses, herpes zoster, cytomegalovirus, they were all on the screen. About 10 years ago, Alberto Ascherio at the Harvard School of Public Health did an initial large study on a military database. And there was already a signal that Epstein-Barr virus was a necessary condition for getting multiple sclerosis.

Lawrence Steinman, MD (guest speaker):

The problem, and it will come up repeatedly as we talk about it, is that more than 95% of us have been infected with Epstein-Barr virus, and we're fine, or at least we don't have MS. And that is a hard one to explain. If the score were 100 to nothing, it would be an open-and-shut case. But when the score is essentially 100 to 95 or to 97, what is it that's going on that the vast majority of people who are infected with EBV? And that is the vast majority of people don't get MS. And there are some relevant explanations from the work that we've done at Stanford that takes us many steps along the way to understanding that quandary.

Ruth Adewuya, MD (host):

Thank you for giving that background. What I'm understanding is that EBV has been on the radar for a while in terms of a possible trigger for MS.

Lawrence Steinman, MD (guest speaker):

That's right.

Ruth Adewuya, MD (host):

Fast forward to today, what's our current understanding of EBV as a cost of MS? And could you maybe even outline what the potential pathophysiological connection between EBV and MS would be?

Lawrence Steinman, MD (guest speaker):

Yes. Let's start out with two studies published in January 2022. One from the Harvard School of Public Health from Professor Ascherio, an epidemiological study published in Science, and the other paper published in Nature from our group at Stanford. The epidemiology is going to tell us what virus is associated with multiple sclerosis, and the Stanford paper will tell us, can you explain the molecular mechanism that the epidemiology may be describing? What Ascherio did was go to that same military database that he had been mining for 10 years, and now he comes up with really definitive answer. He had 10 million samples, approximately a thousand of the 10 million had multiple sclerosis while serving in the military.

Lawrence Steinman, MD (guest speaker):

When you go into the military, they draw your blood at regular intervals during your service. They found out that there were approximately 801 individuals that they could study. And of those 801 who had multiple sclerosis, I mentioned a thousand but they had only 801 that were fully valuable, 800 of the 801 were positive for EBV. That's a big deal. They had another piece of information that was very valuable. They had individuals who started out in the military that had no antibody to EBV in their blood. EBV serology detecting antibodies to EBV is done routinely. And if they did it on you or me or a hundred of us in Stanford Medical community, they again would find 95% of us had those antibodies. But of the 35 who went from negative and then developed MS, 34 of them had a conversion from EBV negative to EBV positive.

Lawrence Steinman, MD (guest speaker):

And they did one other thing. There's a marker of inflammation in the brain called neurofilament light chain, NfL, and it shows up in the blood. They made that measurement. And before they came into the military, when they looked at the NfL level in the blood, it was essentially zero, but after diagnosis of MS, it went up. You had to convert from being EBV negative to EBV positive, and then you had this concomitant data arise in that blood biomarker called NfL. So, over the years, my lab at Stanford and some of the really bright people who have worked with me have had a project to see if we could identify what the antibodies, those clonal antibodies in the CSF, cerebral spinal fluid, were recognizing.

Lawrence Steinman, MD (guest speaker):

And the people involved, the leader was Bill Robinson, who 20 years ago did a postdoc with me and now he's head of our division of rheumatology at Stanford. And another luminary in all of this was a young man named Tobias Lanz, who was a postdoc. He came to Stanford 14 years ago and spent time in my lab being trained by Dr. Peggy Ho, who also plays a very large role in this work. I'm the white-haired old guy who gets to marvel it, the amazing energy and skill of these younger investigators. So, what did they find?

Lawrence Steinman, MD (guest speaker):

Bill Robinson patented a technology to be able to take single cells that make antibody, and we sort them on a flow cytometer, a fax machine fluorescence-activated cell sort sorter, again, invented at Stanford. We could start single plasma blasts, the cells that actually make the antibody, and determine what the sequences were of the antibodies. And there's an amazing thing that happens with antibodies as they recognize their target. They start making stronger and stronger antibodies. It's a process called somatic hypermutation. The somatic hypermutation is the ability of the antibody to really lock in on a target and make a higher affinity antibody.

Lawrence Steinman, MD (guest speaker):

They isolated over a hundred of these antibodies and they started to look for exactly what viruses they were recognizing. One of the strongest signals came from Epstein-Barr virus. So, something was happening that in MS patients, antibody-making cells are getting into the spinal fluid and they continue throughout our life to make antibodies. We spent a lot of time tracking down what piece of the Epstein-Barr virus was you being recognized by a lot of the antibody clones, and it was a piece in EBNA-1. So then, another toolkit was available. You can now scan the whole human proteome with a toolkit and determine, okay, you have an antibody, it binds a virus. Does it bind any human protein? And it did. It bound a human protein called GlialCAM, glial cell adhesion molecule.

Lawrence Steinman, MD (guest speaker):

So now, GlialCAM really lit up with one of the antibodies. We then asked what part of GlialCAM. And GlialCAM is a molecule that's almost all of it is expressed in the brain, that's on the cells that make myelin, the key constituent of white matter. And we found a region of GlialCAM that was being bound, and it had a strong amount of identity in its amino acid sequence with EBNA-1. There were essentially six amino acids, five were identical and one was substituted. Further studies were done to show that when some of those regions, when they were phosphorylated, the binding even got a hundred times stronger. So, there was a couple of notable facts. One is, before the antibodies did that hypermutation, they still were able to bind EBNA-1 pretty well. After they did the somatic hypermutation, that binding became even stronger. And after GlialCAM was phosphorylated in that region of similarity, it's called molecular mimicry, the binding went up even more.

Lawrence Steinman, MD (guest speaker):

This begins to answer one of the questions. We all have antibodies to EBV, including EBNA-1, but maybe you get MS if your GlialCAM happened to be phosphorylated. Maybe you needed this additional... We talked about it as the initial fuse that has to be lit is getting infected with EBV. But then, since 95% of us who are infected aren't with MS, other stuff has to happen. So, some of these events that I just call other stuff would include phosphorylation of GlialCAM, and there's probably several other subtleties which remain to be elucidated.

Lawrence Steinman, MD (guest speaker):

There's another very interesting observation. And I was mentioning that in MS and in a few other neurologic diseases, there's increased synthesis of antibody within the central nervous system. So, about 30 years ago, discovery came out of our lab that in order for an antibody-making cell to get into the brain, they had to have a Velcro-like molecule, a sticky molecule called integrin alpha-4. And we found that the plasma blasts on the outside of the brain had high alpha-4, but when they got into the brain, they lost alpha-4. So, there's a drug, a very powerful approved drug for MS. It's been out there for 20 years, about 300,000 individuals with MS that have taken it, and it is life-changing. So, we've always been very interested in this integrin story, but here, a new wrinkle in it is the cells with high alpha-4 can get in, but they don't seem to be able to get out. They sit there and they make more and more antibody, and that perpetuates the disease. That's very interesting.

Lawrence Steinman, MD (guest speaker):

Now, I mentioned a third individual. Bill Robinson, who's really the captain of the ship and, I'm not retired but I feel like one of those old admirals who you see talking on the news stations. Tobias is the rising superstar. Peggy Ho, I mentioned, so Peggy and I have been working side by side in the lab for 20 years. She met Bill Robinson in the lab. She's married to Bill Robinson. It's another nice feature, but there's this great animal model of multiple sclerosis called experimental autoimmune encephalomyelitis.

Lawrence Steinman, MD (guest speaker):

You can't infect a mouse with EBV. It's very much restricted to humans and nonhuman primates, but we took the GlialCAM peptide sequence and put it into a mouse that was anyway going to get paralyzed in this experimental system. And when she added that peptide, they got much worse paralysis. That is important if you want to identify the suspect that's causing the disease. That's something that a pathologist in the 19th century, Robert Koch, said. You would have to infect an experimental animal and show you could recapitulate the disease. It's a variation on that theme. It fulfills those famous Koch postulates. There is that added feature that was all in the paper. Now, there's much more to be done, but the implications of all this are very inviting.

Ruth Adewuya, MD (host):

It sounds like it. And thank you so much for breaking down the two articles that are relevant that came out, both from what was published out of Harvard and what came

out of Stanford. I have a couple follow up questions that if you'll permit me to ask. So, some of it might be dumb questions, Dr. Steinman.

Lawrence Steinman, MD (guest speaker):

Before you ask your questions, I always tell people that scientists are very arrogant and they are afraid to ask. What you just called as a dumb question, we probably have the same question, so fire away, but don't expect me to give a scintillating answer. I'm probably going to tell you that wouldn't we all like to know the answer to your question.

Ruth Adewuya, MD (host):

Okay, great.

Lawrence Steinman, MD (guest speaker):

The ball is in your court.

Ruth Adewuya, MD (host):

In fact, it makes me feel so much better. I was listening to you expound on the connections between EBV and MS. And I was trying to create a picture in my head to summarize the connections and scientific information that you provided. Let me know if I'm anywhere close. It sounded to me that from the work out of Harvard, there was an understanding, 95% of us have EBV and then 5% are potentially positive for MS. Is that correct?

Lawrence Steinman, MD (guest speaker):

It's probably less than 5%, the 100% and 95%... It's probably 1%, something like that.

Ruth Adewuya, MD (host):

Okay.

Lawrence Steinman, MD (guest speaker):

It's a low number.

Ruth Adewuya, MD (host):

What I gathered is that phosphorylation of GlialCAM is a potential connection as to why people get MS.

Lawrence Steinman, MD (guest speaker):

That's right. The initial switch is if you're not infected with EBV, if you're in that 5% who never got infected, you're not going to get MS.

Ruth Adewuya, MD (host):

Yes.

Lawrence Steinman, MD (guest speaker):

That's good to know, but you have to be really lucky. This is called the kissing disease, so perhaps there wasn't enough kissing in one's history and you didn't get infected, but generally, there have to be other things going on. This is so interesting because the target of the antibody starts mining much more strongly, particularly if that GlialCAM molecule got phosphorylated. So, when they do these genetic associations to MS and other diseases, they have these very strong hits, but they also have some very modest hits. It may be one of these modest hits and some of them are genes that include phosphorylation events. Maybe you have to have this double bad luck. You get infected and then you also have a phosphorylation on one little residue on your GlialCAM molecule. And that could be the ultimate answer that takes you from the necessary getting infected or the sufficient. But, your question is the same kind of question that I'm asking. I think that you have a very good understanding at least of what I'm trying to get across.

Ruth Adewuya, MD (host):

Fantastic. Another clarifying question around whether researchers have looked into whether markers of nerve degeneration present before infection with EBV, or is that a challenge given that, again, going back to what you said, most of us do have EBV already?

Lawrence Steinman, MD (guest speaker):

Well, we've done some studies. There's a Stanford graduate, an electrical engineer whose wife passed away. She got MS after a severe bout of EBV. He's been applying engineering principles and looking at the latency of infection and how soon after you convert, and has some very interesting data. We've published together, and he comes from a completely different orientation. His name's John Andreas, and he really has done some exquisite work, but there looks like there's a period of time after the infection and it's like a clock. If you're going to get MS, it will hit you like a clock. And later infection is less important than during this critical period of infection, which happens to coincide with enlistment in the military.

Ruth Adewuya, MD (host):

You mentioned the latency of time between EBV infection and potential MS. Has that period been defined?

Lawrence Steinman, MD (guest speaker):

People are working on it. We had a guest earlier this week from the Karolinska, that's another hotbed of research. The Swedes are doing a very good job of archiving samples and specimens. Whereas the military specimens that Ascherio started with comprised 10 million, they have about 7000 MS specimens in the banks of the Swedish repositories. The whole country of Sweden has a population of about 10 million, but that's another place. The U.S. is problematic other than organizations like the military. We're really disorganized for better and worse but I think for understanding these kind of questions, since we don't have a national healthcare system. We're always going to be in a catch up situation.

Ruth Adewuya, MD (host):

It really seems like we are making much progress in learning about multiple sclerosis. In terms of treatments, I know that vaccines are a key development. Do you mind talking about their importance and the potential for treating something like MS? I'm also curious if there are any other type of treatments being researched that might hold potential therapies.

Lawrence Steinman, MD (guest speaker):

My sister had polio in 1951 I remember as a four-year old, she's a little older than I. So throughout my life, vaccines, particularly the Salk vaccine for polio, has been one of the great glories. If she only had that vaccine, her life would've been different. But, we may have the opportunity with a vaccine to EBV to put multiple sclerosis in the rear-view mirror in the same way we put polio in the rear-view mirror. And indeed, no less than Moderna, famous for their RNA vaccine to COVID, started a phase one clinical trial with a vaccine to EBV a couple of months ago, January 2022. NIH is also developing a EBV vaccine. We might be able to eradicate the disease.

Lawrence Steinman, MD (guest speaker):

We better be sure that the vaccines don't trigger MS in those who are susceptible. We're keeping a sharp eye out to make sure that the vaccine does not have a dangerous component. So far so good. And the other opportunities are with an antiviral. HIV, for instance, hepatitis C, for instance, are really controlled with antivirals. So, would an antiviral also make MS go away? And there's another more subtle type of therapy that's I would put in third place in being able to carry out. Could we just shut down an immune response that's not wanted to GlialCAM? That would be another possibility from this research. So, it's a very exciting time. I don't think we're going to have the speed of progress that we had with COVID-19. So if people are waiting for the definitive vaccine and an answer in a year, this is going to be back to the old slog of slow development, methodical one step at a time.

Lawrence Steinman, MD (guest speaker):

And then, there are some other related diseases. Infectious mononucleosis is something that we might be familiar with. And one of the devastating parts of infectious mono is fatigue. One of the devastating parts of multiple sclerosis is fatigue. And there are other syndromes, chronic fatigue syndrome, that ought to be taken ever more seriously. These may be examples of diseases caused by EBV, and an EBV treatment could be life-changing. And it's been a lot of hard work that it's paid off with these really exciting answers, which only opened the door for the need to do even more research to answer these very subtle questions. But, it's exciting.

Ruth Adewuya, MD (host):

It sounds like it. You alluded to some of this earlier. What we know right now about EBV and MS potentially translate to SARS-CoV-2 and MS?

Lawrence Steinman, MD (guest speaker):

Well, really good question. One thing is some of the therapies that people with MS take to make their disease milder, do they make them more susceptible to a virus? The good news is that we have more than antibody immunity. We have T-cell immunity. And generally, although MS patients on some of the disease modifying therapies, particularly the anti B-cell therapies, don't get very good antibody responses. They do make adequate T-cell responses, but still they have a higher incidence of COVID-19 infections and hospitalizations, but they've done pretty well. They don't act as if they have no immune system left. They do have an immune system left, but they're the ones who have to be particularly careful. I think one of the things that we've learned about COVID is how versatile the immune system is and how better understanding of immunity has really kept us from having even a worse run with the pandemic. Those vaccines came up in such a timely manner. So, I remain optimistic that the vaccine technology may eradicate multiple sclerosis.

Ruth Adewuya, MD (host):

That's exciting. And I know that you mentioned earlier that we're back to the slog of development of a vaccine for MS. But, if you had a crystal ball and you wanted to predict when you think something viable in this space an MS vaccine, what would you say? Are we thinking 10, 20 years or something like that?

Lawrence Steinman, MD (guest speaker):

I was going to try to be more optimistic, five to 10.

Ruth Adewuya, MD (host):

Okay. I like that.

Lawrence Steinman, MD (guest speaker):

Well, especially since we're using the mRNA technologies. One of the problems with other vaccines is manufacturing production. It's not a trivial problem by any means with mRNA, but it's a lot simpler than having to grow the virus. EBV is a problematic, complicated virus, but if it can be done with using mRNA for some of the envelope and capsid proteins as they're doing, it's going to be much faster. It's going to be a slow in following more conventional timelines, because we don't have the Sword of Damocles of a pandemic hanging over our head, and that will turn things back to normal.

Lawrence Steinman, MD (guest speaker):

And then, there're some issues. If we're all doing so well with the infection, if we didn't get infected, would that have any negative consequences? There's a whole concept, the hygiene hypothesis, that in the developed world, we're too clean and therefore we're more susceptible to allergies and autoimmune diseases that you don't see in parts of the world where there are lower levels of hygiene. That's interesting. It's sort of a concept that is attractive, but it's I think too bulky of a concept to have other than an impetus for clever thinking.

Ruth Adewuya, MD (host):

As we wrap up our conversation, I have few more questions. One is around patients and the implications for what we're learning about EBV and MS. For patients that currently have MS, I know there's the future work and the anticipation to eradicate it. Any thoughts on its implications for existing patients that have MS?

Lawrence Steinman, MD (guest speaker):

To use an example, if I were in a family where a sibling or a parent had multiple sclerosis, and I were a young college student and I got infectious mononucleosis, would I be freaking out? Would I want to get an antiviral, even though they're not specifically approved? And I think therefore clinical trials ought to begin really looking at targeted populations. A lot of people get infectious mono, but if you're in a family where you already have risk factors for MS, maybe it would be a good idea to head it off.

Ruth Adewuya, MD (host):

What sparked your interest in this research area?

Lawrence Steinman, MD (guest speaker):

I don't know for sure. I think the brain's the most interesting organ, and maybe the future is not just in neurology but in molecular psychiatry. There're some great work going on at Stanford with Karl Deisseroth and his studies in molecular psychiatry. But, I think probably my sister's polio got me into the whole area. It's very gratifying. And by the way, my sister, though she had polio as a seven-year old, has two children. One is a Stanford-trained cardiologist, M.D., Ph.D. Another is a lawyer. And so, she was able with a lot of hard work and praises and all of that to have motherhood, family life, and produce a Stanford-trained cardiologist.

Ruth Adewuya, MD (host):

That's fantastic. Thank you for sharing that. And then, final thoughts for clinicians who are navigating the unknown and who might be walking alongside patients that have MS. What are your thoughts in terms of what they should think about or consider as they are caring for MS patients?

Lawrence Steinman, MD (guest speaker):

Well, I think, having a better understanding of what it is to have illnesses like this, when people tell you that they're fatigued. Just imagine if we had to walk around with 30-pound barbells in each arm all day long. The challenges are just so immense. I also tell young doctors that they ought to spend a few hours once a year in the life of somebody. I didn't take care of him, I had a colleague passed away with MS, but he ran a shoe repair shop at Penn Station in New York. He called me up once and said, "You cure a lot of mice. Can I be one of your mice? Who were you studying?" I said, "But who is this?" He said, "I'm Don Burbeary. I run the shoe repair shop, Track 12, where people get off the Long Island railroad." I said, "I'm in New York a lot. I'm going to come see you." I did.

Lawrence Steinman, MD (guest speaker):

One day, he said, "Let's go to lunch in Little Italy." His last name was Burbeary. How do you get from the bowels of Penn Station to Little Italy? There's no way, he couldn't walk very well. He was in a wheelchair. We had to go through the catacombs that Port Authority police let us through one door or another. We finally get up to street level, and I'm with him in his motorized wheelchairs going along. I'm responsible. There're supposed to be curb cuts at every intersection, and there's not any curb cuts, so I'm worrying like, "What's going to happen if he tips over? Do I know how to get somebody back into their wheelchair so easily?" And he's not a small man. Then, we had a good lunch. We had probably some beverages, and the gentleman has to pee. So he says, "I'll need some help." We go to the men's room and he's got to catheterize himself. And he says, "Could you hold this?" We go back. We finish. I take him back down to the shoe repair shop, and my mind's blown.

Ruth Adewuya, MD (host):

Wow.

Lawrence Steinman, MD (guest speaker):

So, my recommendation is that everybody have to spend two hours inside the life of somebody with the illness that they take care of. We can talk about phosphorylation of serine residue 376 very calmly, but it's all about people who actually have this disease, and their life is not easy.

Ruth Adewuya, MD (host):

Thank you so much for sharing that profound story and that consideration for all of us to understand each other's experience. Thanks for sharing that. Thank you so much for chatting with me today, Dr. Steinman.

Lawrence Steinman, MD (guest speaker):

It's been a pleasure. Thank you.

Ruth Adewuya, MD (host):

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