Why Can't I feel My Feet? : Antibodies Playing on the Nerve Floor

Mr. Debprasad Dutta*

Email: debdutta.bio@gmail.com

It was a Sunday morning.

I approached Ramaswamy (name changed) to collect a blood sample with a consent form.

"Can I walk again?" Ramaswamy asked.

"Why is this happening with my husband?" distressed, his wife asked.

I tried to explain in simple terms:

"It all starts with tingling and numbness in the tips of the fingers of both hands and legs and they slowly become floppy. The patient can no longer feel their limbs. And, physical independence eventually gets lost. When inactivity reaches the lung region, suffocation begins and one needs a ventilator at times. This setback to the peripheral nervous system is called Guillain Barre Syndrome (GBS).

"The rare condition affects 1 in 1 lakh people of any age or gender worldwide. While full recovery is achieved by 80% of the sufferers, but for the rest, nerves refuse to recover. Among them, 15% lead a chair-bound life, and about 5% are unable to survive the ordeal."

I had to make a concoction of Kannada, Hindi and English to make them understand what happens in GBS. In lieu of my effort, the patient and his wife forgave me for my inability to speak their mother tongue, Telugu.

The path of destruction

In GBS, the immune system fails and attacks its own molecules on peripheral nerve surface (self-antigens). GBS either affects nerve axon or devours its coat, myelin. When

---

* Mr. Debprasad Dutta, PhD Scholar from National Institute of Mental Health & Neurosciences, Karnataka, is pursuing his research on "Guillain Barre Syndrome: Comprehensive Profiling of Infectious Triggers, Genetic Predisposition, Ganglioside & Ganglioside Complex Autoantibodies". His popular science story entitled "Why can't I Feel my Feet? : Antibodies Playing on Nerve Floor" has been selected for AWSAR Award.

axon damages, nerves cannot send messages to the muscles and the situation is called *a*cute motor *a*xonal *n*europathy (AMAN). On the other hand, because of myelin sheath destruction, the flow of electrical signals slows down due to lack of insulation, leading to another variant condition called *a*cute *i*nflammatory *d*emyelinating *p*olyneuropathy (AIDP). The symptoms range from mild weakness to full-blown paralysis. There are environmental and genetic contributors that mislead our defense system against the nervous system to push the patient towards illness.

The demystifying mission

To find out how the environment and gene work jointly in developing risk and progression of GBS, we have set three major goals. First, to explore the infectious agents that trigger disease in the Indian population. Second, to evaluate different types of self-antibodies in GBS and to assess genetic inclination in GBS. And, finally, how influence of external (infections) and internal (auto-antibodies) environment will relate to the impact of genetic components. One hundred and fifty patients with GBS and same number of healthy individuals are recruited to pursue these goals through a case-control study.

The rare condition affects 1 in 1 lakh people of any age or gender worldwide. While full recovery is achieved by 80% of the sufferers, but for the rest, nerves refuse to recover. Among them, 15% lead a chair-bound life, and about 5% are unable to survive the ordeal.

Who pulls the trigger?

About two-thirds of the patients have a preceding infectious illness, hence, GBS is regarded as a prototype of a 'post-infectious' disease. In most patients, the time gap between the infectious illness and the first neurological manifestations of GBS is one to three weeks. The clinical manifestation in the patients suggests a respiratory or gut infection. Usually, patients recover from the infectious illnesses before the onset of neurological symptoms. Overall, the incidence of infections has not been determined in a large cohort of GBS patients and has not been contrasted with an appropriate control group.

A remarkable diversity of infectious agents has been identified including viral and bacterial species. Dengue virus (DENV), chikungunya virus (CHIKV), influenza virus are prevalent in India. Zika virus (ZIKV) was recently reported to cause outbreaks of GBS in Brazil and French Polynesia. So we were enthusiastic to Fig. out the incidence of Zika virus in GBS. In collaboration with the Center for Disease Control and Prevention (CDC), we examined the presence of ribonucleic acids of those viruses in patients with GBS using real-time Polymerase Chain Reaction (RT-PCR) assay. However, Zika virus was not detected in our study population. Meanwhile, we found antibodies against dengue, chikungunya and Japanese B encephalitis have by IgM capture ELISA in serum and cerebrospinal fluid of patients. Our investigation found that nearly one-third patients develop GBS after *Campylobacter jejuni* infection; C. *Jeuni* is the major GBS-inducing bacterium globally.

The incidence of infectious events in GBS is often underweighed because some infections undergo a subclinical course whereas others are challenging to diagnose since they precede GBS by weeks. With this, we could reasonably say that infections are a vital inducing mechanism in GBS pathobiology.

Molecules can mimic ॥

Upon invasion of pathogens, the immune

system of the body produces antibodies against the glycolipid component of the microbial surface. But the antibodies react with ganglioside of peripheral nerves. This is because ganglioside looks similar to microbial antigens and patient's antibodies get fooled. The behaviour of anti-microbial antibodies as auto-antibodies and their accidental encounter with neural gangliosides is known as molecular mimicry. To find out about auto-antibodies, we developed an in-house Enzyme Immuno Assay (ih-EIA) and the presence of anti-ganglioside antibodies, namely, anti-GM1, GM2, GD1a, GD1b, GT1b and GQ1b have been confirmed in patients with GBS. M, D, T and Q denote monosialic, disialic, trisialic and quadrisialic acid residues that added to ganglioside (G). Interestingly, like two gangliosides can jointly act as a single antigen and, hence, their antibodies are called Ganglioside Complex (GSC) antibodies. Our study supplies evidence for this notion. Presence of singular antibody against GM1 and GM1-containing complex antibodies is most common in our investigation.

Mimicry among chemically different epitopes has renewed the theory that a single antibody should always encounter only one antigenic epitope. Molecular mimicry is not a nonspecific immune reaction rather a part of the adaptive immune system and assumes a pivotal role in response to infectious triggers. Pathogens might stimulate the immune system and result in a tolerance breakdown, which could permit proliferation of cross-reactive B and T lymphocytes. Ultimately, molecular mimicry leads to the induction of auto-antibodies and, thereby, induces GBS pathogenesis.

About two-thirds of the patients have a preceding infectious illness, hence, GBS is regarded as a prototype of a 'post-infectious' disease. In most patients, the time gap between the infectious illness and the first neurological manifestations of GBS is one to three weeks. The clinical manifestation in the patients suggests a respiratory or gut infection.

Proof in polymorphism

Genes play a dictating role despite the synergy of all environmental impactions different people show different vulnerability to GBS. Candidate gene approach can provide valuable insight into the onset and progress of the disease. The genetic susceptibility of patients to infectious agents contributes to variation in clinical outcomes of the patients. Research in this area, however, is insufficient and inconclusive.

Finely controlled signaling of an innate immune receptor called Toll Like Receptor (TLR) is needed for the host to respond to the challenge of infections. If TLR signaling is impaired it can prompt susceptibility to infection. Several single nuclear polymorphisms (SNP) within individual TLRs have been identified and some connections are found with the progression of autoimmune disorders.

TLR2 acts as a ganglioside co-receptor. TLR4 recognizes lipopolysaccharide of *C. Jejuni* TLR3 encounters viral nucleic acids. Therefore, we hypothesized variation among those TLR genes will alter their expression pattern and, thereby, modulate the risk and severity of GBS. Our results suggest two particular polymorphisms in TLR2 increase risk up to 24-fold. Not only that, one of the variants significantly elevates the creatine kinase (a muscle-acting enzyme) level. Overall, the evidence provided by those polymorphisms highlights the role that TLRs play in GBS.

Epilogue

Data on the infectious pathogens have public health importance as it gives an epidemiological insight into triggering infections

in GBS. Our largescale case-controlled study essentially unravels the genetic enigma of this life-threatening illness. A point to note, this comprehensive investigation of GBS is the first and largest in India. Functional analyses of glycol-epitopes of ganglioside complexes in membranes supply new knowledge on antibody-antigen interaction in GBS and shed light on micro-domain function through carbohydrate-carbohydrate interactions. And, therefore, based on the antibody profile, immunological sub-typing of GBS can be done. The current work promises to open new avenues for immunogenetic understanding of GBS. The findings are useful to discover novel biomarkers for better diagnosis. In fact, we have successfully initiated ganglioside antibody testing as a part of routine diagnosis of GBS in NIMHANS.

After my long narration, Shreya (the new intern) looked a little sleepy. So I felt appropriate to stop the converstaion.

But immediately her eyebrow rose, "What happened to Ramaswamy?"she asked.

I resumed my storytelling.

"Ramaswamy started on the road to recovery after a few weeks. He gained strength in his fingers and could hold a pen and write his name again.

"Now I have got my feet back!" his facial expression match relayed his feelings.

"He can walk without support," his wife smiled.

"If many such Ramaswamys will find better diagnosis and treatment in future, this would be my best gift," I said.

She stood up from her seat, "You tell it like a tale; why don't you write for common people," she suggested.

I promised her that I would share this story in 'Vigyan Prasar' magazine. If they publish it, I will get an opportunity to inspire research among the students of my village.