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University of Nevada, Reno

**Guillain–Barré Syndrome: Identification of Acute Motor Axonal Neuropathy and  
Representative Case Study**

A Thesis submitted in partial fulfillment  
of the requirement for the degree of  
Bachelor of Science in Biology and the Honors Program

by

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We recommend that the thesis  
prepared under our supervision by

**Joshua A. Rooney**

entitled

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be accepted in partial fulfillment of the requirements for the degree of

**Bachelor of Science, Biology**

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May 2013

***Abstract***

Guillain–Barré Syndrome is an autoimmune disease that leaves up to 20 percent of patients severely disabled and approximately five percent dead(Yuki,2012). This is often due to a misdiagnoses which stems from a lack of knowledge about the variants of GBS and their pathology. Molecular mimicry is one possible pathogenic mechanism of auto-immune diseases such as Guillain–Barré(Blank, 2007). Acute Motor Axonal Neuropathy GBS is often linked to C.jejuni infections. This is attributed Anti-GM1 IgG Antibodies Affect on the Nodes of Ranvier which is clearly outlined in a stepwise manner starting from infection and ending with treatment. This is a possible explanation of the link between C.jejuni and AMAN GBS.

A case study of a 46 year old patient presenting with GBS-like symptoms is used to demonstrate how to correctly diagnosis AMAN GBS.

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## Chapter 1: Introduction

### Symptoms

There are two types of Guillain-Barré (GB) syndrome, Acute Inflammatory Demyelinating Polyneuropathy(AIDP) and Acute Motor Axonal Neuropathy(AMAN). The only difference between them as far as symptomology is concerned is that demyelinating polyneuropathy is more common among individuals of European descent while axonal neuropathy has a higher prevalence among Asian and Latin ethnic groups. Up to 20 percent of patients remain severely disabled and approximately five percent die, despite immunotherapy(Yuki,2012).

Most of the symptoms and diagnostic tools are consistent for both types of Guillain–Barré. The first symptoms of the Guillain–Barré syndrome are numbness, paresthesia, weakness, pain in the limbs, or some combination of these symptoms(Yuki, 2012). The main feature is progressive bilateral and relatively symmetric weakness of the limbs, where the weakness progresses over a period of twelve hours to twenty-eight days before a plateau is reached. This weakness is often noticed as a “pins and needles” sensation as well as unsteady walking. Around seventy-five percent of patients have a history of preceding infection of the respiratory or gastrointestinal tract usually within three days to six weeks of this muscle weakening. Infections that have been linked to Guillain–Barré include: C. Jejuni; Epstein Barr; cytomegalovirus; mycoplasma; and HIV.

Although acute areflexic paralysis is the chief symptom it can provide many complications. One of the most deadly complications is that paralysis can spread to the

muscles that control breathing, thus causing the temporary need of a respirator. Because paralysis can leave patients stationary for long periods of time, blood clots and pressure sores are also complications. The last thing to be weary of is relapse, it has been shown that up to 10 percent of patients suffering from Guillain–Barré experience a relapse at some point in their lives.

**Diagnosis:**

The initial diagnosis of Guillain–Barré is difficult due to the wide variety of symptoms. The first step is to recognize the major symptoms as discussed above, with the most important being progressive bilateral and symmetrical weakness of limbs. This, combined with a history of a recent infection, is indicative of Guillain–Barré. Although hyporeflexia, or areflexia (reduced or absent reflexes), are common signs of Guillain–Barré, roughly 10 percent of patients do have normal reflexes during the course of the illness so the diagnosis based upon these signs should be excluded in a patient with normal or brisk reflexes (Pritchard, 2010). Because the disease is located in the peripheral nerves, not the brainstem, muscles, spine or cauda equina, the presence of distal paresthesia greatly increases the likelihood that Guillain–Barré is the correct diagnosis. One syndrome that is often confused with GB is Miller Fisher (MF) Syndrome. The best way to eliminate MF as a possible diagnosis is to check for extra ocular muscle weakness which is present in Miller Fisher Syndrome but absent in Guillain–Barré.

There are several procedures that can be used to confirm the diagnosis of Guillain–Barré and rule out causes, such as vasculitis, beriberi, porphyria, toxic

neuropathy or Lyme disease. A lumbar puncture is usually performed in order to remove both infectious and malignant diseases, such as Lyme disease and lymphoma. A cerebral spinal fluid white count of over 10/ul tends to indicate that the patient is suffering from leptomeningeal malignancy, HIV, or another infectious disease(Winer, 2008).

One of the things that is looked for in the diagnosis of Guillain–Barré syndrome is the presence of albuminocytologic dissociation. This increase in protein in the CSF is a strong indicator of Guillain–Barré, however this only occurs in approximately fifty percent of early cases of Guillain–Barré. This marker does, however, increase over the course of the disease. This means that if the lumbar puncture is taken early in the disease it is very possible that proteins have not had a chance to accumulate in the spinal fluid.

Another helpful procedure is Neurophysiology because abnormalities are found in eighty-five percent of cases. These abnormalities usually consist of prolonged ‘f’ wave latencies, and dispersed motor potentials. Neurophysiology can also be used to help classify which subgroup of Guillain–Barré syndrome is affecting the patient.

Another key molecule that shows up in blood tests and is often associated with Guillain–Barré are antiganglioside antibodies. Twenty-five percent of patients with acute inflammatory demyelinating Guillain-Barre and fifty percent of patients with the large amounts axonal variant of Guillain–Barré possess anti-GM1 antibodies(Pritchard, 2010).

There have been a few cases of what appears to be familial Guillain-Barre syndrome. The prevalence however is so low that there appears to be no clear gene mutation linked to Guillain–Barré.

**Treatment:**

In developed countries, only five percent of patients suffering from Guillain–Barré syndrome die. These deaths are usually related to complications such as sepsis, pulmonary emboli, and heart attack(Winer, 2008). The best way to avoid these complications is to begin treatment soon and remain under observation. Unfortunately there is little that can be done to treat Guillain–Barré as a disease. Most current treatment involves treating complications that arise from Barré. Many patients with Guillain–Barré syndrome are not ambulatory and are often treated with subcutaneous heparin and compression stocking to protect against deep-vein thrombosis. Mechanical ventilation is also often required when muscle weakness spreads to the muscles involved in respiration. An early assessment of swallowing will help to identify a patient’s risk for aspiration and if a nasogastric tube might be required.

Immunotherapy is often used in addition to the above precautions. Although plasma exchange is not a cure for Guillain-Barre it was the first treatment shown to effectively hasten patient recovery. Plasma exchange has been found to be most effective within the first 2 weeks of disease onset. Plasma exchange functions by nonspecifically removing antibodies and complements and appears to reduce nerve damage as well as improve clinical improvement. The classic treatment involves five exchanges over two weeks(Yuki, 2012).

Intravenous immune globulin has also shown to be about as effective as plasma exchange if initiated within two weeks of disease onset. Although it is unclear why

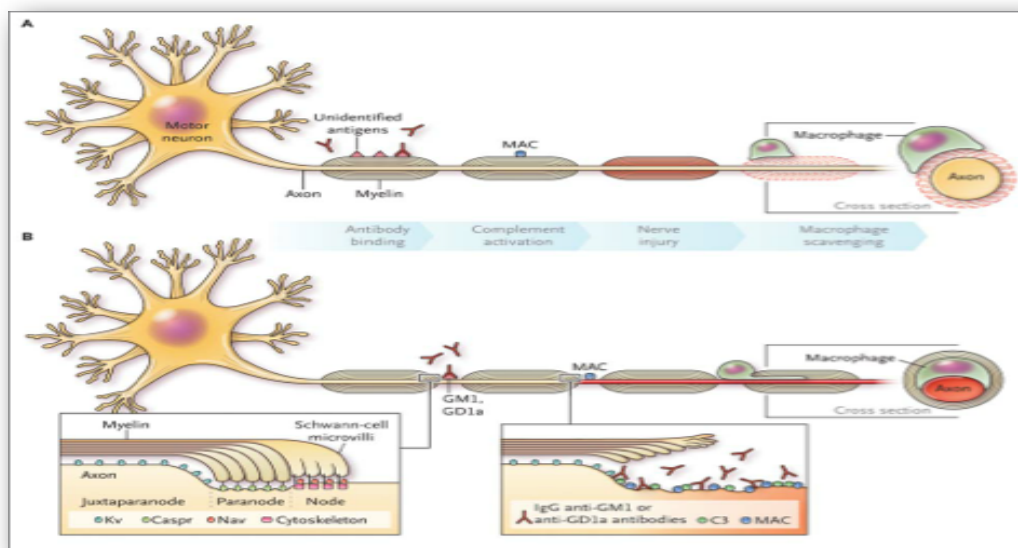
immune globulin helps it is believed that it may act by neutralizing pathogenic antibodies and inhibiting autoantibody-mediated complement activation. Although both treatments are effective there has been no conclusive evidence that a combination of both is any better than either treatment alone.

**Recovery:**

Recovery begins, on average, four weeks after the onset of the disorder.. Many patients show complete recovery within a year, although minor areflexia might persist. Unfortunately, upon recover, five to 10 percent of patients still have severe disabilities such as sensor axonal damage. All patients have to spend months, or longer, in physical therapy regaining muscle strength which is lost due to of long periods of inactivity.

**Pathology:**

The exact pathology of Guillain–Barré is still under close examination. At its core GB is an autoimmune neuropathy, a group of paralytic syndromes often characterized by peripheral nervous system inflammation. Although both types of Guillain–Barré have similar symptoms they are accomplished through different means.



**Fig 1. Immunopathogenesis of Guillain-Barré Syndrome AIDP vs AMAN**

Panel A demonstrates the possible immunopathogenesis of AIDP

Panel B demonstrates the possible immunopathogenesis of AMAN

Guillain-Barré pathology is still not well understood. In this version of Guillain-Barré there is demyelination of peripheral nerves caused by macrophage-mediated stripping of the myelin sheath (Hafer-Macko et al. 1996b). Macrophages are attracted to inflammatory infiltrates, as shown by the peripheral nerves. It is possible that this may be in part mediated by antibodies and complement deposition on Schwann cells and myelin membranes. Unfortunately, at this time, the antigenic targets that might be involved in this are unknown. In AIDP, demyelination can be extensive throughout the length of the nerve, especially in proximal nerve roots and distal intramuscular nerve segments where the blood-nerve barrier is weak. (Olsson, 1968) Recovery is possible in most AIDP cases because resting Schwann cells proliferate and then migrate to damaged sites to remyelinate axons. Axons are generally unaffected in AIDP, although may suffer so-

called bystander injury, the mechanisms for which remain unclear and deserve further study (Willison, 2005).

Acute motor axonal neuropathy involves the targeting of primarily the axolemmal membrane. Like AIDP this inflammatory process occurs mostly in the nerve roots or distal nerve terminals. In AMAN, IgG and its activated complement bind to the axolemma of motor fibers at the nodes of the Ranvier, followed by the formation of the membrane-attack complex (Hafer et al, 1996). This nodal lengthening is followed by axonal degeneration of motor fibers without lymphocytic inflammation or demyelination unlike AIDP.

Immune attack can lead to reversible axonal conduction block due to reversible axonal injury or complete axonal transection other, myelin however is generally unaffected. Depending on the site of transection (proximal or distal), axonal recovery may be poor or good, owing to the distance over which regeneration is effective (Willison, 2005).

Unlike AIDP, recent research has shown significant indications toward possible causes of AMAN as well as their pathology. The next chapter is going to focus on these causes with specific attention given to bacterial infection and carbohydrate mimicry induced autoimmune reactions.

## **Chapter 2: Anti-GM1 IgG Antibodies and their Affect on the Nodes of Ranvier**

During 1976 a mass immunization against A/New Jersey/1976/H1N1 “swine flu” occurred in the United States. People who received the vaccine were found to be at increased risk for the development of the Guillain–Barré syndrome (Yuki et al, 2012). Although other seasonal influenza vaccines have not been associated with the same increase in risk, This finding case caused research into the connection between infections and Guillain–Barré. By the end of this chapter it will be clear that the production of anti-GM1 IgG antibodies in response to *c. jejuni* infection and their disruption of myelin binding and sodium channel cluster formation in at the Nodes of Ranvier is the pathology behind AMAN Guillain-Barre.

### **Molecular mimicry:**

Molecular mimicry is one possible pathogenic mechanism of auto-immune diseases such as Guillain–Barré. Molecular mimicry was originally coined by R. Damian in 1964, when he suggested that antigenic determinants of micro-organisms could resemble the antigenic determinants of their host (Blank, 2007). He suggested that this system might be a defense mechanism of the microorganism in the hope that its host would mistake it as self and not mount an immune response. Today however molecular mimicry is mostly associated with antigenic determinants of microorganisms that might create an auto-immune response in its host.

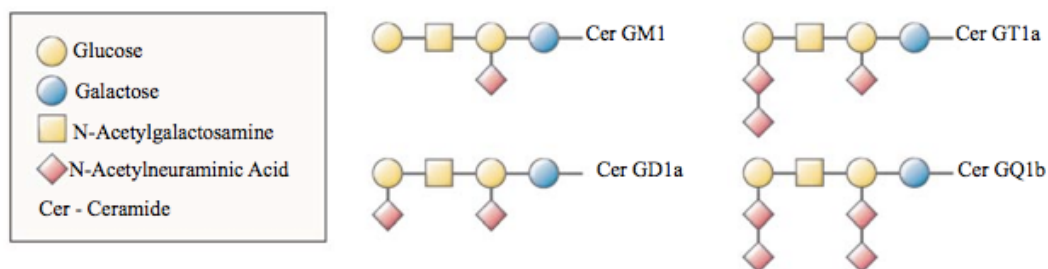


A classic example of molecular mimicry can be observed between *Streptococcus pyogenes*, responsible for rheumatic fever, and auto-immune disease in humans.

Rheumatic fever is often associated with heart damage, which is in fact caused by host B-cells. This happens even though *S. pyogenes*, is an infection of the upper respiratory air ways. This infection allows presentation of the infectors antigens which activate B-cells that cross react with heart proteins such as actin (Blank, 2007)..

### Carbohydrate Mimicry as Acute Motor Axonal Neuropathy Cause:

Ganglioside are important components of the peripheral nerves. GM1, GD1a, GT1a, and GQ1b are four gangliosides that differ with regard to the number and position of their sialic acid (Fig 2.) IgG auto antibodies of GM1 and GD1a are associated with AMAN.



**Fig2. (Yuki et al, 2012)** IgG autoantibodies to GM1, and GD1a are associated with AMAN while GQ1b, and GT1a are associated with Miller Fisher Syndrome.

IgG autoantibodies to GQ1b, which cross-react with GT1a, are strongly associated with the Miller Fisher syndrome. The localization of these target ganglioside

antigens has been associated with distinct patterns of ophthalmoplegia, ataxia, and bulbar palsy. GQ1b is strongly expressed in the oculomotor, trochlear, and abducens nerves, as well as muscle spindles in the limbs (Chiba et al, 1993). Motor and sensory nerves have similar quantities of GM1 and GD1a, which offers a possible explanation of the differences between AMAN Guillain-Barre and Miller Fish Syndrome.

Anti-GD1a IgG antibodies first were detected in two AMAN patients (Yuki et al., 1992). Thereafter, significant associations were found in 37 patients between the presence of anti-GD1a IgG antibodies and the prolonged artificial ventilation with poor recovery after 3 months (Yuki et al., 1993b).

As mentioned earlier *C. jejuni* infections have often been associated with Guillain-Barré syndrome. Because of this *C. jejuni* was proposed to be a source of molecular mimicry leading to GB. To investigate if *C. jejuni* infection does elicit GB, serial electrodiagnostic studies were conducted on *C. jejuni*-positive GBS patients (Kuwabara et al., 2004). Of these 22 patients with *C. jejuni* infections prior to GB symptoms, 16 were classified as having AMAN (n 1/4 16, 73%), while only 5 were classified as having AIDP (n 1/4 5, 23%). The patients with AIDP showed small abnormalities but rapidly normalized in less than two weeks, with all patients eventually showing AMAN pattern (Yuki, 2007). It became clear that *C. jejuni* is not related to the AIDP variety of GB. Later studies have shown that Epstein-Barr and cytomegalovirus might be associated with AIDP however more research is necessary. To further clarify the link between *C. jejuni* and AMAN to anti-ganglioside antibodies there was a study of 86 Japanese patients with GBS (Ogawara et al., 2000). Roughly three fourths of the

patients clearly suffered from the two main types of GB. Of these patients thirty-eight percent suffered from AMAN and thirty-six from AIDP. Tests were run to determine the presence of anti-ganglioside IgG antibodies. In forty percent of the patients anti-IgG antibodies to GM1 were found and anti-IgG antibodies to GD1a were found in another thirty percent of patients. In nearly all cases patients that had either antibody had the AMAN pattern of GB.

### **Why *C. jejuni*?**

*Campylobacter jejuni* is a gram-negative bacterium present in the intestinal tracts of a wide range of animals that lacks homologs of virulence factors found in other pathogens (Poly et al, 2008).

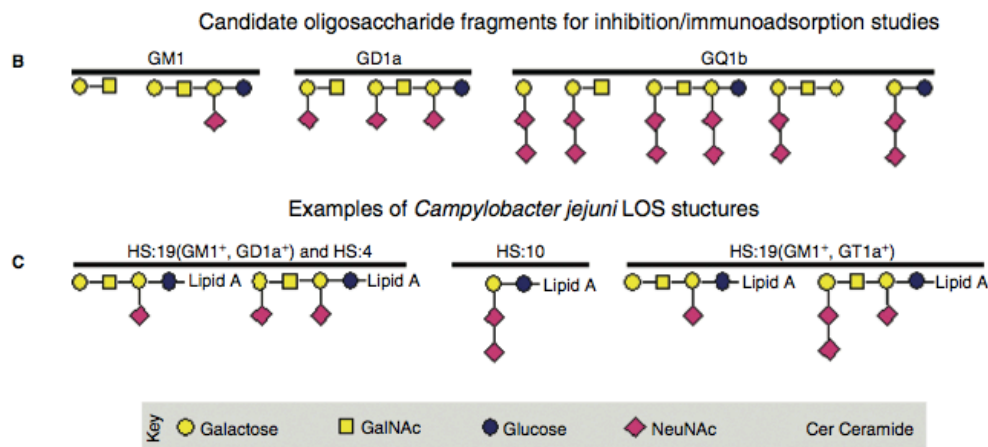
Of the many structures expressed by *C. jejuni*, LOS or Lipooligosaccharide, is often considered the most important. Lipooligosaccharides are variants of the glycolipid, lipopolysaccharid and is commonly found in bacterium that colonize mucous surfaces. LOS are highly variable molecules created through the use of many different bacterial genes and enzymes.

### **A stepwise process of pathogenesis of AMAN Guillian-Barre with *C. jejuni***

#### **Step 1: Infection by *C. jejuni* with GM1 like Lipooligosaccharide chains.**

There are two important prerequisites required for a *C. jejuni* infection to trigger AMAN Guillain-Barre. The first prerequisite is that the *C. jejuni* bacteria must contain LOS sialylated lipooligosaccharides (LOS) on the cell surface that mimic GM1 or GD1a.

Comparison of the LOS loci of various *C. jejuni* strains has demonstrated that only the class A, B and C LOS loci contain the genes that are necessary for the biosynthesis of these ganglioside mimics (Parker 2005). An experiment was conducted by Islam et al. in order to determine which loci are responsible for AMAN. When *C. jejuni* samples from patients suffering from AMAN were compared to control samples from patients with enteritis, they indicated that while most *jejuni* strains can be characterized as one of the five LOS locus classes, the class A and B LOS locus was significantly associated with GBS-associated strains compared to the controls. Fifty seven percent of *C. jejuni* from GBS patients contained LOS locus A compared to seven percent of patients suffering from enteritis (Islam, 2009). *C. jejuni* with LOS locus A contains *cst-II* (Thr51) which can express GM1-like or GD1a-like lipooligosaccharide (LOS).(Fig. 3) GM1-like LOS are similar to a specific fragment of GM1. This fragment consists of 5 molecules of importance. These molecules can be found in figure 3, B.



**Fig3.(Willison, 2005)** HS:19 and HS:4 are two LOS structures common to Locus A. They are structurally similar to GM1 and GD1a oligosaccharide fragments found in

several locations in the human axon. The similarity of GM1 consists of two molecules of galactose, one of which is bound to NeuNAc as well as a molecule of GalNAc and a glucose cap.

The second prerequisite involves why so few *C. jejuni* infections trigger AMAN. Anti-LOS/ganglioside antibodies exist within the natural antibody repertoire, acting as innate defense against bacteria (Martin et al., 2001). Despite this however, ninety nine percent of humans infected with ganglioside-mimicking strains of *C. jejuni* neither develop anti-LOS/ganglioside antibodies nor GBS (Nachamkin, 2001). Being carbohydrates, gangliosides elicit T cell-independent (TI) humoral responses (Martin et al., 2001). To prevent autoimmune reactions, their level and affinity are controlled by tolerance. Auto reactive B cells are regulated both by a variety of extrinsic mechanisms, which limit their encounter with self-antigen, restrict growth factors and reduce immunogenic costimulation, and by antigen-specific mechanisms, which limit T cell help and eliminate auto-reactive B cells (Ferry, 2006). However, even when autoantibody levels are generated, they often cause little or no complications.

In the absence of this B-cell tolerance antibodies can be formed through the secondary response in the following manner. The GM1 like chain of the *C. jejuni* is recognized as foreign and the cells begin to proliferate and differentiate in response to the antigen. This takes 5 to 7 days which explains why Guillain-Barre does not affect patients right at infection. Next the antibody concentration increases exponentially as the stimulated B cells differentiate into plasma cells and secrete antibody. These formed antibodies are immunoglobulin G (IgG). These protein complexes are composed of four

peptide chains, two of which are heavy chains and two that are light chains(Thomas, 1996).

It is the combination of this breakdown of B cell tolerance and the presence of *C. jejuni* with GM1-like LOS chains that leads to AMAN GBS. The immune system produces anti-GM1 antibodies in response to the *C. jejuni* infection which are not properly recognized as auto-reactive because of faulty B-cell tolerance. These antibodies are the molecules responsible for the onset of AMAN.

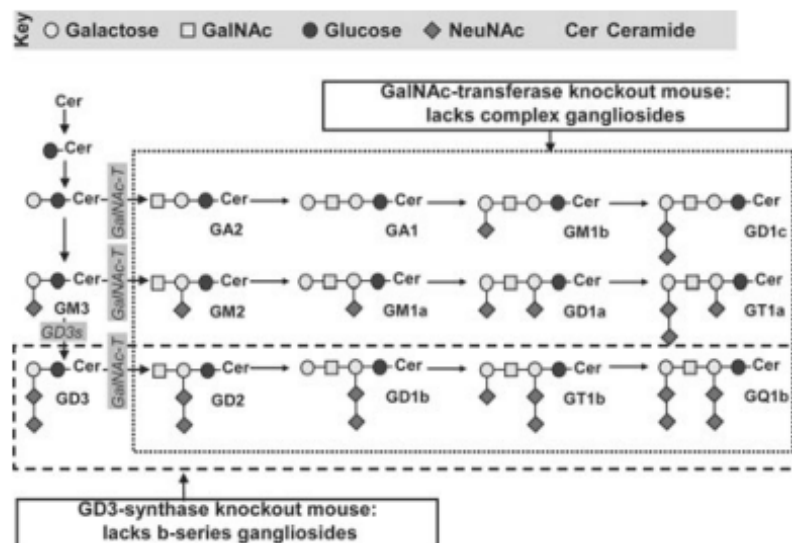
## **Step 2: Anti-GM1 antibodies bind to the nodes of Ranvier.**

Nodes of Ranvier are 1 micrometer long gaps between two adjacent myelin segments and contains clusters of Nav(Na<sup>+</sup>) channels (Suski, 2008). These nodes functions to regenerate the action potential of an axon allowing rapid and efficient action potentials. Schwann cells bind to these nodes and produce multilamellar lipid rich myelin. This myelin increases membrane resistance and decreasing membrane capacitance helping to conserve ionic charge during axolemma depolarization. In the peripheral nervous system the nodal axolemma is contacted by the microvilli of the Schwann cells. The microvilli embed in a specialized ECM around nodes containing proteoglycans syndecan-3, V1 isoform of versican, and NG2. (Suski, 2008).

In the peripheral nervous system the nodal axonal cell adhesion molecules (CAMs) and the glial-derived extracellular matrix (ECM) mediate the initial Nav channel clustering. Glial ECM molecule called gliomedin mediates the initial NF186 clustering in the PNS (Suski, 2008). Gliomedin is a glial ligand for NF and NrCAM and is expressed by myelinating Schwann cells

and accumulates at the Schwann cell microvilli and surrounding nodal ECM. After cleavage, gliomedin assembles into multimers, and is incorporated into the Schwann cell ECM by binding to heparan sulfate proteoglycans. The olfactomedin domain of gliomedin mediates its interaction with NF186 and NrCAM (Davis, 1996).

The total model of PNS node formation involves the clustering of NF and NrCAM preceding the addition of ankyrinG and Nav channels. At these sites gliomedin binds causing the initial clustering. The nerve channels then bind to the ankyrinG and mediate the currents necessary for action potential propagation (Suski, 2008). In the PNS the GM1 glycolipid is found in high concentrations. This glycolipid allows of the binding of the anti-GM1 IgG antibodies to bind direct axolemma, cell membrane of the axon, disrupting the binding of the schwann cell microvilli to the axon nodes.



**Fig 4.(Willison, 2008)** This is from an experiment involving the knockout of b-series gangliosides. It can be used to demonstrate the typical chains found on the axons. The LOS of *c. jejuni* targets the GM1 section of the chain.

The anti-ganglioside antibodies produced after the *C. jejuni* infection have the potential to bind to any membrane that contains gangliosides. AMAN GBS involves then binding of these antibodies to the exposed axolemma at the nodes of Ranvier, predominantly at the motor nerve terminals and only occasionally at the spinal anterior roots.

### **Step 3: Formation of membrane attack complex**

After the IgG is deposited at the nodes they activate their complement, and the C3 components are deposited at the nodes. C3 is a central protein of the complement system, which is important to the immune defense and provides a link between innate and adaptive immunity (Janssen, 2007). This protein functions by helping the immune system recognize and eliminate pathogens and as is the case in AMAN elicits inflammatory responses. The final product of these components, the membrane attack complex(MAC), is formed at the nodal axolemma. Anti-GM1 immunoglobulin G antibodies IgG Ab cause complement-mediated attack with MAC formation at the nodal and paranodal axolemmas. These clusters alter the ability of NF of the axon to bind with the NrCAM of the Schwann cell microvilli. Despite the presence of gliomedin the anti-GM1 IgG antibodies get in the way of the ligation of NrCam with NF.

Nav channel clusters are altered by the destruction of structures that mediate their stabilization, including the axonal cytoskeleton at nodes, Schwann cell microvilli, and paranodal junctions. As auto-immune-mediated destruction spreads, Nav channels and other components at and near nodes disappear (Yuki, 2007). This disappearance leads to



reduced ability of impulse transmission as well as a detachment of paranodal myelin loops. Although the myelin is not being removed from the axon it is no longer able to bind from the Schwann cell to the axon because of the interfering antibodies. In a serious enough case this leads to consequences much like that of demyelination.

#### **Step 4: Sodium channels and Muscle weakness**

Voltage-gated sodium channels are essential in the generation and propagation of action potentials (APs) in excitable tissues such as muscle, heart, and nerve. Proper activity of these channels is crucial to the initiation of APs which ultimately lead to muscle contraction or neuronal firing (Simkin, 2011). The lengthening of the complement-deposited nodes leads to the disappearance of sodium channel clusters. Another important change is the detachment of paranodal myelin terminal loops, which mimics paranodal demyelination. This explains the similarities between AMAN and the demyelinating version of GBS despite their extremely different pathology (Yuki, 2007). Anti-ganglioside antibodies and the resulting complement activation initially would affect the node, after which axonal lesion would spread to the paranodal region. Caspr and contactin are axonal proteins expressed on the paranodal axonal membrane. When immune attack involves the paranode, the axoglial junction is disrupted. These changes have effects on nerve conduction similar to those of paranodal demyelination (Yuki, 2007). Like demyelinating GBS the reduced resistance in the axons causes a reduction in the speed at which impulses can propagate along demyelinated axons or in the case of AMAN, axons with damaged sodium channels.

The Nodes of Ranvier in combination with the myelin sheath allow for the rapid and efficient saltatory propagation of action potentials. This is the process which allows signals to be sent all around the body through the nervous system. Because of the elimination of sodium channels the action potentials begin to slow down. They are no longer as efficient. The paranodal region of the axon is lacking properly connected myelin causing the action potential to occur outside of just the Nodes of Ranvier while the lack of sodium channels reduces the speed at which the nodes can be repolarized. This slows down the action potentials and can be perceived as muscle weakness. If this damage is allowed to continue long enough the axons can become damaged to the point that so little of the signal gets properly transmitted that there is complete paralysis. Luckily once the concentration of the anti-GM1 antibodies is decreased they are replaced on the NF of the axon by the Schwann cells over time. This allows for the slow rebuilding of the axonal pathways.

### **What this means for treatments**

Currently GBS is not preventable and as of now it seems unlikely that it ever will be. Despite several studies done there has yet to be any genetic link found in GBS patients that can explain why some patients develop the right type of B-cell control abnormality that allows them to be affected. There have been a couple cases of familial GBS but not enough to indicate that the capability of developing it is genetically linked.

Knowing more about AMAN GBS however helps to optimize acute therapy. One theory presented by Dr. Willison is that a treatment aimed at antibody neutralization or the removal/suppression of antibody effector function should be investigated (Willison, 2005). One way of accomplishing this would be to neutralize the antibodies by exposing them to soluble oligosaccharides which would bind to the antibodies in the system reducing the amount that are available to bind to axonal nodes. This is hopeful because later studies have indicated that the concentration of antibodies, not just their presence, affects the severity of symptoms.

## **AMAN GBS Case Study**

### **Chief Complaint**

David King is a 46-year-old male who was brought in by his wife complaining of 1 week h/o weakness.

### **HPI**

David is a 46-year-old man complaining of shortness of breath who appears alert but is unable to walk without assistance and complains of pain in his limbs. His wife gives the recent history.

David was well until the past week. Healthy enough to have gone on a backpacking trip with his sons and their scouting troop the weekend before. He came back from that trip and had to take off a couple days from work due to a severe case of diarrhea. After two days off he went back to work but his wife states that he seemed sore.

This morning when his wife got up to go to work she found David on the floor next to the bed. When he got up in the morning he was too weak to stand up and instead fell to the floor. David was coherent but in obvious and extreme pain. He was so weak that his wife had to get their son to help him to the car so she could bring him to your office. David's wife states that "My husband has never been like this before he is normally a healthy strong man."

### **PMH**

David's wife provides a brief medical history. David is an active father of three with no current medications. He lives an active life style involving regular camping a

basic exercise. David is rarely ill and even when sick he isn't likely to take off work. He has yearly physicals required by his job and has never had any problems noted. David rarely drinks, occasionally having a beer at football games or holiday parties, and has no history of drug use. The only time David has ever had any type of medical procedure was a appendectomy as a child.

### **Family History**

David is the oldest of 4 siblings. None of his siblings have any form of medical condition, with the exception of childhood asthma. David maintains a good relationship with his siblings and mother. His father died when David was young to heart disease. Davids wife mentions that diabetes runs on both sides of his family and heart disease on his fathers. There are no other diseases that run in the family.

### **Social/Sexual History**

David is a former marine and current police officer. He has been married to his wife for the last 20 years of his life and has three children. His wife says that although they have the occasional argument their family life is good and both her and David state that he has had no other sexual partners and neither of them have an STDs. His job is very active and despite his busy hours he still manages to work out frequently and do scouting activities such as hiking and camping with his children. David admits to drinking only occasionally, averaging less than a beer a week. He denies recreational drug use and has annual drug screenings for his job. David has never smoked, takes a multivitamin daily, and is rarely ill.

**Medications/Allergies**

David is not currently on any prescription medications and has never had any negative reactions to prescription drugs. David also does not have any environmental allergies that he is aware of.

**Physical Exam / ROS**

David's vital signs, as recorded by the nurse, are as follows:

Temp: 98.1°F BP: 121/80 HR: 75 bpm

Physical Exam findings for David King:

**General:** Thin pale male that appears to be in moderate respiratory distress with the use of accessory respiratory muscles

**Skin:** No Rashes, lesions, or evidence of jaundice

**HEENT:** Pupils equal round and reactive to light and accommodation. No sclera icterus. No injection of conjunctive. Auditory canals clear, TM's clear. Both nostrils patent. Nasal mucosa moist and pink. Mucous membranes moist and pink. No erythema of the posterior pharynx. No tonsillar hypertrophy or exudates. Neck has no masses. Trachea midline. No thyromegaly

**Pulmonary:** Evidence of retractions and use of accessory respiratory muscles. Normal AP diameter. Lungs clear to auscultation bilaterally without wheezes, rales, or rhonchi. Chest wall is nontender. Normal resonance to percussion.

**Cardiovascular:** Normal S1 and S2. No murmurs rubs, or gallops. Carotid, radial pulse 2+ bilaterally

**Abdomen:** Soft. Nondistended. Tender to palpation. No hepatosplenomegaly. Normoactive bowel sounds. No rebound tenderness. No guarding.

**Genitals:** Circumcised male with both testes descended. No lesions present.

**Musculoskeletal:** Strength 3/5 in proximal and distal muscles of the bilateral lower extremities. Strength 4/5 in bilateral upper extremities. No evidence of atrophy in bilateral upper or lower extremities. Bilateral lower extremities (BLE) tender to palpation diffusely. Normal muscle bulk and tone. Full range of motion in all extremities. No joint deformities or swelling.

**Neurological:** Mental status alert and oriented to person, place, and time. Decreased pinprick, light touch, and vibratory sensation in bilateral lower extremities greater than bilateral upper extremities. Patellar tendon reflex absent bilaterally. 1+ biceps and triceps reflex bilaterally. Vision 20/20 right and left eyes with normal visual field testing. Cranial nerves 1-6 intact. Evaluation of cranial nerve 7 reveals diffuse weakness in facial muscles. Evaluation of cranial nerves 9 and 10 reveal absence of gag reflex. Normal CN 11 and 12. Unable to evaluate gait secondary to weakness. Normal finger to nose testing. Normal rapid alternating movements. Normal heel to shin testing.

**Extremities:** Warm and well perfused. No clubbing cyanosis or edema.

### **Dauids Lab Results:**

**Chart 1.** David Kings complete blood count results

<b><u>Complete Blood Count:</u></b>		<b><u>Normal</u></b>
<b><u>Range</u></b>		
Hemoglobin (g/dl)	15.2	13.5 - 16.5
Hematocrit (%)	45	41 - 50

RBC's (x 10 <sup>6</sup> /ml)	5	4.5 - 5.5
RDW	10	<14.5
MCV	85	80 - 100
MCH	30	26 - 34
MCHC (%)	34	31 - 37
Platelet count	325,000	100k to 450k
Glucose, fasting (mg/dl)	135	60 - 110
Iron (mcg/dl)	140	65 - 150
Cholesterol, total	190 mg/dl	< 200 mg/dl

**Electrolytes:**

Calcium dl	8 mg/dL	8.8 - 10.3 mg/
Chloride L	90 mEq/L	95 - 107 mEq/
Magnesium L	1.6 mEq/L	1.6 - 2.4 mEq/
Phosphate dL	2.8 mEq/L	2.5 - 4.5 mg/
Potassium L	3.5 mEq/L	3.5 - 5.2 mEq/



Sodium	125 mEq/L	135 - 147 mEq/L
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**WBC:**

WBC (Cells/ml)	7,000	4,500 - 10,000
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Basophils (%)	0.6	0 - 1
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Eosinophils (%)	3	0 - 3
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Lymphocytes (%)	30	24 - 44
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Monocytes (%)	4	3 - 6
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Neutrophils (%)	60	54 - 62
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**Chart 2. David Kings CSF Analysis:**

Gross Examination	Turbid and colorless	Turbid and Colorless
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Glucose(mg/dL)	80	40 - 85
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Protein (mg/dL)	200	15 - 45
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WBC count	4 cells/mm <sup>3</sup>	0 - 5
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RBC count	0	0
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Gram Stain	Negative	Negative
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Syphilis Serology	Negative	Negative
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**Chart 3. David Kings GM1 and GQ1b Antibody Screen:**

GM1 IgG	89 IV	0
GM1 IgM	73 IV	0
GQ1b IgG	0	0
GQ1b IgM	0	0

### **Nerve Conduction Study Findings:**

Nerve conduction studies were conducted bilaterally. Distal motor amplitudes are low bilaterally, reflecting conduction failure. There is no sensory nerve involvement or peripheral nerve demyelination. F waves are normal. No temporal dispersion, and no significant slowing of conduction velocities or increase in distal latencies.

### **Assessment / DDX**

GBS is a clinical diagnosis arrived at following exclusion of other mimics and with supportive tests (Pritchard, 2010).

**Bilateral Strokes:** Patient's ability to understand and clearly respond to verbal commands makes presence of bilateral strokes unlikely.

**Cerebellar Damage:** Patient demonstrates weakened muscles yet is able to sit up straight and does not appear to have balance issues, making cerebellar damage unlikely.

**Spinal Myelopathy:** Patient does not have bladder, bowel, or sexual dysfunction indicating an intact spinal column.

**Transverse/Acute Myelitis:** Patient complains of pain/numbness in the extremities and does not demonstrate radicular pain radiating from his back. Lack of WBC in the spinal fluid makes viral infections such as CMV unlikely.

**Toxic Neuropathy:** Patient is at low risk for repeated contact with toxic materials, sudden onset of symptoms with increasing severity makes long term toxic exposure an unlikely cause.

**Diphtheria:** Lack of fever and autoimmune response makes infection an unlikely cause of patients symptoms.

**Tick Paralysis:** Symptom onset inconsistent with expected if affected by a Tick during his camping trip. Skin examination showed no possible tick bite locations. Patient also demonstrated no ophthalmoplegia.

**Porphyria:** Lack of mental disturbances as well as vomiting and constipation make acute or hepatic porphyrias unlikely.

**Lyme Disease:** Lack of rash upon skin examination makes Lyme Disease and unlikely cause of the patients symptoms.

**Botulism:** Possible cause of patients symptoms. Later ruled out as a result of GM1 antibody tests and nerve conduction studies.

**GBS Variants:**

The presence of protein and lack of WBC in the CSF served to both eliminate many possible diagnosis's as well as strongly indicate GBS as the correct diagnosis. There are several different variants of GBS and although similar it is important to determine which one the patient is suffering from.

**Acute inflammatory demyelinating polyneuropathy:** AIDP is the most common form of GBS and is often the diagnosis when no other variant is identified.

**Miller Fisher Syndrome:** MFS is unlikely as paralysis would manifest first in the eyes and descend to the periphery. Anti-GQ1b antibodies were also not present in the patient.

**Acute motor axonal neuropathy:** Sensory nerves are not affected. Identified by nerve conduction studies and GM1 antibody tests. Often associated with C.Jejuni infections

**Assessment:**

David was diagnosed with Acute motor axonal neuropathy. Patients with AMAN begin to show symptoms within a couple weeks of recovering from a C.Jejuni infection. This fits the time line of David's sickness following his camping trip. AMAN is also supported by the presence of GM1 IgG and IgM in his blood and the lack of GQ1b antibodies. Lastly the bilaterally low motor amplitudes and lack of sensory nerve involvement in his nerve conduction studies also illustrate AMAN.

**Treatment Plan:**

David was then admitted into the ICU under for close monitoring of respiratory, cardiac, and hemodynamic function. It is recommended that frequent measurements of vital capacity and NIF be taken. David is fit with support stockings in order to increase circulation. Neuropathic pain is managed with Gabapentin in combination with analgesics. Rehabilitation is recommend, involving isometric, isotonic, isokinetic, and manual resistive and progressive resistive exercises. After the acute phase, disabled patients should be treated by a multidisciplinary rehabilitation team and it is recommended that David come in for follow up appointments once a month.

## References

- Blank M, Barzilai O, Shoenfeld Y. Molecular mimicry and auto-immunity. *Clin Rev Allergy Immunol.* 2007;32(1):111-8.
- Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I. Serum anti- GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. *Neurology* 1993;43:1911-7.
- Davis JQ, Lambert S, Bennett V. Molecular composition of the node of Ranvier: identification of ankyrin-binding cell adhesion molecules neurofascin (mucin+/third FNIII domain-) and NrCAM at nodal axon segments. *J Cell Biol.* 1996;135(5):1355-67.
- Ferry H, Leung JC, Lewis G, et al. B-cell tolerance. *Transplantation.* 2006;81(3):308-15.
- Janssen BJ, Gros P. Structural insights into the central complement component C3. *Mol Immunol.* 2007;44(1-3):3-10.
- Hafer-Macko C, Hsieh ST, Li CY, Ho TW, Sheikh K, Cornblath DR, McKhann GM, Asbury AK, Griffin JW (1996a). Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann Neurol* 40:635–644.
- Islam Z, Van belkum A, Wagenaar JA, et al. Comparative genotyping of *Campylobacter jejuni* strains from patients with Guillain-Barré syndrome in Bangladesh. *PLoS ONE.* 2009;4(9):e7257.
- Kim CS, Seo JH, Cha HJ. Functional interaction analysis of GM1-related carbohydrates and *Vibrio cholerae* toxins using carbohydrate microarray. *Anal Chem.* 2012;84(15):6884-90.
- Leopold SS. Editor's Spotlight/Take 5: Have Bilateral Total Knee Arthroplasties Become Safer? A Population-Based Trend Analysis (DOI: 10.1007/s11999-012-2608-9). *Clin Orthop Relat Res.* 2012;
- Martin F, Oliver AM, Kearney JF (2001). Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity* 14:617–629.
- Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M, Yuki N (2000). Axonal Guillain-Barre syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. *Ann Neurol* 48:624–631.
- Olsson Y (1968). Topographical differences in the vascular permeability of the peripheral nervous system. *Acta Neuropathol* 10:26–33.
- Paek S, Kawai F, Choi KJ, Yeo HJ. Crystal structure of the *Campylobacter jejuni* Cj0090 protein reveals a novel variant of the immunoglobulin fold among bacterial lipoproteins. *Proteins.* 2012;80(12):2804-9.

- Parker CT, Horn ST, Gilbert M, Miller WG, Woodward DL, et al. (2005) Comparison of *Campylobacter jejuni* lipooligosaccharide biosynthesis loci from a variety of sources. *J Clin Microbiol* 43: 2771–2781
- Poly F, Guerry P. Pathogenesis of *Campylobacter*. *Curr Opin Gas- troenterol* 2008;24:27–31.
- Pritchard J, Hughes RA. Guillain-Barré syndrome. *The Lancet*. 2004;363(9427):2186-2188.
- Simkin D, Bendahhou S. Skeletal muscle ion channel disorders. *Front Pharmacol*. 2011;2:63.
- Susuki K, Rasband MN. Molecular mechanisms of node of Ranvier formation. *Curr Opin Cell Biol*. 2008;20(6):616-23.
- Thomas FP. Antibodies to GM1 and Gal(β1-3)GalNAc at the nodes of Ranvier in human and experimental autoimmune neuropathy. *Microsc. Res. Tech*. 1996;34(6):536-543.
- Willison HJ. The immunobiology of Guillain-Barré syndromes. *J Peripher Nerv Syst*. 2005;10(2): 94-112.
- Willison HJ, Plomp JJ. Anti-ganglioside antibodies and the presynaptic motor nerve terminal. *Ann N Y Acad Sci*. 2008;1132:114-23.
- Winer JB. Guillain-Barré syndrome. *BMJ*. 2008;337:a671.
- Yuki N, Yoshino H, Sato S, Shinozawa K, Miyatake T (1992). Severe acute axonal form of Guillain-Barre syndrome associated with IgG anti-GD1a antibodies. *Muscle Nerve* 15: 899–903.
- Yuki N, Yamada M, Sato S, Ohama E, Kawase Y, Ikuta F, Miyatake T (1993b). Association of IgG anti-GD1a antibody with severe Guillain-Barre ´ syndrome. *Muscle Nerve* 16:642–647.
- Yuki N, Kuwabara S. Axonal Guillain-Barré syndrome: carbohydrate mimicry and pathophysiology. *J Peripher Nerv Syst*. 2007;12(4):238-49.
- Yuki N, Hartung HP. Guillain-Barré syndrome. *N Engl J Med*. 2012;366(24):2294-304.