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GBS100 Celebrating a Century of Progress in Guillain-Barré Syndrome

GBS100

Celebrating a Century of Progress in Guillain-Barré Syndrome

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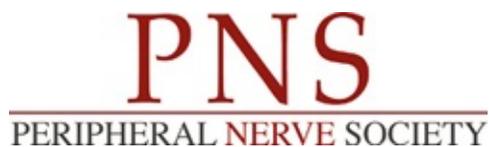
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Foreword

Richard A.C. Hughes
Arthur K. Asbury

The lifetime risk of Guillain-Barré syndrome (GBS) is one in a thousand, not common, but not rare either. It punches above its weight in the hierarchy of diseases because its onset is dramatic, its effects potentially devastating, its pathology fascinating, its mechanisms tantalising and its treatment so far only partly satisfactory. One of us (AKA) studied a large number of cases postmortem and noted the similarity of the usual pathology to the animal model, experimental autoimmune neuritis [1]. This prompted the other (RACH) to search for the antigen responsible for the animal model [2] and to look for better treatments [3]. Interest in GBS grew to the extent that by the early 1990s two books on GBS appeared almost simultaneously, one with one author and the other with three [4,5]. A generation later and a century since the famous description by Guillain, Barré and Strohl in 1916, this new book commands 63 chapters and over 100 authors. It is a fascinating read.

In the 1800s, the site of pathology in acute ascending paralysis was still unclear. In the first two-thirds of the 1900s, pathological studies showed inflammation and tissue damage throughout the nerves and spinal roots. The clinical picture appeared rather uniform. In the mid-1950s, C. Miller Fisher described his eponymous syndrome of eye movement paralysis, loss of tendon reflexes and ataxia which may overlap with GBS [6]. In the last quarter of the 1900s, American and Chinese investigators identified a paralytic syndrome due to predominantly motor neuropathy [7]. Others have described *formes frustes* and more chronic clinical courses. Increasingly sophisticated electrophysiology has distinguished predominantly demyelinating disease from forms due to primary axonal degeneration or, most recently, forms due to nodal conduction block without either demyelination or axonal degeneration. With increasingly refined diagnostic criteria [8], epidemiological studies have shown that GBS is a worldwide problem but with a preponderance of different clinical forms in different geographical regions. The ongoing massive international GBS outcome study (IGOS), with more than 1,000 records, will give new insights into these variations.

The first pathological studies emphasized cellular infiltration by macrophages as responsible for damaging the myelin sheaths with T cells directed against myelin proteins as instigating the process. This mechanism is certainly the cause of experimental autoimmune neuritis. It arises spontaneously in mice lacking specific genes which control T cell regulation. However, some human pathological studies have shown deposition of immunoglobulin and complement on the surface of myelin sheaths, suggesting

antibody rather than T-cell mechanisms in GBS. In mouse and rabbit models, antibodies to glycolipids, especially gangliosides, cause conduction block and axonal degeneration closely mimicking the predominantly motor axonal neuropathy form of GBS. Although there is little evidence of T-cell responses to myelin proteins, antibodies to gangliosides are present in the acute phase of GBS, and their antigenic target defines the clinical pattern of the disease. Thus antibodies to glycolipids have become the favoured mechanism causing GBS. With the latest microarray technology, antibodies to complex arrays of glycolipids are detectable in the more common demyelinating form of GBS as well as in the axonal forms of the disease and Fisher syndrome.

Campylobacter jejuni infection is the commonest of several known antecedent events precipitating GBS. Some genetic strains of this bacterium have lipopolysaccharides in their membranes which trigger antibody responses in genetically susceptible individuals. The antibodies react with gangliosides on the surface of the axolemma or myelin sheaths in parts of the peripheral nervous system accessible to soluble factors. One of the intriguing mysteries about GBS is that so many different infections trigger a clinically similar disease. Case reports and small series report hundreds of preceding illnesses. Case control studies implicate cytomegalovirus, Epstein-Barr virus and hepatitis E as well as *Campylobacter jejuni*, and the IGOS study is likely to add more. Are all these diseases the same? Or does the peripheral nervous system have only a limited number of ways of expressing a disease due to an inflammatory reaction? Does the occurrence of disease reflect the specific virulence of the organism or the failure of immune regulation by the patient? Or, more likely, both? This book should bring us closer to answering these questions.

Even in the absence of a full understanding of the mechanisms of GBS, neurologists have been testing empirical treatments. Spontaneous improvement is usual, which has made it impossible to judge whether many claims of successful treatment reflect anything other than the natural history of the disease. However, recovery is often slow and incomplete, which has stimulated the launch of randomised controlled trials. Cochrane systematic reviews contain convenient meta-analyses of their results. Surprisingly, corticosteroids, the panacea for many inflammatory diseases, turned out to be ineffective. The first treatment shown to be able to hasten recovery and shorten the disease was plasma exchange. Intravenous immunoglobulin is as effective and more convenient and so more commonly used where it is available. The success of these two treatments has made further progress more difficult because it has become unacceptable to omit treatment with one of them. It is encouraging that there is an ongoing trial of the complement inhibitor eculizumab founded on observations in a relevant animal model. We hope that this book will stimulate others to test some of the many available newer immunomodulatory agents to provide the improvement in treatment which our patients need. Our patients and their support organisations constantly remind us that they need more consistent rapid diagnosis, prompter treatment and greater attention to the disability, pain and fatigue which GBS may leave behind. This book will set the stage but much remains to be done.

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GBS Centenary

Hugh J. Willison, John A. Goodfellow and David R. Cornblath

It has been a century since the famous description was first published in 1916 by Guillain, Barré and Strohl of an acute onset polyneuropathy with albumino-cytologic dissociation in the cerebrospinal fluid (CSF) and clinical recovery. This new disorder was distinguished from acute poliomyelitis which also caused acute onset weakness but exhibited asymmetry, variable recovery, and an inflammatory CSF. As time passed, the disease became best known by the names of its first 2 authors. Strohl's name receded from the roll-call, and Landry's prior description also became increasingly overlooked as the current eponym took its place in the medical hall of fame. Thus the Landry-Guillain-Barré-Strohl syndrome became the Guillain-Barré syndrome, more commonly referred to just as GBS. GBS today is known worldwide where the incidence is a steady 2 cases per 100,000 of population per year. On this basis, we estimate there are over 100,000 cases per annum worldwide. GBS is now the most common cause of onset flaccid paralysis, as polio has receded due to vaccination.

As part of the Inflammatory Neuropathy Consortium/Peripheral Nerve Society meeting in Glasgow, 22–23 June 2016, it was decided to have a GBS Centenary Symposium and celebration on 24 June 2016 and to publish this book. Rather than a conventional textbook, a more reflective format was considered appropriate. Modern bibliographic databases make access to the primary literature a click of the mouse away, and there was no particular desire to create a comprehensive summary of 100 years of published work. Authors were thus assigned the brief of choosing roughly 10 publications, their 'Top 10', that influenced their thinking around the subject of GBS. A wide range of authors from emeritus professors to junior medical students from around the world were invited to contribute their thoughts on particular topics, each in their writing style. The result is a collection of 63 essays from 110 authors presented in a highly eclectic format. Some contributors have stuck firmly to the Top 10 format, while others have written conventional accounts of their topic. Some are poetic, yet others prosaic. Contents range from history of the syndrome to the future of GBS. There are many personal reflections by those who have made important contributions to the understanding and treatment of the disease. The reader can take in one or more chapters of special interest or just read any chapter for pleasure. The book is readily accessible to those with highly specialized interests in selected areas of research, the general neurologist looking for an update, and the patients and their families trying to understand this mysterious illness. For all, it should be a road map for the next 100 years.

The book's foreword was written by 2 of the most important GBS researchers, Professors R.A.C. Hughes and A.K. Asbury, who have made seminal contributions to the field over a lifetime of

achievement. It provides an overview of the first 100 years in a few pages. We open with an English translation of the original 1916 paper. A facsimile reprint and re-typed French version are included as an appendix. The History section includes reflections from the Hôpital de la Pitié-Salpêtrière, the home of many famous French neurologists, and moves through a timeline of both famous and forgotten papers.

The Epidemiology section includes a broad worldwide view of GBS. Highlighted is the International GBS Outcome Study (IGOS)—a multinational collaborative effort led by the Rotterdam GBS group involving over 1,000 GBS patients, all contributing to a massive collection of clinical, electrophysiological, biospecimens, and outcomes data. This will be a rich source of study for years to come. Important advances in GBS have come from studies in the Indian subcontinent, China, Asia and Japan, and these are well covered.

The Clinical section highlights those disorders that originally were not thought to be classic GBS, an acute ascending sensory-motor polyneuropathy, but that were eventually realized to be part of the spectrum of GBS. These disorders, ranging from Miller Fisher syndrome to acute motor axonal neuropathy, and lesser-known syndromes have indeed taught us much about the classic syndrome. Investigations of these ‘variants’ have provided important clues about epidemiology and pathogenesis, and we await more from further study of them.

The Clinical Investigation section shows how understanding the many areas that confirm the clinical diagnosis of GBS, shown in the Clinical section, have advanced our knowledge. Pathological studies led to the first inkling that this was an autoimmune disorder and supported both the humoral and cellular hypotheses of the disease. Physiology has helped refine the pathophysiological understanding of GBS, leading to the more recent concepts of the node of Ranvier as a major site of action in the evolution of the disease. Imaging promises to provide new insights, as it can look at the whole nervous system or concentrate on specific areas of interest. Lastly, there remains great interest in what we can learn from CSF aside from disease confirmation.

The sections on the many aspects of more basic research (animal models, basic science, antibodies, and antecedent events and susceptibility) show how much has been done but also how much more there is to do. Taken together, these sections begin with the observations from the 1950s in experimental allergic neuritis and move through an enormous research effort. These studies include important observations on diarrhoea, *Campylobacter jejuni* and GBS, gangliosides and GBS, auto-antibodies and GBS, and genetics and GBS. These and others have led to the current understanding of GBS as an autoimmune disorder in which a genetically susceptible individual comes into contact with a specific inciting agent and then develops antibodies against that agent which mistakenly identify the nerve as similar and attack it.

The Treatment section discusses the current standards of care: plasma exchange and intravenous immunoglobulins. Importance is given to the role of supportive care as critically necessary in the overall management. Noted is the fact that no new treatment has been approved for GBS since the 1990s. There are several clinical trials now underway and hopefully, by the printing of this book, their results will be known and new treatment options will be available.

The Outcome section shows how far we have come in the provision of rehabilitation services and our ability to predict outcomes. But we also need better outcome measures for our clinical trials that mimic what patients think about themselves and their recovery.

The last section is a potpourri of Top 10, from bibliographical citations to images to views of medical students and Ph.D. candidates to the patient perspective. It ends with a view to the future.

We wish to thank the GBS CIDP Foundation International for their continuous support and for providing the funds for publication. We thank our many colleagues around the world for embracing this

project with such enthusiasm and demonstrating a remarkable array of talent. Last we dedicate this publication to all those who have had GBS and their families, who provide the inspiration to do ever more to alleviate the natural course of Guillain-Barré syndrome.

HISTORY



Georges Charles Guillain
1876–1961
Professor of Neurology, La Salpêtrière, Paris

1

On a Radiculo-Neuritis Syndrome with Hyperalbuminosis of the Cerebrospinal Fluid without Cell Reaction. Notes on Clinical and Graphical Characteristics of Tendon Reflexes

MM. Georges Guillain, J.-A. Barré and A. Strohl

We draw attention, in this note, to a clinical syndrome which we have observed in two patients. This syndrome is characterized by motor disturbance, abolition of the tendon reflexes with preservation of the cutaneous reflexes, paraesthesia with mild disorders of objective sensation, pain at pressure of muscle masses, slightly accentuated changes of the electric reactions of nerves and muscles and very notable hyperalbuminosis of the cerebrospinal fluid with absence of cytological reaction (albumino-cytological dissociation). This syndrome appeared to depend on a concomitant involvement of spinal roots, nerves and muscles, most probably of infectious or toxic nature. It must be differentiated from simple radiculitis, pure polyneuritis and polymyalgia. Experimental research using a graphic method on the speed of reflexes and their delayed time, on the modalities, the muscular contractility, show that, in reality, the whole peripheral neuro-muscular system is involved in the syndrome. We also particularly insist on the hyperalbuminosis of the cerebrospinal fluid without cytological reaction, which to our knowledge has never been mentioned in similar cases.

OBS. 1. The soldier D ..., of the hussars, twenty-five-years old, arrived on the 20th of August 1916, at the neurological Centre of the VIth Army because of motor disturbances of the lower and upper limb members. The affliction affected him beginning in July the 25th with tingling of feet and weakness of the lower limbs, such that he had to stop walking every 200 to 300 meters. Then during the following days, tingling appeared in the upper limbs and on the lower part of the face; the muscular strength weakened in the upper limbs.

These various disorders evolved without obvious cause: the patient didn't have a recent infectious disease or any pharyngitis (even mild). He didn't have symptoms of food poisoning and no major tiredness. We shall add that, in his past medical history, we didn't find any important facts; the sick person denied any syphilitic infection and any alcoholic habit. The first examination of August 25th allowed us to

notice the following symptomatology.

The muscular strength was globally decreased in the upper and lower limbs but without a total paralysis; this decrease of the muscular strength was especially prominent in the extremities where we noticed an extreme weakness of the flexion and the extension of toes, foot, fingers and hands.

The muscles of the trunk were also weak, so that the patient could not sit independently without support.

Walking was possible for a few steps; we then noticed a certain gait instability and the upright posture of the foot could not be maintained.

There was no disorder of facial muscle power.

The electric examination showed that in the upper limbs, the faradic excitability was normal and the galvanic excitability was good for all the muscles with lively shocks; there was no polar inversion; we only remarked on a light hypo-excitability of the finger extensor; sometimes the shock was slightly slowed down; we noticed polar inversion for the lateral gastrocnemius, but the degeneration reaction was very incomplete.

The patellar, Achilles and medio-plantar reflexes investigated with the hammer were absent, as well as the bicep, radio-and cubito-pronator, and olecranon reflexes.

The plantar cutaneous reflexes led to obvious flexion of the toes with a contraction at distance of the tensor fascia latae. Cremasteric and cutaneous abdominal reflexes were normal. We noted no withdrawal reflexes, either plucking of the instep, or by hyperflexion of the toes.

The neuro-muscular excitability brought about by direct percussion of the muscle mass with the hammer was maintained.

The patient still reported tingling in both feet up to the top of malleolus and in both hands up to over the wrist. There was no clear disorder of objective sensibility, but we found a mild hypoesthesia to touch, heat and pain in the feet and hands. The muscle mass of the upper and lower limbs ached upon pressure.

Pupils reacted to light and accommodation.

There was no sphincter dysfunction.

There was no fever, and no respiratory or gastro-intestinal disorder, and the pulse was normal.

Urine, examined at the Laboratory of Bacteriology and Chemistry of the Army, did not contain either sugar, nor albumin, nor indoxyl; the chemical elements were in their normal proportion.

Lumbar puncture showed clear cerebrospinal fluid, not hypertensive, with hyperalbuminosis (2.5 grams of albumin per litre) without a leukocyte reaction (2–4 cells per field).

The Wassermann reaction in the blood was negative.

Specimens from the pharynx and nasal mucus showed the absence of any diphtheria bacilli.

Treatment consisted of absolute rest, massage of the upper and lower limbs, and injections of strychnine and soda of phenylsalicylate.

On August the 27th, tingling decreased in the lower limbs.

On September the 2nd, some improvement in muscle strength was observed, and there was no more tingling in the feet, but it persisted in the hands. Tendon reflexes were still absent. A repeat lumbar puncture showed a very marked hyperalbuminosis without appreciable leukocyte reaction as in the previous examination.

On September the 19th, motor disturbance was improving; the patient was able to walk for an hour; he could stand on one foot. Paresthesia had completely disappeared in the lower limbs but persisted in the hands although this was attenuated. Tendon reflexes were clinically absent, there were no withdrawal reflexes, and cutaneous reflexes were normal. With direct percussion of the muscle masses with the hammer, neuromuscular excitability seemed normal in the upper and lower limbs and in the face.

The patient, gradually improving, was sent to convalesce on the 30th of September.

OBS. II. The soldier D ..., from the ... Infantry Regiment, thirty-five-years old, arrived on the 5th of September 1916, at the Neurological Centre of the VIth Army because of motor disturbance in the lower limbs which were shown in the following circumstances.

On the 28th of August, after a walk of 15 kilometres, he felt an abnormal fatigue, headache, erratic pain in the upper and lower limbs. He lay down, could not sleep and shivered part of the night. The next morning he walked with huge difficulty to go to the examination; he was exempted from service for four consecutive days. The paretic state began with the lower limbs and then reached the upper limbs. On the fourth day he wanted to leave at five o'clock with his comrades, got dressed, but fell with his bag and could not get up. Transported to an aid station, he was then discharged to the neurological centre of the Army. These deficits had developed without an apparent cause: he had had no recent infectious disease, had no symptoms of food poisoning or other; moreover, he was very convinced that he had never contracted syphilis.

On the 5th of September, we noted the following findings.

The patient could do, with effort, small flexion and extension movements of the toes, flexion of the knee and of the hip. The same difficulty existed for movements of the upper limbs, especially distally. The head was usually rotated to the left and the patient encountered difficulty turning it to the right; he could open and close the mouth, but slowly and incompletely.

The examination of electrical reactions showed a slight hyper-excitability of nerves and muscles to faradic current. With galvanic current, excitability increased slightly, especially for the nerves of the upper limb; there was no reaction of degeneration.

The patellar reflexes were very difficult to elicit due to muscle hypertonia; they seemed to be present. The Achilles and medio-plantar reflexes were absent. The upper limb reflexes could not be assessed because of the hypertonia and the impossibility of a complete muscular relaxation. Plantar cutaneous reflexes brought a frank flexion of toes; cremasteric and abdominal cutaneous reflexes were normal. There was no withdrawal reflex either when the back of the foot was pinched or by hyperflexion of the toes, but the patient perceived the sensations caused by these excitations.

The neuromuscular excitability brought about by direct percussion of the muscle mass with the percussion hammer was present.

The patient complained of tingling in the extremities. There was no disorder of objective sensitivity and only a light touch, pain and temperature hypo-aesthesia in the feet and hands.

Muscles of the calf and forearm were painful upon pressure.

The pupils reacted equally to light and accommodation. The patient urinated independently; he knew when he needed to but could not feel the flow of urine.

He had no fever, no Kernig's sign, no nausea, no vomiting. The urine examined by the Bacteriology and Chemistry Laboratory of the army contained neither sugar nor albumin or indoxyl; the chemical elements were in their normal proportion.

It is necessary to note that a skin rash appeared 3 or 4 days previously, mainly localized in the upper thorax and in the lower abdominal region. The eruption was characterized by erythema and papulous spots. Except for these regions the eruptive elements were disseminated on the rest of the thorax and the abdomen, whilst no component was seen on the lower and upper limbs. The lumbar puncture showed

clear cerebrospinal liquid, not apparently hypertensive, with hyperalbuminosis (more than 0.85 grams of albumin with the albuminometry of Sicard), without leukocyte reaction (3–4 lymphocytes per field).

The findings noted during the first examination had a slight tendency towards improvement. However, on the 20th of September, we still noticed the muscles' weakness in the distal limbs, the absence of all the tendon reflexes apart from the left biceps reflex, the maintenance of cutaneous reflexes, muscle pain on pressure, and parasthesia of the extremities with a light hypoesthesia. We also observed intermittent myoclonic jerks in the muscles of the calf and of the thigh. A repeat lumbar puncture was unchanged from the previous examination: clear liquid, not hypertensive, with an accentuated hyperalbuminosis without leukocyte reaction (3–4 lymphocytes per field).

The patient was evacuated to the back lines on the 1st of October.

Discussion

Both observations which we have just recounted are completely similar. These two patients, without detectable visible cause, developed a clinical syndrome, characterized by disorders of all the muscles of the upper and lower limbs, worse distally, the loss of tendon reflexes with preservation of all the cutaneous reflexes, paresthesia with mild disorders of objective sensation, pain when pressure was applied to muscle masses, small modifications of the electric reactions of nerves and muscles and the distinct finding in the cerebrospinal fluid of a marked hyperalbuminosis without cytological reaction.

The hyperalbuminosis of the cerebrospinal fluid without cellular reaction is a peculiarity which is important to emphasize. This albumino-cytological dissociation (Sicard and Foix) is observed most of the time in certain medullary compression, in Pott's disease and in certain cases of syphilis of the central nervous system, but we do not believe these apply here, as it seems to us that our patients have pure radiculitis and polyneuritis.

In the second patient, it is also important to note that a certain hypertonia accompanied the paralytic state of certain muscles. When the patient was resting, the muscle tone was clearly higher than that of a healthy individual in the same situation. Passive movements remained normal. The limited number of voluntary movements, as described above, were rigid and marked with a certain lethargy. Examination of the tendinous reflexes proved difficult since the required stretching of the relevant muscles was prevented by the ongoing contraction taking place in the antagonists. Despite these findings, which we encounter frequently enough in meningitis cases, the patient was able to assume a seated position by stretching the arms out almost completely, whilst simultaneously applying light support to the knees to prevent any bending. The lower limbs could rise almost to a right angle with the trunk, and bend like those of a normal subject. Consequently, the sign of Kernig did not exist in our patient. This state of hypertonia was consequently in no way attributable to meningitis, but rather a particular state of the muscular contractility apparently caused by damage to the peripheral nerve.

We have already highlighted the fact that states of hypertonia can occur during some peripheral neuritis and in incomplete nerve injuries, and specify at this opportunity that spasms frequently observed during certain facial palsies are not an exception to the peripheral wounds of the nerves, as it is classically believed.

All the disorders observed in these two patients belong to a simultaneous pathology of spinal roots, peripheral nerves and muscles. The major hyperalbuminosis in the cerebrospinal fluid testifies to meningeal involvement; the character of the paralytic disorder prevailing distally in the limbs and the muscle pain on pressure indicate the involvement of the peripheral nerves and of the muscles. Moreover, it seems to us that it is a too great a simplification in neurology to segregate polyneuritis and polymyositis;

in one very large series of cases of infectious or toxic polyneuritis, the intramuscular nerve endings, the muscular fibres can themselves be affected and in reality it is more often polyneuromyositis than pure polyneuritis.

In the first patient, the experimental findings made by the graphic method allowed us to identify certain new characteristics in the study of reflexes and muscular contractility. The graphic method can inform the interpretation of symptoms and injuries.

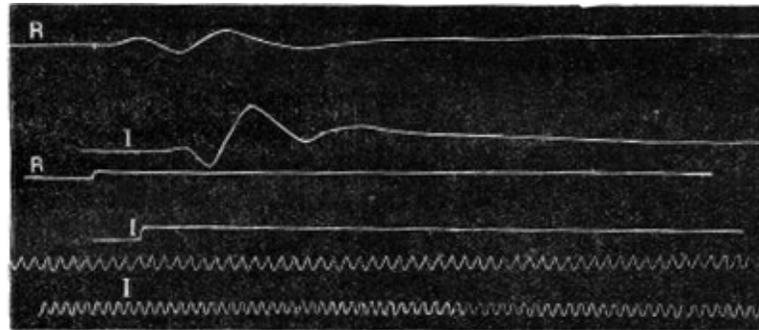


Figure 1.1 R, R, Myographic curve of quadriceps femoris during the patellar reflex, with a Desprez signal indicating the time of percussion and time in 1/100 second. I, I, I, The same plots for direct percussion of the quadriceps femoris muscle. Recorded on 21 August 1916. Note the almost total absence of contraction 'reflex' following percussion of the patellar tendon, while it is clearly present for direct percussion of the muscle.

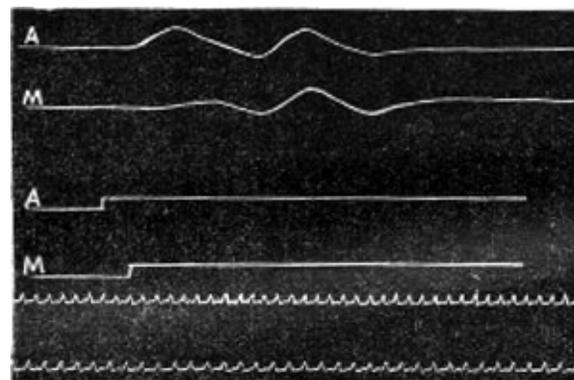


Figure 1.2 (A) Myographic curve of the gastrocnemius muscle in the ankle reflexes. (M) The same appears in the medial plantar reflex. Recorded on 21 August 1916. The first rise of the curve (A) is a mechanical shock; the second is a 'muscular' contraction. The 'reflex' part, which does not exist in the case of ankle reflexes, is, however, very visible low on the curve of the medial plantar reflex.

In this patient, while the tendon reflexes appeared absent during clinical examination throughout the course of the disease, the graphic inscription showed some contraction of quadriceps, femoral and gastrocnemius muscles under the influence of direct muscle percussion; the tendons of these muscles and their muscle masses showed interesting peculiarities. From the onset of the disease, the search for the patellar reflex brought a contraction after mechanical shock which we see clearly in Figure 1. This contraction, strikingly weaker than that obtained in a healthy subject, occurs after a delay of approximately 0.056s, and is not followed by the second, more ample and longer contraction normally seen as part of a delayed reflex. We noticed 0.152s after the beginning of the excitement a very light uprising of the curve, indicating the vestige of the reflex contraction. The patellar reflex is also almost completely reduced to an idiomuscular reflex until the cure of the disease. During this period the percussion of the mass of quadriceps provoked a beautiful muscular contraction occurring with a delay of

0.051s, followed by the second contraction, having all the characters of a contraction of reflex origin (Figure 1) and occurring 0.150s after the beginning of the excitement.

The muscles partially respond to mechanical excitement of the tendon, which is transmitted by spread via the muscular fibres, and results in a nearly normal double contraction when it is directly percussed. It seems to be affected by a mechanical hypo-excitability which makes it excitable only for abrupt blows directly on the body of the muscle.

The Achilles reflex, at the beginning, was also greatly modified and reduced almost completely at the mechanical shock. That shown in Figure 2 is of very low amplitude, occurs after an extreme delay—that is approximately 0.110s—and is not followed by the normal delayed reflex contraction. But, instead of what occurred for the patellar reflex, these changes partially reversed, and, already on September 5th (see Figure 3), repeat testing revealed a more ample, brisker, faster muscular shock (0.055s), followed by a delayed second reflex contraction arising after a delay of 0.140s. The direct mechanical shock of the gastrocnemius followed a parallel evolution and gradually resumed a shape closer to normal.

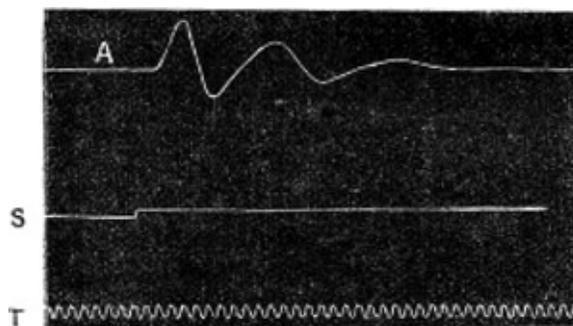


Figure 1.3 An external myographic curve in the ankle reflexes. Recorded on 5 September 1916. The reflex is present with its three characteristic elevations. However the 'muscle' contraction and especially the 'reflex' contraction are lower than in a normal subject.

It is worth noting that, at the beginning of the disease, although the percussion of the Achilles and gastrocnemius tendons didn't provoke any muscular contraction, the investigation of the medio-plantar reflex brought the second contraction, with a 0.144s delay constituting what we have to consider as a reflex contraction of low intensity (see Figure 2).

Furthermore, whereas a simple clinical examination revealed only the abolition of the tendinous reflexes, a detailed analysis of the myographic curves, by revealing which elements of the reflex are abnormal, leads us to a series of worthwhile remarks. Firstly, the complete disappearance of the reflex part of the myographic curve or, when it remains, its extreme delay and reduced amplitude and speed, shows us the deep and dominant change of the nervous drivers or of the central part of the reflex. In addition, the muscular shock is also modified, decreased in height, slowed, and delayed in its appearance; this causes us to think that the muscular element was also touched by the process of poisoning. Finally, the comparison of curves obtained after percussion of the patellar tendon and from the Achilles' tendon allows us to notice a different evolution for these reflexes. Whereas the first one was quickly abolished and didn't return until the the patient had left the hospital, the second, although seeming abolished clinically, was detectable using the graphic method and had characteristics getting gradually closer to normal earlier. *We insist on this important fact that the graphic method allows much more precise assessment of the state of tendon reflex than an examination with the hammer.*

The pathogenesis of the syndrome of radiculoneuritis observed in our patients was not able to be determined. An infection or poisoning must without doubt be suspected, but we were not able to identify

them. The prognosis did not appear to be very grave, if we judge it by the evolution of the affliction in our two patients: the first one was almost cured and the second in the process of improvement when they were evacuated by the Army.

(1) This observation was briefly analyzed by one of us in a medical meeting of VI Army at Villers-Cotterets in August 1915.

2

GBS: The Early Years in Paris

Jacques Philippon and Jean-Marc Léger

The 19th Century: Landry, Stohl and Others

Landry's Acute Ascending Paralysis

Jean Baptiste Octave Landry, born in 1826 (a year before J.M. Charcot), as a young medical student voluntarily treated patients during the cholera epidemic of 1850, and then trained during his residency alternatively in medicine and surgery. However, his medical thesis in 1854, *General considerations on pathogenesis and therapeutic indications of nervous diseases*, showed a decided interest in neurology [1]. In 1859 he published a paper entitled *Acute ascending paralysis* in which he gave the following description.

The sensory and motor systems may be equally affected. However the main problem is usually a motor disorder characterised by a gradual diminution of muscular strength with flaccid limbs without contractures.... The paralysis moves rapidly from lower to upper areas. The progression can be more or less rapid. When the paralysis reaches its maximum intensity, the danger of asphyxia is always imminent. However in eight out of ten cases death was avoided. When there is a reversal of the paralysis, the recovery period involves phenomena opposite to those indicated in the development period. Patients then either recover very quickly, or the disease becomes chronic with slow improvement [2].

What Should Be the Exact Name of the Syndrome? Guillain-Barré or Landry-Guillain-Barré?

The description of a neurological disease as provided by Octave Landry in 1859 is very close to that set forth by Guillain and Barré 57 years later. The main difference is related to the fact that biological and cytological studies of the cerebrospinal fluid (CSF) were not possible in Landry's time, the first lumbar puncture having been introduced in 1891 by Quincke in his search for a method of draining hydrocephalus. If the hallmark is indeed albumino-cytological dissociation, it is evident that the syndrome described by Landry cannot fulfil this definition. G. Guillain and J.A. Barré, when completing their description in 1936, added, "We do not accept to include in our syndrome the acute ascending paralysis described by Landry considering that the Landry cases mixed diagnoses which may have included other causes of paralysis such as poliomyelitis or acute encephalomyelitis" [3,4]. Today, however, it seems fair to recognize that the clear description offered by Landry is very similar to the clinical entity of GBS. The importance of Landry's work has been underlined by Haymaker and Schiller, considering in their book [5] that he was among the 133 founding fathers of neurology.

Confirmation of the importance of the work of Landry was given by Jules Dejerine (1849–1917),

future professor of neurology at the Salpêtrière Hospital, who wrote in his medical thesis (1879), *Studies on nervous system lesions in the acute ascending paralysis*, “Landry was the first to draw attention to a specific form of paralysis that he called extenso-progressive.... Based on certain symptomatic particularities, including the fact that sensation was relatively unaffected, he proposed designating these paralysees by the general name of acute centripetal or ascending paralysis”.

And Strohl?

Why did the name of André Strohl appear only in the first paper and disappear in the later publications? Had he been forgotten on purpose because he was the youngest (29 years old) and less known than the 2 other co-authors? There is a more rational explanation: Strohl’s participation was limited to the study of reflex and nerve conduction, and did not concern the clinical aspects. His academic career (professor in Paris in 1925) was mainly concerned with physical medicine, a subject in which he published more than 200 papers [6].

Dumenil and Chronic Ascending Neuritis

In 1864 Louis Stanislas Dumenil (1823–1890), a surgeon working in Rouen, described one case (and 3 more in 1866) of acute and symmetrical ascending paralysis which according to him might be caused by an atrophy of the peripheral nerves. He said, “Not least important nor least interesting in the history of these peripheral paralysees is their extension to a large part of the nervous system—one could speak of generalisation—to the point of compromising life through the invasion of the most essential nerves such as the vagus nerve” [7]. He was the first to carry out an electric exploration, using Duchenne de Boulogne’s new machine, noting that after a phase of depression, the excitability of the nervous trunk improved progressively from the centre to the periphery. It demonstrated the existence of peripheral paralysis, with the possibility of extension to the nervous centres: for Dumenil, they could be called chronic ascending neuritis.

Even if some observations of similar patients appear by the end of the century, nothing new concerning clinical or pathological aspects was worthy of note until the description of Guillain, Barré and Strohl [8,9].

Neurology in Paris at the Turn of the Century

After the death of Charcot in 1893, the main subject of discussion among neurologists remained what constituted the true nature of hysteria. Within this debate a turning point appeared with the studies of Joseph Babinski (1857–1932). Babinski had been a senior resident under Charcot, and his first conception was directly inspired by his master, but he progressively drew away from the Charcot school of thought, creating a new definition of hysteria (1901) and suggesting as a substitute the term ‘pithiatism.’ At the same time, he pursued his own work on organic symptoms, but returned to psychological problems during the war, writing *Hysteria-Pithiatism and reflex nervous disorders in the neurology of war* [10] with J. Froment (1878–1946).

After the death of Charcot, the chair for nervous diseases at the Salpêtrière was held by Raymond (1844–1910), then by Jules Dejerine (1849–1917). Married to an American student, Dejerine would have an efficient teammate in his wife, especially on their masterpiece *Anatomy of the central nervous system*. Among their other numerous papers, 2 are eponymous: the description of the Dejerine-Roussy syndrome,

caused by a lesion in the posterior thalamus, and Dejerine-Sottas neuropathy.

Pierre Marie (1853–1940) succeeded Dejerine in 1917 until his retirement in 1923. He described acromegaly and hereditary cerebellar ataxia. He worked on aphasia, though his ideas were opposed to those of Paul Broca and Karl Wernicke. He started the *Revue Neurologique* in 1893 and the Société de Neurologie, being its first general secretary.

French Neurology during the First World War

More than half of the members of the Société de Neurologie served in the armed forces; some continued their activities in their department partially militarised, like Babinski and Froment at the Pitié Hospital or Dejerine and A. Thomas at the Salpêtrière. Others were posted in neurological military centres, such as G. Roussy at the centre of the 10th Army or G. Guillain and J.A. Barré at the 6th.

Besides a heavy traumatic pathology affecting the central nervous system and peripheral nerves, combat conditions and social attitudes during the war resulted in a considerable number of mental disorders. The distinction between emotional stress and psychic trauma directly related to fighting and simulation was a major concern for army physicians. The Société de Neurologie and representatives of allied medical centres held a joint meeting in 1916. Many of the works from this meeting were published after the war, such as the book by Babinski and Froment, *Hysteria and pithiatism and reflex nervous disorders in the neurology of war* (1917) or *Neurological works during war* by Guillain and Barré (1920). It is remarkable that in such conditions, just after the Battle of the Somme, the 3 authors were able to publish a short note on 2 paralysed soldiers they encountered in 1916 [8].

The Academic Careers of G. Guillain and A. Barré

Georges Guillain classed first at the resident exam in 1898 and had the possibility of working among prestigious mentors in neurology, completing his residency with P. Marie. It was under Marie's direction that Guillain carried out his anatomical works (on the pyramidal tracts and internal capsule), emphasising in a special lecture the importance for a neurologist to think as an anatomist, a physiologist and a biologist. In this way he started with the study of cerebrospinal fluid (focusing on the benzoin colloidal reaction in nervous syphilis).

Jean Alexandre Barré, a resident under Babinski in 1909–1910, defended his thesis on tabes arthropathies. He started a long collaboration with Guillain during the war and became his friend. While a professor of neurology in Strasbourg, beginning in 1919, Barré published several hundred papers. In 1925 he welcomed Joseph Babinski in Strasbourg with great warmth, ensuring a large audience for his conference on the importance of asking the right questions and in detecting subjective symptoms; he was responsible for the introduction of Guillain to Babinski.

The Conception of GBS after the War

After the original description of the syndrome in 1916, no other mention of GBS appeared until Guillain and Barré published *Neurological Work in War Conditions* in 1920. This relative reserve persisted until 1936 with a complete clarification at the Société de Neurologie, following a paper by Théophile Alajouanine on a case of 'acute polyneuritis followed by death'. Guillain and Barré clearly defined the nosographic limits of their syndrome, insisting particularly on the importance of the albuminocytological

dissociation in the CSF, otherwise encountered only in spinal compressions, Pott disease and syphilis of the nervous system. This characteristic was for them absolutely different from infectious polyneuritis. They rejected the hypothesis of any similarity with the acute febrile neuritis recognised by Osler in 1892 and Holmes in 1917, due to the presence of fever in their syndrome. They admitted, however, with additional experience, to some modifications in the original description: cranial nerves may be involved and difficulty with micturition can occur. Furthermore, some clinical subdivisions may be described such as spinal, spinal and brain stem, brainstem, mental signs. They added ataxia with cerebellar involvement but rejected definitively polyneuritis with normal CSF protein and high cellular count [3,4].

The Unknown but Well-known Patient

Harvey Cushing, along with many other American physicians, took part in the First World War. In August 1918, after an episode of flu, he suffered from an illness characterised by a progressive weakness of the legs with paraesthesia, then his hands, and lastly associated with bilateral facial paresis. He wrote on the 3rd of November (cited by J.F. Fulton): “My hands now caught up with my feet—so numb and clumsy that shaving’s a danger and buttoning laborious”. At the end of the war, Cushing showed some signs of improvement. Back in Boston in February 1919, he resumed his operative schedule, but remained exhausted after operations. He attributed this fatigue to his previous ‘polyneuritis’.

The exact diagnosis of his illness was unknown and particularly difficult to establish due to an association with peripheral vascular disorder. For S.C. Reich [11], the combination of progressive weakness of the limbs, together with areflexia and bilateral facial paralysis substantiates the diagnosis of GBS. This seems to be confirmed by the evolution: rapid deterioration, plateau phase and slow improvement albeit incomplete.

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3

GBS 100: The Eponym

Alastair Compston

When, in 1916, Georges Guillain (1876–1961), Jean-Alexandre Barré (1880–1967) and André Strohl (1887–1977) described 2 soldiers admitted to the ‘Centre neurologique’ of the 6th Army based near Amiens with tingling and progressive weakness in the limbs, electrical inexcitability of the reflexes and ‘hyperalbuminose ... sans reaction cellulaire’ in the cerebrospinal fluid, the condition was initially known as ‘acute febrile or ascending polyneuritis’ [1]. Abe (AB) Baker (1908–1988) responded to the hint dropped by Guillain, who soon started referring to ‘our syndrome’, and described cases of encephalomyeloradiculitis as the ‘Guillain-Barré disease’. Generally left out in the cold thereafter, the 1977 obituary notice of Strohl refers to the syndrome of ‘Guillain, Strohl and Barré’. Also jostling for a place on the podium when speaking of ‘acute ascending paralysis’ is the description in 1859 by Jean Baptiste Octave Landry (1826–1865) [2]—an omission partly corrected by Webb Haymaker (1902–1984) and James Kernohan (1911–1981) when reviewing their experience of the ‘Landry-Guillain-Barré syndrome’ [3]. (Sir) William Osler (1849–1919) wrote on acute ascending (Landry’s) paralysis [4] and drew attention to the series reported by (James) Ross (1837–1892) resolving the ambiguity of whether this is primarily a disease of the spinal cord or peripheral nerve [5]. But, in recent years, neurology has settled for the abbreviated GBS (Guillain-Barré syndrome) when referring to acute post-infectious polyneuritis.

Thomas Willis entitled the last of his 12 treatises *Pharmacutice rationalis* (1674–1675), emphasising that treatment in medicine should be mechanism-based [6]. But that ideal presupposed a concept of disease and a system for nomenclature [7]. By the late 17th century, as far as the nervous system was concerned, not much had changed for over 2 millennia since the Greeks and Romans rationalized existing concepts. Magic medicine ignored the sick individual as a source of information. Plato (427–327 BC) believed in health as a state of harmony; disease was an excess, alteration or relocation of body (earth, fire, air and water) and soul. Hippocrates (460–377 BC) internalized medicine, relating disease to the individual and illustrated his ideas with case histories describing the onset, duration and outcome of symptoms. For Galen (AD 130–200), disease had a locus and a pathological process; function was affected first and alterations in structure then followed. It was a short step to extend the concept of physiology (Francis Glisson, 1597–1677) to that of a pathological process that encapsulated primitive origins of the concept of immunology (Girolamo Frascatoro, 1478–1553). Although previous images had shown appendages attached to the brain and spinal cord, Andreas Vesalius (1514–1564) first accurately depicted the nervous system—brain, spinal cord and peripheral nerves—as one structure in his “drawing of the nerves, which shows the origin of the 7 pairs of nerves that arise from the brain and the beginning of the spinal cord, and superbly explains the ordering and succession of all the pairs that take their origin

from the spinal cord that is contained in the bones of the back”. Thomas Sydenham (1624–1689) advanced the concept of natural history, detected by observation of untreated disease over time. There followed studies on the anatomical seat of disease (Giovanni Morgagni, 1682–1771); and the final transition to the notion of cellular (dis)organization as the basis of all pathology (Rudolph Virchow, 1821–1902). Thus, when Guillain, Barré and Strohl studied medicine, disease consisted of a pathological process affecting one or more parts of the body, with a natural history and a cellular basis for alterations in physiology underlying the characteristic symptoms and signs.

But how best could one disease be distinguished from another, and appropriately named? As clinical descriptions proliferated, nomenclature switched from reference to the most obvious manifestations—the ‘falling sickness’, the ‘sick headache’, the ‘scrofulous palsy’—to hagiography and the medical eponym. Famous doctors were remembered through diseases named after them. Later, a mechanism-based taxonomy seemed preferable, and pathology started contributing to the classification of disease. Now, acronym competed with eponym in medical nosology. In the present context, a variety of abbreviations emerged for the Guillain-Barré syndrome based on different patterns of the natural history and variations in the pathology responsible for acute, recurrent and chronic inflammatory demyelinating and axonal polyneuropathies—the family of AIDP, CIDP, AMAN, AMSAN and their many first-, second- and third-degree relatives. Nonetheless, custodians of the eponym fought back, as the celebration of this book concedes. In the preface to *Neurological Eponyms*, Peter Koehler, George Bruyn (1928–2002) and John Pearce argue that medical eponyms are again in vogue, and the feeble attempt of would-be scientific doctors to emulate the ‘real’ sciences of mathematics and physics has suffered a *volte face* in which the silent revolution of molecular biology in identifying mutations, deletions and frame-shifts has reconciled the inferiority complex allowing medicine, once again, to luxuriate in the eponym [8].

It usually takes a while for eponyms to settle down and the designated heroes to emerge. Inevitably, many eponymous attributions attract rival claims for priority on behalf of others who provided earlier accounts of that particular disorder. Indeed, a brief wander through any reasonable library easily identifies examples appearing before the eponym-defining publication(s). In writing *A Practical Treatise of Painful Distempers with Some Effectual Methods of Curing Them, Exemplified in a Great Variety of Histories* (1739), Theophilus Lobb (1678–1763) considered “some of the most frequent and common painful distempers which afflict human bodies: they are many, and some of them very dreadful, both on account of the exquisite torment they give, and the hazard into which they put the lives of those who fall under them” [9]. Mrs M-y W—r, aged 24 years, seen on 26 April 1733, complained that for several days she had noticed pains in the bottom of her feet and pricking in the ankles such that she could not tell how to walk. A complete cure of the complaint, caused ‘by acrid particles brought into contact with the extremities of the nerves of her feet’, was soon effected through the judicious administration of various tinctures. As a general conclusion, Dr Lobb anticipated subsequent elucidation of the pathology and mechanisms of demyelinating peripheral neuropathies: ‘If the covering membrane of the nerves happens to be corroded, and the extremities of them made naked, acrid particles ... by striking thro’(ugh) the common covering membrane of the nerves, may ... excite pain’.

John Pearce [10] has drawn attention to the epidemic of acute sensorimotor polyneuropathy in Paris, described by Auguste François Chomel (1788–1858) [11], and observed by Robert Graves (1796–1853) during his Parisian visit in the summer of 1828—the illness characterised by “sensations of pricking and severe pain in the integuments of the hands and feet, accompanied by so acute a degree of sensibility that the patients could not bear these parts to be touched by the bed-clothes ... [followed by] a diminution or even absence of sensation ... the power of motion declined ... advancing with progressive pace .. over the whole of both extremities ... and finally ... to become altogether paralytic” [12]. This and the

subsequent work of Louis Duménil (1823–1890) of Rouen [13] went largely unnoticed. (Sir) William Gowers (1845–1915) considered that only when “fresh facts were brought forward by [Alex] Joffroy [1844–1908], (Ernst von) Leyden [1832–1910] and [Sir] Thomas Grainger Stewart [1837–1900] was attention generally directed to the subject of peripheral neuritis” [14]. Grainger Stewart reported 3 examples of the illness already described by Graves [15]. He noted loss of the tendon reflexes (which Grainger Stewart had introduced into routine medical practice in the United Kingdom), and electrical inexcitability of the limbs. One patient died. Whereas the brachial plexus was normal, the axis cylinders in nerves of the forearms had undergone degeneration. Between 1861 and 1876, Grainger Stewart progressed steadily from lecturer and consultant in pathology and medicine to professor of physic at the Royal Infirmary of Edinburgh. His most influential books were *The Teaching of Medicine in Edinburgh* (1877) and *An Introduction to Disease of the Nervous System* (1884). Attendance grew steadily at his lectures in the Extra-Academical School in Edinburgh. The 2 manuscript notebooks in which Grainger Stewart drafted these 141 lectures cover 594 pages [16]. Lecture 98 includes a section on “Paralysis and other conditions due to disease of nerve endings or nerves (neuritis, peripheral neuritis, multiple neuritis, alcoholic paralysis)”. Amongst other details, the attentive medical students were told:

Paralysis and other cond[itions] due to dis[ease] of n[erve] endings or nerves. We noted that there was paralysis in hands & feet spread up to arms and legs & that there was interference of sensation and motion. There is no change in brain and cord. 1. Very often the dis[ease] affects the fib[rous] tiss[tissue] bet[ween] the fibres accounting for the parenchymatous changes. 2. There is a change in the n[erve] medullary sheath centre of n[erve] and sens[ory] struct[ure]. Often the changes proper to the n[erve] begin at periphery and spread up towards centre. Occasionally there are patches on the n[erve] involved, normal p[ar]ts and then involved portions. The proc[ess] may originate in the n[erve] (sic) or in the trophic cells. Symptoms. It very seldom happens that peripheral n[erve] is confined to one n[erve] but gener[ally] occurs in hands and feet—spreads up. There is a peculiar sensation in hands at first there is awkwardness in the hands at first. Hands get glossy, nails and hair get altered. There are changes in bl[ood] distribution, eg pallor or congestion. These cases go on till pat[ient] is unable to help himself but with treat some good improve sets in always and then there are felt various morbid sensations and espec[ially] tenderness on press[ure] along line of nerves. Often serious sym[toms] arise viz by involving vital n[erve]; h[ear]t and resp[iratory] nerves may be attacked & death.

Pasted alongside is the printed galley summarising the lecture with the annotation: “Some get a peripheral neuritis recurring y[ea]r after y[ea]r”. Despite this emphatic formulation of the diseases of peripheral nerve, Lecture 56 (pages 220–21) describes “acute ascending paralysis (Landry)” as a disease of the spinal cord.

This account of competitors for the GBS ‘victor ludorum’ is incomplete, and not all the descriptions provide information on natural history with recovery and pathological findings sufficient to distinguish the disorder named after Guillain and Barré from other forms of rapid onset multiple neuritis. Wijdicks and Ropper credit James Wardrop (1782–1869) and Charles-Prosper Ollivier d’Angers (1796–1845) with original contributions to the subject [17]. Readers of this book will have little difficulty in identifying additional, equally deserving celebrants. But if we are to stick with eponym for describing patients with acute post-infectious polyneuropathy, why not the ‘Lobb-Wardrop-Ollivier-Chomel-Graves-Landry-Duménil-Grainger Stewart-Ross-Guillain-Barré-Strohl syndrome’?

[This text is based partly on passages that appeared in various editorials for issues of *Brain* written between 2004 and 2015.]

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4

GBS100: Some Literary and Historical Accounts

A.J. Larner

Introduction

The importance of hearing patient narratives of disease—‘hearing the patient’s voice’—is increasingly recognised as a complement to the technical narratives of disease produced by clinicians. As well as being of intrinsic interest, the patient perspective may broaden medical sensibility to, and perception of, the experiential aspects of disease, and rightly give the impression that clinicians are actually listening to their patients rather than simply shaping their narratives for their own purposes.

As for other neurological diseases, there are many patient accounts of GBS (see, for example, patient accounts on www.gaincharity.org.uk), only a few of which are discussed here, alongside some fictional accounts and some possible historical cases.

Patient Narratives of GBS

The American author Joseph Heller (1923–1999), most noted for his 1961 novel *Catch-22*, developed GBS in late 1981 and subsequently wrote an account of his illness, *No Laughing Matter* (1986) [1]. The book consists of alternating chapters by Heller and his friend Speed Vogel, so that both patient and collateral narratives are provided, although it might be argued that Vogel is simply Heller’s alter ego (page 220), since during the author’s illness Vogel lived in his house, wore his clothes, forged his signature on cheques, dealt with his fan mail, and ‘more or less assumed his identity’ (page 72).

In the Heller/Vogel account, neurological problems began with weakness (difficulty pulling open a door, removing a heavy sweater over his head), along with dysphagia (trouble swallowing a meal after the first few mouthfuls, a symptom calculated to ring alarm bells in a ‘prodigious eater’) and cacogeusia (food tasting metallic). At the gym, simple stretching exercises proved difficult:

Lying supine on a mat... Bending each leg in succession, I was supposed to wrap my arms about the shin and lift my head to touch my chin to my knee. I could not come close, on either side. (page 22)

Heller could only do 7 of his usual 15 push-ups. The following day, a Sunday, Heller again noted food tasted metallic, as well as chewing his food more slowly than usual, and he reflected that

something neurologically unpleasant was taking place inside me, something I could not control and could not fathom. All of my limbs felt tired. (page 19)

He spoke to his physician to report his symptoms, who diagnosed GBS over the telephone and arranged to see him, followed shortly thereafter by a neurological consult (with Dr Walter Sencer) and admission to the medical intensive care unit of the Mount Sinai Hospital in New York (Sencer published a number of articles in the *Journal of the Mount Sinai Hospital*, later the *Mount Sinai Journal of Medicine*, in the 1950s and 1960s but none relate to GBS). At no time did Heller experience numbness or pain (page 21).

During his 22-day ICU admission he did not require ventilation, although a tracheostomy was mooted at one point. His weakness was profound, with dysarthria and dysphagia requiring a nasogastric tube, and respiratory and cardiac monitoring. His major gripe was sleep deprivation, and then a fear of not waking from sleep, which led to low mood and psychiatric consultations. Treatment was entirely supportive, Heller's illness predating effective immunotherapies for GBS.

There was some doubt about the diagnosis because 2 lumbar punctures returned normal results and it was with relief (according to Heller) that a third puncture (all were reported to be painless) showed a raised protein, confirming the suspected clinical diagnosis.

Despite a weakness so profound that he could not lift his head or roll over, Heller commented that

I never once throughout the entire experience thought of myself as weak... I was paralyzed, not weak. And in truth, I wasn't weak. My *muscles* were weak. (page 162; italics in original)

The corner was turned shortly after Christmas 1981, with gradual neurological improvement thereafter such that after 3 months and 3 days he left Mount Sinai Hospital for a rehabilitation facility (the Rusk Institute at the New York University Medical Center) until mid-May 1982.

In passing, Vogel gives an answer to the perennial question of why we refer to 'Guillain-Barré syndrome' and not 'Guillain-Barré-Strohl syndrome', despite the tripartite authorship of the original 1916 paper. One of Heller's friends was the novelist Mario Puzo (1920–1999), author of *The Godfather* (1969), later made into a celebrated film. Informed of Heller's diagnosis by a mutual friend, Puzo apparently blurted out

"My God, that's terrible!"

"Hey Mario, you know about Guillain-Barré?"

"No, I never heard nothing about it [*sic*]," Mario replied. "But when they name any disease after two guys, it's got to be terrible!" (page 44)

By extrapolation, then, perhaps naming a disease after 3 guys (Guillain-Barré-Strohl) rather than 2 would render it simply too awful to contemplate (e.g., Gerstmann-Straussler-Schenker disease?).

It was also in 1981 that Tony Benn (1925–2014), an English Labour Party politician and socialist, developed GBS just at the time that he was campaigning (ultimately unsuccessfully) for the deputy leadership of his party. Brief notes on his illness appear in his political diaries [2], beginning with an entry on 5th May:

I wasn't feeling very well today. I have had this tingling in my legs and now my hands, and my face has been very hot and my skin has been rough.

After consulting a fellow member of Parliament who was medically qualified and being reassured, Benn then consulted his general practitioner on 14th May:

I reported the fact that I have got this tingling in my legs. At the moment, walking is like having on wellington boots full of water with a sponge in the feet. I don't have any feeling in my feet and my hands tingle.... He thinks it might be some nerve condition.

By 1st June, referral to a neurologist was made, and on 4th June

I was taken to see Dr Clifford Rose [1926–2012], who examined me. I hadn't got reflexes in my legs or arms.

He told me, "I think I know what this is. If this was only a medical consideration, I would recommend you came into hospital at once."

I said, "Well, I'm perfectly happy to do that because I am simply incapacitated."

Benn was then in hospital from 6th to 17th June, when there are no diary entries [2]. Following discharge from hospital, he noted on 24th June that "I have to rest in the afternoon, and it's still painful to walk", but there do not seem to be any other comments on possible sequelae in the abridged diary entries, other than "very tired" on 26th September, although this could have been related to his political campaigning prior to the party conference which started that day. Overall, therefore, it seems that Benn's GBS was a mild episode, with prominent sensory symptoms, unlike Heller who seems not to have had any sensory features.

An account of recovery from 'Guillain-Barré disease' by an ex-patient, Lucile Marie Hoerr Charles, PhD, a college professor, appeared in 1961 in the journal *Psychosomatic Medicine* [3]. Her illness followed a routine smallpox vaccination (and was indeed written up and published as such [4]). She had been affected 2 years earlier and was paralysed for 6 weeks. Looking back, she said

It has been a tremendous experience of both body and soul; a slow, painful, miserable, uncertain, frightening business; often wonderful, and full of amusement and beauty also.

Pain was a significant factor in this illness:

In a few days my hands became numb and, when I held them under the faucet, I could not tell the difference between hot and cold water. Soon, severe pain came. My whole body seemed to be just one cramp.

Despite making a good physical recovery, she found

convalescence after leaving the hospital was also a dark, trying period—in some ways worse than acute illness. No more support by institutional routine, nor by the constant bustle, energy, authority and tender-loving-care of medical people who were pushing hard to make me well.

I am sure many if not all neurologists have encountered GBS patients who report feeling 'abandoned' after hospital discharge, despite good or excellent neurological recovery.

Fictional Accounts of GBS

Paralysis and the recovery from it are subjects calculated to attract writers for their dramatic potential.

Although not perhaps amounting to a genre, a number of classic novels, primarily intended for children ('improving literature'), feature characters who develop paralysis: Katy Carr in *What Katy Did* (1872) by Susan Coolidge; Clara Sessman in *Heidi* (1880) by Johanna Spyri; Colin Craven in *The Secret Garden*

(1909) by Frances Hodgson Burnett; and Pollyanna Whittier in *Pollyanna* (1913) by Eleanor H Porter [5,6]. All these novels predate the original description of GBS, but the fictional possibilities of this paralysing disorder have subsequently been exploited on occasion.

Likewise, playwrights have sometimes featured characters with paralysis, for example *The Sacred Flame* (1928) by W Somerset Maugham (himself medically qualified), and *Whose Life Is It Anyway?* (1978) by Brian Clark, featuring post-traumatic paraplegia and quadriplegia, respectively.

In *Solomon's Porch: The Story of Ben and Rose* by Jane Riley [7], Ben Windham, a college professor in his 50s, develops a neurological illness which is labelled Guillain-Barré syndrome. Whether the author had any experience of the disease, firsthand or otherwise, is not clear, but there are certainly elements in the description of the disease within the novel which jar the clinical reader. The patient suffers progressive weakness over a few weeks (contrary to the blurb, "... gone to a party ... when he left, he was crippled"), but despite his seeing numbers of clinicians and undergoing a lumbar puncture, no clear diagnosis emerges, other than polyneuropathy, possibly related to his underlying diabetes. Despite this lack of diagnostic clarity, a referral to rehabilitation services is made, before transfer to another medical centre where the diagnosis is immediately made by a physician and confirmed by a neurologist who labels some of the previously consulted professionals 'irresponsible'. However, the timeframe of the novel is a little difficult to follow and it may be that neurological decline has been going on for more than 8 weeks; indeed the neurologist considers that this is the 'slow kind' of GBS, and "considers yours chronic inflammatory demyelinating polyneuropathy" (pages 67–68); so, not GBS at all! This may explain the treatment with prednisolone (pages 67, 81) as well as plasma exchange. The patient's wife is still, perhaps justifiably, a little baffled:

I don't really understand the difference between GBS and CIDP. You seem to be somewhere in between the symptoms for those two. (page 88; also 206)

So, subacute demyelinating polyradiculoneuropathy (SIDP), perhaps?

When the patient weakens again, nearly 18 months later, the prednisolone still seems to be continuing (page 106), prior to more plasma exchange, IVIg, and cyclophosphamide. More perplexingly, the patient complains of loss of sensation from the chest down, suggesting a sensory level (pages 41, 54, 85), and eventually is found to have cervical spine stenosis (page 115) requiring surgical intervention, presumably some form of decompression. Whilst the concurrence of 2 neurological diseases is not impossible, it is implausible. Furthermore, the patient's wife is informed by the physician shortly after the GBS diagnosis that the "leading cause of death related to GBS is suicide" (page 69), though how a paralysed patient might achieve this is not made clear.

Although accuracy or consistency is not necessarily to be anticipated in a work of fiction, one seriously worries for any GBS patient or family who might use this book as a source of information about the disease; the frontispiece disclaimer, with direction to Guillain-Barré Foundation International for information regarding the disease, is a welcome inclusion.

In *Thaw* by Monica Roe [8], the narrating voice is that of Dane Rafferty, an 18-year-old skier recovering from GBS in a rehabilitation centre in Florida, far distant from his family and friends in upstate New York. The action takes place over a 2-month period, with flashbacks to premorbid days and disease onset. Hence the focus is more on the recovery phase, particularly the input from a physiotherapist, Anya, and an occupational therapist (no doctor ever darkens these pages!); this is concordant with the author being a "travelling physical therapist" (according to the blurb) presumably with experience of treating GBS patients:

Recovery from Guillain-Barré is a strange process. After you get to the totally helpless point and hang out there for a while, the whole thing begins to reverse itself.... Trouble is, during all that time when you can't move, your joints start to tighten up, so by the time you can actually tell your muscles to move on their own, the joints may be too stiff to let it happen. (page 56)

As Dane's recovery progresses (relatively quickly), some of the techniques of neurorehabilitation are mentioned, for example the tilt table, and the patient's perspective on this:

This table contraption that can be cranked from horizontal to completely vertical, bringing the person on it along like Frankenstein's monster rising from the slab. Supposedly, being upright and putting weight on your feet is good for your bones and joints even if you can't do it yourself.... It also makes you dizzy if you do it for too long at first. (pages 69–70)

Later Anya sets Dane to work with the inflatable ball ("It makes my muscles burn like hell ... but it's really been helping my balance and torso strength"; page 192), and standing using the parallel bars:

The sequence of steps that we always go through to get me standing: plant both feet on the ground, as far back as possible; shift weight forward through my legs. At this point, [Anya] usually pulls my hips forward and up, giving just a little extra power to my upward push. (pages 193–95)

There is something heartfelt and, one senses, personal about the battles of the physiotherapist, Anya, with her recalcitrant patient, Dane, and patient relatives complaining about lack of recovery. When Dane is refusing physiotherapy Anya tells him

You all want to be fixed, want us to perform miracles we can't guarantee and provide answers we can't give.... You'll still turn right around and blame us if it doesn't end up exactly the way you hoped it would. (page 207)

This lament sounds like the voice of experience, but, like the other passages quoted, is seamlessly assumed within the narrative. Most readers will probably be more concerned with whether Dane recovers fully, gets back to skiing, and makes it up with his girlfriend (who ditched him when he was paralysed, intubated and ventilated—some girls surely know how to pick their moment!). But there is much to enjoy in this book if reading with neurological spectacles on.

Historical Diagnoses of GBS

Appeal to the historical record may help to answer the question as to whether cases of GBS occurred before 1916. However, since CSF findings were part of the diagnostic characterisation by Guillain and his colleagues, and lumbar puncture was only performed after 1897, cases conforming to the original clinical and investigational description (*dissociation albumino-cytologique*) occurring before the 1916 publication would seem unlikely. Hence inferences based on clinical features are the only recourse to answer the question of historical cases.

For example, Reich suggested that the renowned American neurosurgeon Harvey Cushing (1869–1939) suffered from GBS in 1918 when an undiagnosed illness characterised (in Cushing's diaries) by symmetrical weakness, numbness and paresthesias of the hands and feet, areflexia, bilateral facial paresis, diplopia, and fever prevented him from operating [9].

Another, more formalised, example of this diagnostic revisionism relates to the diagnosis of Franklin Delano Roosevelt (1882–1945), 32nd president of the United States of America (1933–1945), who suffered a paralytic illness in 1921 which is widely believed to have been due to poliomyelitis. Goldman

and colleagues re-examined the clinical features as recorded in biographies of FDR (he never underwent lumbar puncture or neurophysiological testing as far as is known) and undertook a Bayesian (i.e., probabilistic) analysis of 8 key symptoms [10]. By multiplying prior probabilities by symptom probabilities, Goldman and colleagues calculated that 6 out of the 8 symptoms had posterior probabilities which favoured a diagnosis of GBS over poliomyelitis. It is an interesting analysis, which certainly attracted widespread attention when first published, but essentially inferential. The exact determination of FDR's diagnosis is never likely to be established.

Could other previous fictional accounts of paralysis in fact be describing GBS? Any such claim would of course be entirely speculative. Since both Katy Carr and Pollyanna Whittier develop paralysis following traumatic accidents, GBS would not seem to be a likely diagnosis. Few details are given about the onset of illness in Clara Sessman and Colin Craven, but as they both subsequently recover from these illnesses it might be wondered whether they had GBS, in Colin's case sufficient to produce lower limb atrophy.

Conclusion

It seems implausible that GBS did not exist before Guillain, Barré, and Strohl took to print, thus characterising GBS as a disease entity. This same condition seems to be described, for example, in the reports by Landry [11] (1859), Wardrop [12,13] (1834), and Warrington (1903) [14]. Thus a '100th anniversary' of GBS is in some ways a cultural construct. Clinical knowledge of GBS which has accrued over the century has sometimes transferred into the literary domain, spawning both personal and fictional (or possibly 'factional') accounts of the disease, as well as attempts at retrospective diagnosis, which may inform or frustrate readers, depending on the perspective (lay, professional) from which they approach these documents. At best, these narratives may allow clinicians to hear the patient voice which may ultimately inform their approach to the management of sick patients.

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Some Patient Accounts of GBS

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Guillain-Barré Syndrome: My Worst Nightmare by Byron Comp (2004)

A Solitary Confinement by Robin Sheppard (2007)

Going Full Circle: My Fight Against Guillain Barré Syndrome by Phillip Taylor (2008)

My Wake-up Call—A Survivor of Guillain-Barré Syndrome by Jerry L Jacobson (2011)

Guillain-Barré Syndrome: My Journey Back by Shari Ka (2011)

Blue Water, White Water: A True Story of Survival by Robert C Samuels (2011)

Why Didn't I Die? by Barbra Sonnen-Hernandez (2011)

Happily Ever After: My Journey with Guillain-Barré Syndrome and How I Got My Life Back by Holly Gerlach (2012)

My GBS Story. A Life Changed by Cindy Herron (2014)

5

10 Papers Illustrating the Guillain-Barré Syndrome Timeline

Michael P.T. Lunn

Brilliant minds have peppered the history of Guillain-Barré syndrome (GBS) and brilliant thought has advanced both GBS and wider humanity to where we find ourselves in 2016. Quite frequently serendipity, time or simply posthumous recognition have identified the important moments in the history of GBS and the world. I have chosen papers that illustrate some of the key GBS developments and juxtaposed these against important (and less important) contemporaneous scientific discoveries.

1849–1881: Charles Darwin discovers worms are unable to hear the bassoon

In 1849, Octave Landry published examples of the subsequently eponymous ascending paralysis in his paper ‘La paralysie ascendante aiguë’ [1]. In the same year, Charles Robert Darwin was asking his ten-year-old son to play his bassoon as loudly as possible to garden worms to see if they could hear or not. In his 1881 treatise ‘The Formation of Vegetable Mould through the Action of Worms, with Observations of their Habits’ Darwin wrote, “Worms do not possess any sense of hearing. They took not the least notice of the shrill notes from a metal whistle, which was repeatedly sounded near them; nor did they of the deepest and loudest tones of a bassoon. They were indifferent to shouts ... [and] ... when placed on a table close to the keys of a piano, which was played as loudly as possible, they remained perfectly quiet [2].’ Clearly greater science was to come from his serendipitous, prior trip on the *Beagle*. Landry’s eloquent description of 10 cases of ascending paralysis, however, was the nidus from which our subsequent understanding of Guillain-Barré syndrome derives. He clearly describes acute ascending paralysis with paraesthesia, normal intellect, the absence of bladder or bowel involvement but respiratory involvement leading to 2 deaths. There was no identified abnormality at postmortem.

Whilst Charles Darwin brooded uncomfortably on the *Origin of Species*, biding his time, studying his worms, Landry married a destitute aristocratic beauty, switched his focus to a very lucrative hydrotherapy business and died of cholera aged 39, with Charcot at his bedside. Arguably his scientific observation was far in excess of Darwin’s, his greatness recognised by Charcot. His life was cruelly cut short; what more might there have been?

1870—James Clerk Maxwell—Discovery of Electromagnetism—1873

In the timeline of GBS, Westphal [3] and Erb [4] are credited with the simultaneous description of deep tendon reflexes in patients with spinal cord disease. The recognition and diagnosis of GBS in the 21st century remains clinical, and the history and examination are key. Westphal described the exaggeration of the knee jerk in a patient with multiple sclerosis and in his paper reports that the same phenomenon had been observed by Erb in a paper which he was reviewing. Although Landry had described the ‘flaccid limbs without ... reflex movements of any kind’ [1], he did not recognise reflexes as such. Mitchell and Lewis are probably the first to score reduced reflexes [5]. Their nomenclature graded 0 as absent, and then = as very slight, – slight, N normal, + marked and ++ as very marked. One might think that with 3 grades of reduced reflexes they were keener on lower motor neurone lesions than those of the central nervous system. It was left to Guillain, Barré and Strohl to emphasise the loss of reflexes 30 years later.

1910–1915: Einstein and the Theory of General Relativity

The war gave Guillain, Barré and Strohl the opportunity to describe the cases of 2 paralysed soldiers in the 1916 paper that has seeded this publication [6]. Rather like Landry shifting his focus to hydrotherapy, Strohl decided his career was better placed in physical medicine than neurology. It is said that as a result his name was left off the subsequent eponym for our disease. One would hope that today such a lack of acknowledgement would not occur. However, the 3 authors drew attention to the doubling of the latency of the knee and ankle reflexes, and deduced therefore that there must be a delay in the conduction time of the reflex arc. They also emphasised the albuminocytological dissociation in the cerebrospinal fluid (CSF) of the 2 soldiers, both of whom had raised CSF protein. The authors of this paper, mentioned innumerable times in this book, had no idea of the causation of their illness. They were very insistent that the soldiers did not have venereal disease, despite syphilis being rife in the trenches, and they remained unsure whether the cause was intoxication or infection. What we remain sure about is that syphilis still doesn’t cause a peripheral neuropathy (although of course it is in the differential diagnosis for a radiculitis).

1940–1941: Howard Florey and the First Use of Penicillin in Humans

Despite the recognition of GBS as an entity, and a number of treatises on GBS from Guillain and others, the pathogenesis remained elusive. Infection remained a strong candidate for the causation of GBS in the early part of the 20th century. Leyden [7] had described an intense cellular nerve infiltration, but Haymaker and Kernohan, in a highly influential paper [8], commenced thoughts on processes other than infectious inflammation. They described the ‘limited pathological material’ of 50 fatal cases. Oedema and nerve root swelling, possibly secondary to infection, were blamed for the pathology seen and a cell-mediated pathogenesis fell out of favour. Perhaps this was the beginning of thoughts of a humoral pathogenesis, before any knowledge of antibodies. They also heralded the future heterogeneity of the Guillain-Barré syndromes with the statement that ‘from the clinical standpoint all forms are initially polyradiculoneuritis’, many forms existed broadly classified into 4, but that ‘the majority ... bridge the gaps between.’

1950–1953: Francis Crick and James Watson Describe the Helical Structure of DNA

In 1956 Charles Miller Fisher described 3 cases of an acute syndrome consisting of ataxia, areflexia and external ophthalmoplegia, without long tract signs or central nervous system involvement and with albumin-cytological dissociation in one case [9]. Guillain himself probably described a similar case much earlier [10]. Miller Fisher syndrome is now recognised as a distinct clinical entity, with varying amounts of overlap to GBS, part of the heterogeneity recognised by others. Perhaps the other important milestone associated with Miller Fisher syndrome is its association with antibodies to gangliosides, thought to be key to the pathogenesis. We now recognise that IgG₁ antibodies to GQ1b are found in almost all cases of Miller Fisher syndrome and related or overlapping syndromes (e.g. acute oropharyngeal palsy and Bickerstaff's brainstem encephalitis) [11].

1970–1971: Federico Faggin and Intel 4004, the First Commercial Microprocessor

One of the most influential papers in our GBS timeline covers just 3 succinct pages in *The Lancet* [12]. In it Richard Hughes describes a randomised placebo-controlled trial of prednisolone treatment for GBS in 40 participants. His conclusion was negative: “Our results provide no grounds for the use of steroids in the management of acute inflammatory neuropathy since the prognosis is not improved, the rate of recovery is slowed and the chance of relapse may be increased”. Looked at simply, this paper sets out a negative trial suggesting that prednisolone is ineffective and possibly harmful. Looking back now, the paper has had a much further range of influence, still reaching into the 21st century. Firstly, it sets a benchmark for randomised controlled trials in GBS. It stimulated the need for other effective therapies beyond supportive care. Lastly, it described a clinimetric approach to GBS studies that became known as the Hughes scale, which some authors continue to use. However, Professor Hughes recognised the scale's inadequacies from an early stage, spawning the Inflammatory Neuropathy Cause and Treatment (INCAT) studies and, subsequently, the Peripheral Neuropathy Outcome Measures Standardisation (PeriNomS) Rasch-built scales that benchmark influential investigation in the 21st century.

1980–1981: The First Flight of the NASA Space Shuttle

Guy McKhann is the only person who appears twice in my timeline. In 1985 the Guillain-Barré Syndrome Study Group, under Dr McKhann's guidance, published the first really significant trial of effective treatment for GBS [13]. This trial was massive for its time, including 245 adults and children with GBS within 30 days of diagnosis. This sort of study size in GBS has only recently been exceeded with IGOS1000, which has already included more than 1,000 participants in 2016. The paper is widely misquoted and misunderstood, especially when it comes to the use of IVIg, shown later to be of equivalent efficacy to plasma exchange. What is seldom recognised is that neither plasma exchange nor IVIg have been shown to terminate the progress of GBS, reduce disability or abort the nadir of disease. The GBS Study Group trial clearly demonstrated a more rapid recovery, approximately halving the time to 1 Hughes grade improvement or to be extubated if ventilated. This translates of course into a massive reduction in complications of GBS.

1990–1994: Andrew Wiles Proves Fermat's Last Theorem

Guy McKhann and colleagues from Johns Hopkins and China described a fascinating epidemic

neuromuscular paralysis in their Lancet paper of 1991 [14]. So frequently in science it is the things that are not expected that are the most interesting. They observed, “On the basis of clinical features alone, many of the children and young adults in China would be considered to have GBS.” but the electrophysiological and epidemic features were not those seen in Western GBS—this was a new, GBS-like disease. From this paper acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN) were conceived. A common infectious initiation with *Campylobacter jejuni* was identified. The involvement of activated complement and targeted macrophage destruction was visualised, and antibodies in the serum of patients to gangliosides and ganglioside like epitopes resulting in immunological molecular mimicry were described. The findings and pathological knowledge from this critical paper opened the door to multiple avenues of investigation, paving the way to our understanding of GBS as an autoimmune disease. The Chinese Paralytic Syndrome was a sensational discovery.

2000–2001: DONUT Collaboration and the Discovery of the Neutrino

For the last 40 years the study of GBS has been littered with attempts to produce a disease model that reproduced an acute inflammatory peripheral nerve disease in animals. Kadlubowski and Hughes’ description of EAN in 1979 by immunisation with bovine myelin protein antigens (mainly P2) was an early successful attempt. Adoptive transfer EAN produced a T-cell model of the disease. More latterly the beautiful demonstration of the development of a spontaneous neuropathy by subverting immunological tolerance with a transgenic mouse producing P0-specific T cells in 2009 added a key piece to the jigsaw to join up the immunological ‘dot to dot’ puzzle from instigation to completion of disease in GBS. Arguably the most striking model description, however, was by Nobuhiro Yuki and colleagues in 2001 [15], with a rabbit animal model caused by sensitisation with GM1 ganglioside. This paper re-ignited the quest for models which had been fallow for some years and re-engaged the anti-ganglioside antibody hypothesis.

2010–2009 Grunting Causes Diplocardia Earthworms to Emerge from the Soil

So the world of worm science turned full circle, and in 2009 Mitra and colleagues demonstrated that exposing earthworms to vibratory frequencies of less than 500Hz caused them to emerge from their burrows. Darwin, in his paper on the ‘Formation of Vegetable Mould’, had actually demonstrated that worms were very sensitive to the vibrations of the piano when they were placed on the sounding board and middle C (256Hz) was sounded. So over 170 years we have discovered that worms can’t hear but they can *feel* middle C! GBS has gone far further. My key paper for this decade is not yet published but derives from the work of Hugh Willison with complement inhibition. Halstead and colleagues demonstrated the complete abrogation of an antibody-mediated acute inflammatory neuropathy in mice [16]. Although the human pilot study in multifocal motor neuropathy was negative [17], at the time of publication 2 trials of eculizumab in GBS are ongoing. If these fulfil their promise and eculizumab can bring to an abrupt halt the damage ongoing at the time of patients presenting with GBS we will have the first ‘cure’ for this most fascinating of diseases.

Conclusion

Man has been to the moon, antibiotics have come (and perhaps are now going), the mystery of our DNA has been solved and the laws of the universe have been cracked open. Worms still can't hear! Many, many great minds have contributed to these developments and in GBS research has not fallen behind. We should be proud of our discoveries, sometimes serendipitous, sometimes deliberate. The unusual is often the most rewarding. We should continue to pursue our goal to cure this disease—we have not done so badly thus far.

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6

10 Papers the World Forgot: A Critical Analysis of Scientific Progress in GBS

Stephen W. Reddel, Todd A. Hardy and Stefan Blum

Introduction

The centenary of Guillain, Barré and Strohl's landmark description of Guillain-Barré syndrome (GBS) provides an opportunity to celebrate the key papers that have advanced our understanding of the condition. It is equally a time to contemplate some of the lesser-appreciated observations about GBS. In this chapter, using esoteric and poorly understood techniques, we have identified 10 GBS papers which in some aspect are forgotten, but which the authors feel are worthy of renewed reflection. We apologise if there are even more worthy forgotten papers that we have overlooked, especially gems hidden within other languages. We hope that by reappraising the past we and the reader can progress from it.

Gardner WJ, et al. Increased intracranial pressure caused by increased protein content in the cerebrospinal fluid; an explanation of papilloedema in certain cases of small intracranial and intraspinal tumors, and in the Guillain-Barré syndrome. *The New England Journal of Medicine*, 1954

The literature on GBS reports several cases of patients with concomitant papilloedema, but the association is not widely known. In 1954, Gardner and colleagues hypothesized that papilloedema in GBS and in other conditions, such as ependymoma of the cauda equina, acoustic tumour and poliomyelitis, arises as a result of elevated CSF protein. In elegant experiments in anaesthetized dogs, they demonstrated that elevated CSF protein can slow absorption of CSF, presumably by partially obstructing the arachnoid villi, leading to increased intracranial pressure (ICP) and papilloedema [1].

This finding accorded with an earlier postmortem observation in a GBS patient who had elevated ICP, papilloedema, a normal ventriculogram and a tendency for CSF fluid to clot after lumbar puncture, of deposition of 'amorphous material' at the Pacchionian granulation of the arachnoid villi [2]. Further support for impaired CSF absorption leading to raised ICP and papilloedema comes from a later study showing deranged CSF flow in a patient with GBS who also had elevated CSF protein and hydrocephalus [3].

Other reports have disputed the hypothesis of impaired CSF absorption for various reasons, chief among them being that, in individual patients, the level of elevated protein does not necessarily

correspond to papilloedema [4]. Case reports of isotope-labelled albumin absorption from the subarachnoid space in patients with GBS also failed to demonstrate a major defect in CSF reabsorption [5]. Moreover, the observation that not all patients with GBS and papilloedema have hydrocephalus has also been used to argue against the impaired CSF absorption hypothesis.

Amit R, et al. Acute, severe, central and peripheral nervous system combined demyelination. *Pediatric Neurology*, 1986.

Historically, numerous cases have been described recognizing central nervous system (CNS) demyelination in patients with GBS [6,7], but the association is rare. GBS and acute disseminated encephalomyelitis (ADEM) are distinct, monophasic, acquired, multifocal, demyelinating diseases of subacute onset which follow in the days to weeks after infection or vaccination. Both are presumed to have an autoimmune basis and seemingly respond to immunotherapies such as plasma exchange or intravenous immunoglobulin (IVIg). Similarly, cases of chronic peripheral and central demyelination—i.e. CIDP and MS—have been described [8].

The similarity between GBS and ADEM, and the fact that they can occur simultaneously in the same patient has implications for their immunopathogenesis. The monophasic nature of these diseases implies that they arise due to a transient breakdown in immune self-tolerance to a common epitope on the myelin of both the peripheral nervous system (PNS) and CNS. Presently, the identity of this common myelin epitope is unknown, but myelin protein P1 in the PNS is identical to myelin basic protein in the CNS, so an epitope shared between the PNS and CNS is conceivable [9].

If a shared epitope hypothesis is correct, then a pertinent question is why do so few patients who contract GBS also acquire ADEM?

Bickerstaff ER, Cloake PC. Mesencephalitis and rhombencephalitis. *British Medical Journal*, 1951; Bickerstaff ER. Brain-stem encephalitis; further observations on a grave syndrome with benign prognosis. *British Medical Journal*, 1975.

Bickerstaff's original description of what came to be known as 'Bickerstaff's brainstem encephalitis' (BBE), includes the characteristic symptoms of subacute onset oculomotor and facial palsy, bulbar impairment and ataxia, followed by obtundation with mixed upper motor and lower motor neurone signs. While these papers, and the syndrome they describe, are far from forgotten, it is usually not appreciated that as many as 7 of Bickerstaff's 11 patients also experienced nausea and vomiting prior to becoming obtunded [10,11].

The reason why BBE patients should complain of nausea is not clear. One explanation is that it is caused by GQ1b antibodies gaining access to the cerebrospinal fluid (CSF) at sites where there is increased 'leakiness' of the blood brain barrier, such as at the level of the chemoreceptor trigger zone in the area postrema of the medulla [12].

If this is correct, then it provides further evidence that BBE not only affects the PNS but the CNS as well [13]. Observations in support of CNS involvement, at least in a proportion of patients with BBE, include abnormal EEG findings with encephalopathy, increased infratentorial T2 signal changes on MRI, inflammatory brainstem lesions at postmortem, absent cortical-evoked responses, and the finding that GQ1b antibodies in the CSF decline as patients with BBE recover [14,15].

Stewart G, et al. HLA antigens in the Landry-Guillain-Barré syndrome and chronic relapsing polyneuritis. *Annals of Neurology*, 1978

Numerous publications on GBS refer to the concept of molecular mimicry converting an allo-antigen response to an auto-antigen response, suggesting an infection triggering an initial adaptive immune response, followed by a cross reactivity to a neural antigen in the context of an interaction between the infection and the individual's HLA characteristics.

Confounding this hypothesis is the lack of a general association of HLA with GBS, unlike most autoimmune diseases. This lack of association in GBS is unlike the situation in chronic relapsing polyneuritis / CIDP [16]. A lack of association in GBS was confirmed in a number of other studies [17,18].

Presuming that GBS is still caused by the broader immune system and is not directly infectious, this engenders several possible considerations.

1. Each subform of GBS may have HLA associations specific to the trigger and ethnicity that are lost in any general study. This is intellectually the easiest consideration because it accepts the hypothesis. There is some limited support for this idea of subgroup analysis in the context of the 1976–1977 influenza vaccine, or for certain infections [18,19,20].
2. Major HLA are not the determinants of this molecular mimicry and other adaptive systems determining nonpeptide antibodies or NK pathways transduce this response. This is discussed in Yoshi (see below).
3. The cross-reactive response may be the norm, but only certain individuals open the blood nerve barrier, permitting the development of a clinical rather than just an immunological process. In EAN the use of a nonspecific approach to opening the blood nerve barrier facilitates the entry of a specific response to a target antigen [21].

As it currently stands the lack of HLA association for GBS casts some doubt on the molecular mimicry hypothesis. This is, however, in keeping with the idea that GBS is not—or at least is not substantially different from—a standard autoimmune disease [18,22].

Yoshii F and Shinohara Y. Natural killer cells in patients with Guillain-Barré syndrome. *Journal of the Neurological Sciences*, 1998

GBS differs in a number of aspects from most classic autoimmune diseases, with lack of a clear HLA association, poor response to corticosteroids and a short-lived monophasic course. These differences could indicate a deviation of the usual immune response very early after the initial trigger in GBS, raising the possibility that the innate immune system plays a role.

In spite of this, study of the innate immune system in GBS has been neglected. Natural killer cells are an integral part of the cellular innate immune system, with important links to the adaptive immune system. Yoshii and Shinohara describe decreased natural killer cell function early in the disease course in GBS patients compared with healthy controls [23]. After plasmapheresis, they found, NK cell function recovered to normal range. In contrast, NK cell numbers did not change.

The role of the innate immune system in GBS pathogenesis has been recently re-explored. The response of dendritic cells of patients with GBS secondary to *Campylobacter jejuni* have been found to have an increased activation to lipopolysaccharides (LOS) [24]. Macrophage migration inhibitory factor

(MIF), a cytokine-inhibiting random migration of macrophages, has been found to be upregulated in the serum and CSF of GBS patients, with upregulated Toll-like receptor 4, a receptor of LOS on monocytes [25]. Furthermore, genetic differences in killer-immunoglobulin-like receptors (KIR) in GBS patients compared to healthy controls have been found [26].

Exley AR, et al. Tumour necrosis factor-alpha and other cytokines in Guillain-Barré syndrome. *Journal of Neurology, Neurosurgery and Psychiatry*, 1994

A role for cytokines in the pathogenesis of GBS has been proposed ever since the detection of the therapeutic effect of plasma exchange, which suggested soluble factors are involved. The role of TNF- α in GBS pathogenesis had been suspected based on the observation of TNF- α mediated changes after intraneural injection in mice as well as the detection of TNF- α positive macrophages around nerves at the time of disease onset in EAN [27,28].

Exley and colleagues studied a range of cytokines in GBS patients undergoing plasma exchange. They found no difference in IL-1 and IFN- γ in GBS patients versus controls. In contrast, TNF- α concentrations were increased in GBS patients, including correlation with severity [29].

More recently TNF- α polymorphisms showed genetic differences in GBS patients versus healthy controls. A meta-analysis examining 12 studies from diverse geographical areas confirmed that TNF- α polymorphism 308 A/G was significantly associated with the risk of developing GBS [30]. Parallel to this, clinical observations linked demyelinating neuropathies with use of TNF- α inhibitors, such as infliximab or etanercept. A range of demyelinating neuropathies, including AIDP, MFS, CIDP and MMN have been described in the context of TNF- α inhibition therapy [31]. Confusingly, TNF- α inhibition was also reported as therapy for CIDP [32].

This points towards a complex role of TNF- α in the pathogenesis of GBS; indeed, such a role has been proposed for a number of other autoimmune diseases [33]. Possibly, this could be explained by interaction of TNF- α with its diverse receptors or downstream effects thereof. TNF- α can act as a pro-inflammatory and activate macrophages by binding to a specific cell surface receptor, TNFR1. However, binding to TNFR2 has been linked to anti-inflammatory and neuroprotective effects [34].

Prineas JW. Pathology of the Guillain-Barré syndrome. *Annals of Neurology*, 1981; Schonberger LB, et al. Guillain-Barré syndrome. Its epidemiology and associations with influenza vaccination. *Annals of Neurology*, 1981.

We include 2 papers, but commend the whole edition on GBS, the very first supplement of *Annals of Neurology*. It reported on a GBS conference sponsored by the Kroc Foundation, endowed by the founder of the McDonald's hamburger chain.

Prineas' paper provides a fascinating review of the early pathological studies of GBS and related diseases [35]. Initially such cases were not accepted as being a disorder of the peripheral nerves, as anterior horn cell changes were present, suggesting this was spinal in aetiology. Charcot's strength of opinion contributed. Slowly this evolved into acceptance that the peripheral nerves were involved, and the anterior horn cell changes were a postmortem artefact.

Later studies reviewed indicated that pathological changes were patchy and particularly where

anterior and posterior roots join to form the spinal nerves. These dissections of large numbers of necropsy GBS cases early in the disease course are unlikely to be repeated. These demonstrated that the time from onset to death is an important contributor to the pathological changes present. Earliest is the presence of oedema and irregularity of the myelin sheaths, with cellular infiltration only 9–11 days after onset.

The paper concludes with a review of Prineas' electron microscopy studies of GBS with wonderful figures of macrophage processes insinuating into the myelin sheaths.

Schonberger and colleagues provide a comprehensive review of the evidence supporting a significant excess of GBS after the A/New Jersey influenza vaccinations administered in the United States in 1976 and 1977. This resulted in the American Academy of Neurology collaborating with a national GBS surveillance system initiated by the Centers for Disease Control. Their paper confirms that the additional GBS cases after the 1976–1977 vaccination program occurred in the first 5 weeks and peaked at 2–3 weeks after vaccination, and that this was not the case in subsequent years [36]. The same temporal association of GBS following any other triggering infection, whether respiratory or gastrointestinal, was also shown.

Guillain G, et al. Sur un syndrome de radiculo-névrite avec hyperalbuminose du liquide céphalo-rachidien sans réaction cellulaire: remarques sur les caractères cliniques et graphiques des réflexes tendineux. *Bulletins et mémoires de la Société médicale des hôpitaux de Paris*. Translation by Brody IA and Wilkins RH, *Archives of Neurology*, 1968 (Brody and Wilkins, 1968).

It may seem strange to end a chapter on things forgotten with a paper that started the book! However, the historical context is now dim. When authors newly describe a syndrome, a distinction is being made from what is already known. Guillain, Barré and Strohl's paper stressed that there was no fever or venereal disease [37]. So when they wrote "the striking hyperalbuminosis of the cerebrospinal fluid without cellular reaction is a feature ... of signal importance ... it has not been described ... in radiculitis and polyneuritis" [38], what were those other cases of sometimes febrile radiculitis and polyneuritis *with* a cellular reaction?

These were doctors working in an army hospital in the Great War, seeing soldiers from the trenches, in northern France. In addition to the war wounds there were numerous infections, arising from poor hygiene, as well as zoonoses, with endemic rats and lice in the fields and forests behind the front, and *Ixodes ricinus* ticks, the European vector for Lyme neuroborreliosis, even now prevalent in this part of France [39].

Febrile polyneuritis was discussed contemporaneously, by Lieutenant-Colonel Gordon Holmes, Consultant Neurologist, British Armies in France [40]. Richard Hughes writes, "It is puzzling to know what modern disease Osler and Holmes were describing since absence of fever is the rule in Guillain-Barré Syndrome" [41]. Polio and diphtheria are discussed therein; syphilis and spinal compressions and/or Pott's disease (tuberculosis) were discussed by Guillain and his colleagues. However, Lyme disease and trench fever may be forgotten differentials for the historical context. Holmes' paper noted an association with trench fever.

True nervous system Lyme disease can present as rapidly progressive radiculoneuritis akin to GBS [42]. Facial nerve involvement is common, and indeed was noted by Holmes[40]. Fever may be absent,

but a cellular pleocytosis is characteristic. It is still the case that “a lumbar puncture is usually performed ... to rule out infectious diseases, such as Lyme disease” [43].

Trench fever, a systemic infection of *Bartonella quintana* transmitted by the body louse, was rife and may cause meningoencephalitis with cellular pleocytosis. Trench fever caused marked loss of fighting man power and consequent investigation [44]: “It is curious that the sniffing up the nose of the infected excreta of lice did not give rise to infection, whereas the placing of the same material in the conjunctival sac did give rise to positive results in 2 cases. The attempt to infect by way of the urethra was unsuccessful.”

The initial presentation can be similar to GBS, although the later course is not. “The men affected by trench fever began ... with a faintness that dropped them in their tracks, headache and backache, with pain and stiffness in the legs.... Many could not bear the pressure of the bedclothes” [45] (Herringham, 1917). Neurologists will recall such histories, and while many cases will be parainfectious myalgia or some other cause of pain, some will be a pain-predominant presentation of GBS, with initially preserved reflexes.

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EPIDEMIOLOGY GLOBAL VIEW

7

The Global Epidemiology of Guillain-Barré Syndrome and the Relationship to Vaccines

James J. Sejvar

Introduction

Although relatively uncommon compared to many other neurologic illnesses, Guillain-Barré syndrome (GBS) has been the focus of some of the most intense and extensive epidemiologic assessments of any illness. This has primarily been driven by an apparent causal association between GBS and a particular formulation of the influenza vaccine. As such, our understanding of the epidemiology of GBS is intimately intertwined with the concern about GBS and vaccines. As a result, this chapter will discuss what we currently know about the global epidemiology of GBS, and how its relationship to a single vaccine has driven much of the overall understanding of GBS epidemiology.

Global Epidemiology of GBS: The Basics

Shortly after the recognition and description of the specific clinical entity of GBS (whether you are in the ‘Landry (1859)’ camp or the ‘Guillain, Barré, and Strohl (1916)’ camp), there was a great deal of focus on, and rapid increase in understanding of the clinical, laboratory and electrophysiologic aspects of this newly recognized syndrome. However, the epidemiology of the syndrome was poorly understood; epidemiology as a distinct medical discipline was still in its early stages at this time, and the relative rarity of the condition made epidemiologic studies challenging. Nevertheless, over time, case reports began to trickle into small case series, and from this trickle, several epidemiologic factors of GBS seemed to emerge: it was relatively uncommon; it seemed to affect males more commonly than females; it could occur at any age; and it was often associated with some antecedent prodrome suggestive of infection, particularly upper respiratory tract infections. Information on incidence, demographics, and other basic epidemiologic data were gleaned from larger studies on the occurrence of neurologic disease in general.

Early assessments of the epidemiology of GBS were hampered primarily by one thing—lack of a standardized set of criteria or common case definition, by which cases could be accurately compared with each other and reliable estimates of incidence obtained. So many different systems of classification and characterization of the syndrome began to evolve that it eventually prompted Guillain himself to state, “I no longer recognize the syndrome J.A. Barré and I described” [1].

The first true descriptive epidemiologic study of GBS was published in 1973 by Lesser and colleagues, who utilized the composite medical diagnostic file of the Mayo Clinic in Rochester,

Minnesota [2]; this is a database that allows for the collection of longitudinal data from a circumscribed population over a long duration. This study retrospectively identified all GBS cases occurring in Olmsted County, Minnesota between 1935 and 1968. The case definition used was a clinical history of acute or subacute onset of bilateral weakness with or without cranial nerve abnormalities or sensory findings, in the absence of concurrent febrile illness; cyto-albuminologic dissociation was assessed for but was not a requisite. This was the first comprehensive study to validate, in a systematic and standardized fashion, many of the epidemiologic features of GBS that we now take for granted. During that 34-year period, 29 patients meeting the case criteria were identified, resulting in a mean annual incidence of 1.6 cases/100,000 population/year; males were slightly more likely to be affected than females. Rates were highest in the 40–59 age group (though, notably, due to small sample size the standard errors in each age group were large). Cases were not clustered in any season, or in any given year. An antecedent respiratory or infectious illness closely preceding neurologic illness onset was reported in 16 (55%) cases.

The Lesser study was followed up by a subsequent assessment using the same methodology and the same database, extending the investigation period through 1976, and included a case-control component [3]. In order to evaluate the possible association between antecedent infections and GBS, controls consisting of persons with acute neurologic illness but not GBS (including meningitis, herpes zoster and idiopathic Bell's palsy) were identified. The additional 8 years yielded 11 additional cases, with a total of 40 cases identified between 1935 and 1976. The findings of this assessment were largely the same as the first assessment: overall mean annual incidence of 1.7/100,000 persons; slight male predominance; no detectable trends by age, sex, season or year; and an increase in incidence in the 40–59 age group. The case control study suggested that GBS patients were statistically more likely to report a febrile or other infectious illness in the 4 weeks preceding GBS onset than the age- and sex-matched controls, but no differences in terms of exposure to prior immunizations, allergic or metabolic disorders, or exposure to toxins, suggesting an important relationship between prior infections and GBS.

Together, these papers solidified many of the basic epidemiologic tenets of GBS that had been reported to that time—at least in a white, homogeneous, largely middle class population in the Midwestern United States. Even the authors of these studies cautioned against generalizing these findings to the entire United States, let alone the world. A population-based study assessing the incidence of GBS in San Joaquin County in California was published at approximately the same time as the publication of the Olmsted County papers [4]; the findings in this study were commensurate with the Olmsted County data with the exception that the San Joaquin evaluation failed to demonstrate a male predominance, most likely due to small sample size ($n = 18$).

Aside from these assessments, surprisingly little work on the basic epidemiology of GBS was conducted from identification of the syndrome until the swine flu (H1N1) campaign of 1976. Globally, what work was being conducted consisted of case reports and case series using varying classification schemes, case ascertainment methodologies, and denominators resulting in a vertigo-inducing variety of estimates of incidence, seasonality versus no seasonality, age distributions and other basic epidemiologic parameters. Essentially, the only consistent feature of these various estimates and assessments was inconsistency. This heterogeneity was demonstrated eloquently in an article by McGrogan and colleagues, who undertook the heroic task of performing a systematic literature review of the epidemiology of GBS worldwide between 1980 and 2008 [5]. An initial review of the literature yielded 511 papers; these were winnowed down to include only epidemiologically sound studies with sufficient data and that conformed to one of the relatively widely accepted case definitions/case criteria for GBS. After this laborious process, a resultant 63 papers survived to the point of full review.

To review the results of McGrogan's assessment is to appreciate the immense diversity of fundamental epidemiologic features of GBS worldwide. Much of this diversity, however, is 'man-made', driven by differences in case ascertainment, case definitions/classifications and reporting methods, rather than true fluctuations in GBS epidemiology. These differences resulted in a vast range of incidence estimates with incidence rates varying between 0.38/100,000/year (95% CI 0.25–0.56) in Finland to 2.53/100,000/year (95% CI 1.87–3.35) in Curaçao. McGrogan's review of the literature also highlighted that most of what we know about the epidemiology of GBS is based upon populations in North America and Europe, where the vast majority (89% of the 63 studies) of studies were conducted; for the rest of the world, so few assessments had been conducted as to preclude comment on geographical trends. An important observation from McGrogan's paper was the influence of study method had on incidence estimates. Invariably, incidence estimates provided by prospective studies and database searches were higher than those found by retrospective studies relying on medical record review.

In an effort to streamline the heterogeneous results of McGrogan's work, several colleagues from the U.S. Centers for Disease Control and Prevention (CDC), including myself, performed a meta-analysis to further refine these incidence estimates in North America and Europe [6]. Beginning with the articles cited by McGrogan, and inclusive of several other studies published subsequent to McGrogan's article, we conducted a meta-analysis of the most thorough epidemiologic papers published up to 2009. To obtain the most accurate incidence estimates, we applied specific and tight criteria. Because so little was understood about the epidemiology of GBS elsewhere, we included only articles estimating GBS incidence in North American and European countries; studies had to include at least 20 cases; data had to be population-based; and a diagnosis of GBS had to be confirmed by a subject matter expert using a widely accepted case definition for GBS. This study identified 1,683 nonduplicative publications, of which 16 met the inclusion criteria. Using regression analysis, we were able to derive an equation to calculate average GBS rate per 100,000 person-years as a function of age ($\exp[-12.0771 + 0.01813 (\text{age in years})] \times 100,000$); this equation provides a utilitarian method of ascertaining 'expected' rates of GBS by age in any given population in North America or Europe, which we hoped would be a useful tool. Our calculation resulted in a median crude incidence rate for GBS of 1.11 cases per 100,000 person-years. GBS incidence increased by 20% for every 10-year increase in age, and consistent with other studies, was slightly higher for males than females. This study was able to harness the power of the meta-analysis not only to calculate a robust GBS incidence estimate, but to provide a tool to estimate background age-specific rates of GBS incidence in comparable populations.

As mentioned, McGrogan's article highlighted the disappointing paucity of good, solid incidence and epidemiologic estimates of GBS outside of North America and Europe that still persists today. These estimates are plagued by less-than-reliable case ascertainment methods (e.g. use of ICD codes only), lack of validation of cases by applicable criteria and other limitations. A notable exception to this general lack of understanding is an assessment of GBS incidence in China published in 2002[7]. Cheng and colleagues were able to perform a prospective, population-based assessment of GBS in Harbin, China (population 5.4 million as of 2001) over a 1-year period. The authors performed enhanced active surveillance for GBS amongst a network of physicians, included a component of admission record review at all hospitals in Harbin to identify GBS patients who may have been missed by the prospective surveillance, and had each GBS diagnosis confirmed by examination by senior neurologists. The authors identified 36 patients in Harbin, resulting in a crude incidence of 0.67 (95% CI 0.47–0.92) for both sexes; male:female ratio was 1.4. Interestingly, when assessing age-specific incidence, the lowest GBS rate per 100,000 person-years was in the 30–39 age group, while the highest was in the youngest age group (<10 years of age; 1.15/100,000/year). The 2 notable findings from this study were the relatively low crude incidence when

compared to other studies using such robust case-finding methodologies, and the finding of a high incidence among children and lower incidence in adults.

The findings of this study continue to perplex me; their study design was sound, they were well-powered to detect their cases, but the age-specific data differs from nearly every other GBS epidemiologic study. The authors make several suggestions as to why this finding might be, including more complete case ascertainment among children, misdiagnosis of GBS in this age group and a true difference in GBS epidemiology in Harbin due to genetic or environmental differences between populations; however, I'm still left with questions.

Of note, a similar finding of high incidence of GBS among children was observed in a well-designed assessment of GBS epidemiology in Bangladesh performed by Islam and colleagues [8]. By applying a standardized case definition to identified acute flaccid paralysis (AFP) cases for 2 consecutive calendar years (2006 and 2007), they calculated a crude incidence rate fell between 1.5 and 2.5 per 100,000 population /year. Seasonality was noted, with a peak in early spring (May) and nadir in February. The high incidence of GBS in children in Bangladesh has been hypothesized to be related to a larger burden of exposure to enteric pathogens, specifically *Campylobacter jejuni*. This study assessed incidence only in children <15 years, precluding a comparison of incidence between children and older age groups as in the Harbin paper. Both these studies demonstrate, however, that it is probable, even likely, that the epidemiology of GBS in other parts of the world differ substantially from that described and well known in North America and Europe. This highlights the need for further, well-designed investigations into the epidemiology of GBS in South and Southeast Asia, Africa, Central and South America, and the Middle East in order to truly obtain a global comprehension of the epidemiology of GBS worldwide, as well as risk factors for GBS in these areas.

GBS and Vaccines

The issue of GBS epidemiology and the association of GBS and vaccines are intimately intertwined. This is because, due to one singular event, GBS went from being an interesting but rare neurologic disorder to a syndrome that has undergone some of the most extensive epidemiologic scrutiny of human medical conditions (at least in North America and Europe). More is understood about the epidemiology and incidence of GBS than about many far more common diseases. However, the apparent causal association between GBS and a formulation of the vaccine against so-called swine flu had far-reaching implications for our understanding of GBS.

An important concept in the discussion of potential adverse events following immunizations (AEFI) is the concept of causality. It is possible to find case reports or case series of the development of nearly any neurologic illness following nearly any vaccine; reports of 'X illness following Y vaccine' permeate the literature. However, substantiation of an etiologic or causal nature of such associations with data from clinical trials or large epidemiologic studies is generally lacking. Thus, the occurrence of many clinical events that are associated with a particular vaccine by virtue of temporal proximity is substantially different than demonstrating a causal relationship.

GBS and Vaccines: The Early Years

Since its initial recognition and description, it was observed that GBS could occasionally occur following a vaccination. In a review of over 1,100 case reports and case series conducted by Leneman and colleagues [1], vaccines were included among the literal litany of events temporally associated with

development of GBS (other conditions ‘associated’ with GBS in this review included penicillin, recent emotional stress, falls and fractures and lightning strikes). However, even in this list, it was recognized that some vaccines anecdotally seemed to be more strongly associated with subsequent GBS than others, on the basis of more GBS cases apparently reported following particular vaccines. The most common of these anecdotal GBS-inducing vaccines seemed to be neurally derived rabies vaccine; other vaccines, including tetanus, smallpox, the Salk poliomyelitis and seasonal influenza vaccines were also included in this comprehensive list. However, like every other association noted at that time, the association between vaccines and GBS was temporal only, and a causal association was unable to be demonstrated, primarily because these investigations lacked appropriate controls against which to compare the experience of a given series of cases.

GBS and the 1976 U.S. Swine Flu Vaccination Campaign: The Shot Heard ‘Round the World

GBS began to be viewed specifically through a vaccine-filtered lens in 1976. This was the year of the U.S. bicentennial—1976 marked the 200th birthday of the nation. Against this backdrop of patriotism, fireworks and celebration, the understanding of GBS was about to be changed forever.

In early February of 1976, the New Jersey Department of Health obtained isolates of influenza viruses from military recruits at Fort Dix in New Jersey, who were suffering from influenza-like illness (ILI). Testing at the CDC indicated that most of these influenza virus isolates were of a seasonal strain that was commonly circulating at that time. Several of the isolates, however, were determined to be an H1N1 virus of swine origin (A/NJ/76[H1N1]). These isolates were antigenically similar to the virus responsible for the catastrophic 1918 ‘swine flu’ pandemic that resulted in millions of deaths worldwide. Surveillance around the Fort Dix area failed to identify the presence of the H1N1 virus outside of the base; surveillance among the Fort Dix military personnel, however, demonstrated sustained person-to-person transmission. In March 1976, a panel of experts was emergently convened, and recommended widespread H1N1 vaccination in anticipation of another epidemic of ‘swine flu’. Within weeks, a presidential order was issued creating the National Influenza Immunization Program (NIIP), and the CDC was charged with its implementation. During that summer, the U.S. government contracted with 4 pharmaceutical companies to produce enough H1N1 vaccine to immunize the entire U.S. population; the manufacturers balked, agreeing to manufacture the vaccine on such short order only if they were deemed ‘immune’ from any litigation from possible adverse events. The U.S. government agreed, and the manufacturers quickly went to work formulating millions of doses of vaccine (predominantly monovalent inactivated vaccine). On 1 October 1976, the NIIP was launched, with great fanfare. Keep in mind, to this point, the H1N1 virus had not been identified off of the Fort Dix base, and no one had died or fallen severely ill from the virus. Over the subsequent 11 weeks, about 45 million persons were administered the vaccine.

Before the campaign was launched, a nationwide passive surveillance system was established to evaluate any possible adverse events following this immunization. A young CDC epidemiologist, Dr Larry Schonberger, was put in charge of overseeing and monitoring this program. Trained in internal medicine and just arriving as a CDC staff member after his studies in epidemiology at Johns Hopkins and anxious to begin applying his epidemiologic knowledge, Dr Schonberger took on the project, never anticipating the storm that was to come. By 2 December, 2 clusters of GBS were reported to CDC from 2 different U.S. states. Out of an abundance of caution, these clusters led to the initiation of active surveillance for GBS cases in these 2 states and 2 additional states, and shortly thereafter 7 additional states, to evaluate the possibility of a causal relationship between GBS and the influenza vaccinations. As

Dr Schonberger crunched the numbers, he became intrigued; as more GBS cases came in, intrigue turned into concern. In this pre-desktop computer era, Dr Schonberger spent hours comparing rates of GBS among vaccinees and nonvaccinees, and became convinced that something was out of the ordinary—there appeared to be a plausible association between the vaccine and GBS. Using person-time analysis, the data suggested that recent vaccinees had a 7-fold greater incidence of GBS compared with those who had not received the vaccine. On the basis of these preliminary findings, and the weakening evidence that a swine flu pandemic was actually going to emerge, the vaccination campaign was suspended on 16 December 1976 (Dr Schonberger recalls the secretary of Health and Human Services telling him in a stern and somewhat irritated voice, “You *better* be right about this.”)

The preliminary findings were published in CDC’s *Morbidity and Mortality Weekly Report* (MMWR) on 24 December [9]. All personnel who were originally deployed to oversee and administer the vaccine were reassigned to conduct active surveillance for GBS nationally to determine as quickly as possible whether the influenza vaccinations were in fact related to GBS and, if so, to determine the risk. CDC and state health departments performed active outreach to neurologists throughout the United States to request reporting of all cases of GBS with an onset between 1 October 1976 (the commencement of the immunization program) and 31 January 1977. This focused surveillance on GBS identified a total of 1,098 cases; 532 (48.5%) occurred sometime after receipt of a swine flu vaccine. Many notable epidemiologic features were identified, including several that provided strong evidence of an etiologic association between the vaccinations and GBS:

1. The attack rates of GBS among adults were significantly higher in vaccinees compared to the rates in the unvaccinated population.
2. The distribution of cases occurring by week after vaccination clustered in the first 5 weeks, particularly in weeks 2 and 3 after vaccination. Compared to the expected rates, the relative risks for GBS during weeks 2 and 3 after vaccination exceeded 12. This pattern of occurrence was biologically consistent with the development of an immune-mediated condition such as GBS.
3. Compared to the unvaccinated GBS patients, the proportion of the vaccinated patients with a history of an acute illness within 4 weeks before onset of GBS was markedly lower—33% versus 62%—suggesting that vaccine and not another antigenic stimulus was resulting in the increased incidence of GBS.
4. The reported relative risk (RR) of GBS for the 6-week period after vaccination in adults was 7.6 (95% CI 6.7–8.6), resulting in an attributable risk (AR) during this period of 0.88 GBS cases/100,000 vaccinees.
5. About 98% of the vaccinations were administered to adults. During the 6-week period after vaccination, the attack rates in each of the 4 adult age groups, (18–24, 25–44, 45–64 and 65+ years) but not for children (0–17 years), were significantly elevated compared to the background rates.

Summary conclusions of this study were that many cases of GBS were directly related to vaccination, with those recently vaccinated having a significantly elevated attack rate in every adult age group. The total vaccine AR in adults was reported as just under 1 case of GBS per 100,000 vaccinations. Dr Schonberger published his final results in the *American Journal of Epidemiology* [10].

Criticisms and Reanalysis

Despite these studies suggesting an epidemiologic link between the 1976 influenza vaccine and GBS, the

results were questioned on a number of different bases: cases of GBS were ascertained and classified by state and local public health officials who may have not have familiarity with the complicated diagnosis of GBS; full evaluation of cases by a trained neurologist was not required, and many non-neurologist practitioners may not have had enough familiarity with the syndrome to arrive at a correct diagnosis; and the intense publicity surrounding the swine flu immunization effort may have led many practitioners to over-diagnose any peripheral neuropathy among vaccinees as GBS. Similarly, the CDC did not conduct an independent medical record review of the 1,098 cases submitted as GBS. It was noted that the characteristics of a number of cases accepted by the CDC did not conform to accepted diagnostic criteria for GBS. Finally, the publicity surrounding the campaign may have biased practitioners towards over-reporting GBS among vaccinees, or alternatively under-reporting GBS among unvaccinated patients. These points were summarized in a scathing editorial published in the *Archives of Neurology* by Kurland and colleagues [11].

As legal claims against the U.S. government for damage caused by the H1N1 vaccine mounted, the pressure on Dr Schonberger similarly increased. Schonberger and others at the CDC were deposed, and Schonberger found himself trying to explain the epidemiologic principles behind the association to litigious lawyers and patients. “It was the most stressful time of my life”, he recalls. Ultimately, the U.S. Justice Department insisted on an independent assessment of the original CDC data by an expert panel comprised of epidemiologists and neurologists. This expert panel was convened in 1982 and chaired by Dr Alexander Langmuir, one of the legends of public health epidemiology and a former CDC director. The CDC was forced to hand over all of the raw data and calculations. In determining risk, the panel decided after analysing available data to exclude many cases that were included in the original study, and to base its risk assessment on the most definite and severely affected cases (those with ‘extensive motor involvement’). It also decided to use 2 different estimates of the expected background rates of GBS. Based on the ‘extensive motor involvement’ cases, the panel confirmed the original findings of a significantly elevated risk of GBS among vaccinees that peaked at weeks 2–3 following vaccination and lasted for at least 6 weeks. The panel reported that this vaccine effect possibly lasted for 8–10 weeks, but not longer. Depending upon the estimated baseline used, the panel concluded that the total vaccine attributable risk of GBS was 0.49 to 0.59 cases per 100,000 adult vaccinees over a 6- or 8-week period after vaccination respectively; thus, although the estimated attributable risk was somewhat lower than that estimated by original study due to more conservative case selection, this investigation again substantiated a causal association between the vaccine and GBS; results were published in the *American Journal of Epidemiology* [12].

The 1976 Swine Flu Vaccine and GBS: Biological Evidence

The multiple assessments demonstrating the link between the swine flu vaccination and GBS essentially proved causality by epidemiologic means. What remained unclear, however, was why this would be the case, biologically. One study assessing the possible association of human leukocyte antigen (HLA) types among cases of GBS identified during the 1976 vaccine surveillance effort [13]. This study identified no specific HLA haplotypes amongst 92 GBS cases and 100 controls that were clearly associated with development of GBS following the 1976 influenza vaccine.

Perhaps the most creative attempt to establish a biological underpinning to this association was performed by Nachamkin and colleagues [14]. This study assessed the potential association of influenza vaccines with anti-ganglioside antibodies. Particular *Campylobacter jejuni* serotypes have been found to have a strong association with the development of GBS, which is hypothesized to be due to molecular

mimicry between bacterial surface lipooligosaccharides which express peripheral nerve ganglioside-like epitopes, resulting in the production of cross-reactive antibodies. It has been hypothesized that, since *C. jejuni* is frequently present in poultry, and since influenza vaccine is produced in chicken eggs, contamination of vaccine-production eggs with *C. jejuni* may have resulted in an association of GBS with the 1976 vaccine or other formulations.

Nachamkin tested this latter hypothesis by obtaining archived lots of the swine flu vaccines as well as several other influenza vaccine formulations that had not been associated with increased risk of GBS. Mice were inoculated with vaccine; additional mice were inoculated with *C. jejuni*—one serotype that displays GM1 ganglioside mimicry as a positive control, and one with no GM1 ganglioside mimicry as a negative control. The immunized mice were found to have no antibodies to *C. jejuni*, suggesting that *Campylobacter* antigens were not present in the vaccine formulations. However, all immunized mice developed anti-GM1 IgM and IgG antibodies, with significant increases in such antibodies observed over time. While the authors offered a possible hypothesis as to how these antibodies might have been generated (involving an interaction between vaccine epitopes and neural sialic acid moieties), the ability of all tested vaccine formulations, even those with no apparent association with GBS, to induce anti-GM1 antibodies calls into question the biological significance of this finding. Additional exploration of the role of vaccine proteins, including use of negative controls of a type not utilized in the study, would be needed to further substantiate these findings.

Subsequent Assessments of the GBS Risk after Receipt of Influenza Vaccines Not Containing the 1976 Swine Flu Antigens

The association of the 1976 influenza vaccine with GBS obviously led to great concern about the potential of subsequent seasonal influenza vaccines to lead to a similar association. Between 1977 and 2009, at least 9 published assessments of the risk of GBS following influenza vaccines were conducted (Table 7.1) [15–24]. Of these 9 studies, 2 involved active, population-based surveillance, medical record reviews and patient interviews [19,24]. Three used searches of large datasets that enabled linking of disease codes and vaccination information and the comparison of GBS attack rates in different time windows after vaccination [20,22,23]. Two involved active sentinel neurologist surveillance [17, 25]. One used hospital discharge data without a link to vaccination information [18] and one assessed reports to a national passive vaccine-adverse event surveillance system [21]. Two of these studies suggested a small but statistically significant increase in risk of GBS following various formulations of influenza vaccine, on the order of relative risks of about 1.6 to 1.7—statistically significant but not particularly concerning, from a clinical standpoint [19, 20]. The other 7 studies did not find a statistically significant overall vaccine-related increase in GBS risk (Table 7.1).

Overall, the preponderance of epidemiologic evidence on the risk of GBS following influenza vaccine formulations since 1976 would suggest that this risk, if any, is much smaller than that observed in association with the 1976 vaccination campaign. Hence, any risk of GBS following influenza vaccines would likely be greatly outweighed in any particular season by the benefits of the vaccinations in reducing influenza morbidity and mortality.

Table 7.1 Epidemiologic features and incidence of Guillain-Barré syndrome following the 1976 swine influenza vaccine, and various formulations of seasonal influenza vaccine, 1976–2009

| Author (First) | Title | Journal Citation | Year | Case Ascertainment Method |
|-------------------------|---|---|------|---|
| Schonberger LB | GBS following vaccination in the national influenza immunization program, United States, 1976–1977 | <i>Am J Epidemiol</i> 1979; 110 (2): 105–123 | 1979 | Outreach to clinicians through network of surveillance personnel based at state/local jurisdictions throughout United States; case information collected by surveillance personnel using standardized case report form. |
| Hurwitz ES | GBS and the 1978–1979 influenza vaccine | <i>N Engl J Med</i> 1981; 304 : 1557–61 | 1981 | Active surveillance by request to practicing neurologists in the United States to report all cases of GBS and vaccine status by completion of a 1-page report form.* Cases reported to CDC by 1,813 members of the American Academy of Neurology (AAN). |
| Kaplan JE | GBS in the United States, 1979–1980 and 1980–1981: Lack of an association | <i>JAMA</i> 1982; 248 (6): 698–700 | 1982 | Active surveillance by request to practicing neurologists in the United States to report all cases of GBS and vaccine status by completion of a 1-page report form.* Cases reported to CDC by 1,648 members of the AAN. |
| Kaplan JE | GBS in the United States, 1978–1981: Additional observations from the national surveillance system | <i>Neurology</i> 1983; 33 : 633–37 | 1983 | Active surveillance by request to practicing neurologists in the United States to report all cases of GBS, as well as vaccine status.* Cases reported to CDC by 1,648 members of the American Academy of Neurology (AAN). Completeness of reporting assessed by direct telephone contact with neurologist offices to identify unreported cases from 1980 to 1981. |
| Roscelli JD | GBS and influenza vaccination in the U.S. Army, 1980–1988 | <i>Am J Epidemiol</i> 1991; 133 (9): 952–55 | 1991 | Database search of Individual Patient Data System (IPDS) codes for hospitalizations associated with specific International Classification of Diseases (ICDA) code for GBS. This was followed up by attainment and review of medical records. |
| Chen RT | Investigation of a possible association between influenza vaccination and GBS in the United States, 1990–1991 | <i>Postmark Surv</i> (abstract) 1992; 6 (1): 5 | 1992 | Active surveillance (not defined) in 13 U.S. states, with confirmation of GBS cases by chart review and patient interview |
| Lasky T [§] | The GBS and the 1992–1993 and 1993–1994 influenza vaccines | <i>NEJM</i> 1998; 339 (25): 1791–1801 | 1998 | Search of hospital discharge databases in 4 U.S. states (IL, NC, MD, WA) for ICD-9 code for GBS (357.0), followed by standardized chart review by reviewer blinded to vaccine status. Patient telephone interview was used to obtain vaccine history and additional clinical information, and providers contacted to obtain exact date of vaccination. |
| Haber P | GBS following influenza vaccination | <i>JAMA</i> 2004; 292 (20): 2478–81 | 2004 | Review of reports of GBS and non-GBS adverse events following influenza vaccine reported to VAERS. Active follow-up of VAERS reports between 1994 and 2003 to verify diagnosis of GBS and obtain additional clinical information |
| Juurink DN [§] | GBS following influenza vaccination in adults: a population-based study | <i>Arch Int Med</i> 2006; 166 : 2217–21 | 2006 | 1. GBS hospitalizations identified by review of Canadian Institute for Health Information Discharge Abstract Database for ICD-9 codes of 357.0 or ICD-10 codes of G61. 2. Vaccinations assessed by review of Ontario Health Insurance Plan database for vaccinations administered between October and November (presumed to be influenza vaccines). GBS cases and ‘influenza’ vaccines linked by health card number |

(continued)

Table 7.1 (continued)

| Author (First) | Title | Journal Citation | Year | Case Ascertainment Method |
|----------------|---|--|------|---|
| Stowe J | Investigation of the temporal association of GBS with influenza vaccine and influenza-like illness using the United Kingdom General Practice Research Database (GPRD) | <i>Am J Epidemiol</i> 2008; 169 (3): 382–88 | 2008 | Search of the national GPRD for READ or OXMIS codes for GBS and for influenza, with subsequent validation by medical record review. Vaccinations identified by same method. By using the self-controlled case control method, risk of GBS following vaccination was divided into 3 risk periods of 0–30 days, 31–60 days, and 61–90 days after vaccination or influenza-like illness. |
| Burwen DR | Evaluation of Guillain-Barré syndrome among recipients of influenza vaccine in 2000 and 2001 | <i>Am J Prev Med</i> 2010; 39 (4): 296–304 | 2010 | Observational study using U.S. national data from the Medicare program. Persons with a claim for influenza vaccine during 2000 and 2001 using Healthcare Common Procedure Coding System codes were identified; potential GBS cases were identified using hospital claims data using ICD-9 code 357.0. Hospitalizations with diagnosis code 357.0 and admission date within 18 weeks of influenza vaccine underwent chart review. Risk interval design was used to compare the rate of GBS within weeks 0–6 of vaccination to rates within weeks 9–14. |

[§] Studies with findings suggesting an increased risk of GBS following influenza vaccination.

* Excludes Maryland (approximate population during this period of 4.22 million), which conducted its own active surveillance for GBS during this period.

| Author (First) | Case Definition | Study Period | Number of GBS Cases in Study | Estimated Background Incidence in Persons > 17 years old |
|----------------|--|--|--|--|
| Schonberger LB | GBS as diagnosed by a clinician | October 01, 1976–January 31, 1977 | 1,098 GBS patients total; 532 with receipt of A/NJ/76 influenza vaccine prior to GBS onset; 15 with receipt of vaccine after onset of GBS; 543 without vaccine; 8 with unknown vaccination status | 1.16 cases/100,000 adults/year |
| Hurwitz ES | GBS as diagnosed by a neurologist, and objective evidence of muscle weakness | September 1, 1978–March 31, 1979 (6 months) | 544 total; 13 vaccinated (12 adults), 495 unvaccinated (393 adults); 15 of unknown age; 21 of unknown vaccination status | 0.46 cases/100,000/year |
| Kaplan JE | GBS as diagnosed by a neurologist, and objective evidence of muscle weakness | September 1, 1979–March 31, 1980, and September 1, 1980–March 31, 1981 | For 1979–80: 528 (437 total adults); 7 vaccinated (all adults); 412 unvaccinated; 18 vaccination status unknown. For 1980–81: 459 total (375 total adults); 12 vaccinated (all adults); 347 unvaccinated; 16 vaccination status unknown. Inclusive total, 1979–1981: 987; 19 vaccinated (all adults), 968 unvaccinated (812 unvaccinated adults) | Not estimated |

(continued)

Table 7.1 (continued)

| Author (First) | Case Definition | Study Period | Number of GBS Cases in Study | Estimated Background Incidence in Persons > 17 years old |
|--------------------------|--|---|---|---|
| Kaplan JE | GBS as diagnosed by a neurologist, and objective evidence of muscle weakness | September 1, 1979–March 31, 1980, and September 1, 1980–March 31, 1981 | 2,575 cases of GBS reported during study period (1,910 among persons who were 18 years or older in 1976) | Although the raw data and exact numbers are not provided, the figure in this paper (Figure 1) indicates a range of approximately 0.35/100,000/year in age groups 20–24 to approximately 0.85/100,000/year in 60–75 year age group in males, and approximately 0.5/100,000/year in the 75–79 year age group among females |
| Roscelli JD | GBS as identified by search strategy (e.g. ICD code 357.0, for GBS) | 1980–1988 (all inclusive months) | 289 total GBS cases identified | A background rate was not readily available. Since influenza vaccine is administered to all service personnel in October of each year, the authors made the presumption that persons developing GBS in months outside of October or November would not have illness attributable to influenza vaccine. The cases of GBS occurring in these 'non-November months' was used as estimated background, which was cumulative. The result was 3.4 GBS cases per 10 ⁶ non-November months (95% CI 3.0–3.8). |
| Chen RT | Not defined | July/August–December, 1990 | 189 adult GBS cases; 11 vaccinated, 178 nonvaccinated | Not estimated |
| Lasky T ⁵ | Definite: Symmetric progressive paralysis in more than 1 limb, areflexia/hyporeflexia in legs and arms, CSF protein > 40 mg/dl with WBC < 10/ml, and died or reached peak of neurologic deficit within 4 weeks, afebrile, and with no alternative diagnosis. Probable: Above criteria with no documentation of CSF or CSF with protein < 40 mg/dl and WBC > 10/ml. Possible: Missing one or more criteria. | September 1, 1992–February 28, 1993 and September 1, 1993–February 28, 1994 | 1,201 hospital discharges with ICD-9 code of GBS, medical records obtained for 1,109 (92%) of 821 individual patients (discounting multiple admissions). After exclusions, 273 adult patients included. Sixty-six percent underwent telephone interview. Overall, 19 vaccinated cases and 141 unvaccinated cases were included, with data adjusted to account for 6 vaccinated cases in which it was not possible to confirm illness onset within 6 weeks post-vaccination. | 0.7 cases/100,000/year |
| Haber P | Not provided | July 1990–June 2003 | 501 VAERS reports of GBS (non-GBS reports not provided). 264/323. GBS reports confirmed by follow-up during 1994–2003 | Not estimated |
| Juurlink DN ⁶ | GBS as identified by search strategy (e.g., ICD-9 or ICD-10 code) | April 1, 1992–March 31, 2004 | 1. Self-matched case series: 1,601 GBS hospitalizations between 1 April 1993 and 31 March 2004; 269 with onset within 43 weeks of vaccination in October or November (presumed to be | Based upon the time-series analysis, the background incidence among adults was calculated at 1.4 cases/100,000/year. |

(continued)

Table 7.1 (continued)

| Author (First) | Case Definition | Study Period | Number of GBS Cases in Study | Estimated Background Incidence in Persons > 17 years old |
|----------------|---|--------------|--|---|
| Stowe J | GBS as identified by READ or OXMIS codes. Validation criteria not specified. | 1997–2004 | <p>influenza vaccines. 65 of these hospitalizations within primary risk interval (defined as the first 7 weeks following vaccination, subtracting the first 7 days following vaccination) and 144 within control interval (defined as the final 4 6-week blocks of the 43-week assessment period).</p> <p>2. Time-series: 2,173 GBS hospitalizations between 1 June 1991 and 31 March 2004. Admissions for GBS prior to and following the initiation of mass vaccination in October/November of 2000 were compared to assess for any differences.</p> | <p>Unavailable, as all age groups were included in this study. (Among all age groups, data from the GDRP assessment suggested an incidence of 2.05 cases/100,000/year. Similar data were obtained from admission rates for GBS as identified from the Hospital Episode Statistics database, which details discharge diagnoses from all National Health Service</p> |
| Burwen DR | <p>Definite: Bilateral weakness and hypoflexia of upper, lower extremities (or cranial nerve muscles); symmetric; nadir within 28 days of onset; CSF with WBC count < 10/mm and protein > 40 mg/dL; electrodiagnostics consistent with GBS/polyneuropathy.</p> <p>Probable: As for Definite, but with symmetry not noted, CSF with WBC count < 50/mm³ or not done, electrodiagnostics not inconsistent with GBS or not done.</p> <p>Possible: As for Probable, but with no data on symmetry, nadir, other competing diagnoses not excluded.</p> | 2000–2001 | <p>652 persons with admission during the 18 weeks after influenza vaccine with code 357.0; medical records reviewed for 637; 83 were considered definite, 152 probable, and 109 possible (293 [46%] classified noncases).</p> | <p>hospitals; using ICD-10 code G610 (GBS) and the population statistic for England as the denominator, these data suggested an incidence of 1.6 cases/100,000/year among all age groups).</p> <p>Not estimated; self-controlled methodology employed.</p> |

*Studies with findings suggesting an increased risk of GBS following influenza vaccination.

Table 7.1 (continued)

| Author (First) | Adult Risk |
|-------------------------|---|
| Schonberger LB | RR 7.6 (95% CI 6.7–8.6) for the 6-week period after vaccination; 6-week attributable risk: 0.88 GBS cases/ 100,000 adult vaccinees; this 6-week GBS risk attributable to vaccination in adults ranged from 3.3 cases/100,000 in persons age 18–24 to 11.4 cases/100,000 in persons age 24–44. A total vaccine attributable risk of 0.95 adult cases/100,000 vaccinations was also reported in order to include a possible additional 0.07 of an extra case per 100,000 vaccinations occurring more than 6 weeks after vaccination. |
| Hurwitz ES | RR 1.4 (95% CI 0.7–2.7) for the 8-week period after vaccination; 0.62 cases/100,000 among vaccinees within 6 weeks of vaccination. |
| Kaplan JE | RR for GBS in the 8-week period after vaccination for each season individually: 1979–80: 0.6 (95% CI 0.45–1.32) ; 1980–81: 1.4 (95% CI 0.80–1.76) . RR for the 2 seasons combined, by age groups (CI not provided): 18–49 years— 1.0 ; 50 years or older— 0.8 . |
| Kaplan JE | None calculated. |
| Roscelli JD | Attributable risk: 0.2/100,000 vaccinations; The rate of GBS occurring during the ‘November months’, during which GBS in vaccinees was presumed to be attributable to influenza vaccine, was: 0.33 cases /100,000 for ‘November months’ (95% CI 2.0–4.6) . Chi-square when comparing to non-November months: NS (p = 0.90). |
| Chen RT | RR 1.1 (95% CI 0.5–2.2) for the 6-week interval following vaccination. |
| Lasky T [§] | For the 1992–93 season alone, the RR was 2.0 (95% CI 1.0–4.3, p = 0.07); For the 1993–1994 season alone, the RR was 1.5 (95% CI 0.8–2.9, p = 0.2); For both seasons combined, the RR was 1.7 (95% CI 1.0–2.8, p=0.04) . |
| Haber P | None calculated. |
| Juurink DN [§] | RR for GBS within the 6-week risk period based upon the self-controlled assessment was 1.4 (95% CI 1.05–1.99, p=0.02) when compared with the control period. |
| Stowe J | Adult risks not available, as all ages included. For all ages, RR for influenza vaccine: 0–30 days after vaccination: RR 0.58 (95% CI 0.18–1.86). 0–90 days after vaccination: RR 0.76 (95% CI 0.41–1.40). RR for influenza-like illness: 0–30 days after vaccination: RR 16.64 (95% CI 9.37–29.54); 0–90 days after vaccination: RR 7.35 (95% CI 4.36–12.38). |
| Burwen DR | In 2000, IRR among definite and probable cases based on GBS rate in vaccine-exposed period vs. comparison period: 0.86 (95% CI 0.52–1.41); for 2001 IRR was 1.21 (95% CI 0.79–1.86); for the 2 seasons combined, IRR was 1.04 (95% CI 0.76–1.43). Results were similar when possible cases were included. Small numbers of cases < 65 years (n = 16), but no difference in results when analysis was restricted to > 65 years. |

[§]Studies with findings suggesting an increased risk of GBS following influenza vaccination.

I think it is also important to point out that, in 1976, had an influenza pandemic actually occurred, the GBS risk associated with the vaccine would have become a minor issue, as the morbidity and mortality prevented by the vaccine would have undoubtedly eclipsed the small but real risk of post-vaccine GBS. It is because the pandemic never materialized that this association with GBS took on the focus that it did.

2009 pH1N1 Influenza Virus: Déjà vu All Over Again

During the spring of 2009, the world witnessed the emergence of yet another H1N1 influenza virus of swine origin. Unfortunately, unlike the 1976 H1N1 influenza virus, the 2009 virus was definitely a serious public health threat; in April 2009, the virus was identified in specimens obtained from 2 epidemiologically unlinked patients in the United States. Similar viruses were rapidly identified in Mexico, Canada, and subsequently other countries throughout the world; by the summer of that year, 94,512 confirmed cases with 429 deaths were reported to the World Health Organization (WHO), leading to the declaration of a pandemic.

The global emergence of the pandemic (H1N1) 2009 virus (pH1N1), and its rapid global spread associated with community-wide outbreaks, hospitalizations and deaths prompted rapid development of new influenza A (H1N1) 2009 monovalent vaccine that could be produced in sufficient quantities to be used globally. The association of GBS with the 1976 swine flu vaccine had left public health officials shell-shocked, and immediately led to questions about a similar association of neurologic disease with vaccines against the p(H1N1) 2009 virus, also partially of swine origin.

Although the safety and efficacy of the influenza A (H1N1) 2009 monovalent vaccine was to be assessed through a small number of limited clinical trials, the interval between vaccine

development/manufacturing and widespread use of the vaccine was extremely short, pre-licensure safety data was quite limited, and post-licensure safety surveillance was going to take many months to collect and assess. Such was the angst about a repeat of the 1976 situation that a group of us at CDC huddled together on a Saturday afternoon shortly after recognition of the pandemic, deliberating the need to conduct surveillance for GBS in the face of the vaccination campaign, and if so, how. Ultimately, we decided to implement real-time, active, population-based surveillance for GBS following p(H1N1) 2009 monovalent vaccine in order to inform public health recommendations regarding the relative benefit and harm associated with the vaccination program. Several other countries, recounting the 1976 incident, decided to do the same.

In the United States, in rapid fashion an active, population-based surveillance system for GBS cases was implemented (published by Wise and colleagues) [26]. This study differed from the 1976 assessment in that it primarily compared risk of GBS following p(H1N1) vaccine to regular seasonal vaccine and to estimated baseline rates of GBS. Surveillance commenced on 1 October 2009 (coincident with the introduction of the U.S. vaccine), and continued through 31 May 2010. The assessment was overseen by the CDC, and conducted amongst CDC's 10 Emerging Infections Program (EIP) sites; the EIP sites constitute a platform to perform population-based real-time surveillance for a number of different conditions in 10 U.S. states, encompassing a population of approximately 49 million residents. Learning from the criticisms of the 1976 evaluation, each EIP site established a surveillance network comprised of neurologists and other healthcare providers that was queried weekly to stimulate reporting of suspect GBS cases; hospital discharge data were also reviewed (ICD-9 code 357.0) to capture additional cases not reported through the provider network. Cases were stratified into 2 broad age groups (0–24 years; ≥ 25 years) to ensure ascertainment of sufficient person-time. Trained surveillance officers reviewed medical records and conducted telephone interviews with suspected cases to obtain basic demographics, risk factors and vaccination status, and determined date of receipt of p(H1N1) and seasonal influenza vaccines. Cases were then classified according to Brighton criteria case definitions for GBS (Appendix) [27]; patients meeting Brighton Levels 1–3 (akin to suspect, probable and confirmed) were included in analysis. The observed number of GBS cases was compared with the number of expected cases, which we estimated by applying age-specific GBS background rates to the EIP population. GBS background rates were estimated by modelling published population-based GBS rates.

Among 44.9 million persons under surveillance from 1 October 2009 to 31 May 31 2010, study personnel identified 707 suspect GBS cases, with 411 cases ultimately meeting inclusion criteria. Eighty-four percent of GBS cases were ≥ 25 years, 52% were male, 68% were white, 15% required mechanical ventilation, and 3% died. The median weekly number of GBS cases by date of onset was 12 (range 1 to 19); the frequency of cases was similar across the surveillance period for both age groups.

The 411 GBS cases in the EIP catchment was similar to the expected number for the surveillance population (age-adjusted observed/expected ratio: 1.21, 95% CI 0.91–1.74), as well as among persons 0–24 and ≥ 25 years old. Data suggested 11 total excess GBS cases during the surveillance period and an estimated 0.74 (95% CI: 0.04–1.56) excess cases of GBS per million p(H1N1) vaccine doses. GBS incidence was not significantly elevated following receipt of seasonal vaccine. Antecedent events were less common among cases who received p(H1N1) vaccine in the 42 days prior to onset compared with those who did not (59% vs. 79%, $p = 0.02$). Examination of specific antecedent event types showed that upper respiratory or influenza-like symptoms were the only category that was significantly less common among cases receiving p(H1N1) vaccine than those who did not (38% vs. 67%, $p < 0.01$).

Thus, the estimated excess occurrence of GBS associated with p(H1N1) vaccine, less than one case per million p(H1N1) doses administered, was similar to that associated with some previous seasonal

influenza vaccine formulations and was 10-fold lower than the excess risk associated with the 1976 vaccine.

In contrast to the finding of less than one excess GBS case per million doses of p(H1N)1 vaccine administered, numerous studies have demonstrated the effectiveness of p(H1N1) vaccine in preventing pandemic influenza A (H1N1) 2009 infections; the CDC estimated that the use of p(H1N1) vaccine prevented 713,000 to 1.5 million cases, 3,900 to 10,400 hospitalizations, and 200 to 520 deaths in the United States during the same time period [28]. Thus, the small but real increased risk of developing GBS following the p(H1N1) vaccine was dwarfed by the benefits of receiving the vaccine [29–31].

Multiple additional analyses of the potential association between p(H1N1) vaccine and GBS were performed in various countries, and included at least one international consortium study [32–35]. Results of course varied between country and study, but none demonstrated a risk of GBS vastly different from the U.S. surveillance results.

The bottom line, then, regarding GBS and influenza vaccines is that the 1976 swine influenza vaccine was associated with a small but real increased risk of developing GBS in the 6–8 weeks following receipt of vaccine. Subsequent seasonal influenza vaccines, including the 2009 p(H1N1) swine influenza vaccine, have not demonstrated such increased risk, with rare exceptions. In the 3 assessments that did demonstrate a slightly increased GBS risk, the risk was many magnitudes lower than that associated with the 1976 vaccine. In any given season, the small but potential risk of GBS is greatly outweighed by the morbidity and mortality associated with influenza illness.

... And Everything Else: Association between Other Vaccines and GBS

To this point, as you may have noticed, the entire discussion of GBS and vaccines has centred on influenza vaccine. That is because nearly every other vaccine that has been associated with GBS has been done so on a temporal basis only—a person receives a vaccine, several weeks later GBS occurs—and that is about all we can say. These ‘temporal’ associations are made even more challenging by the fact that it is nearly impossible to exclude the possibility that another antigenic stimulus (e.g. a clinically silent infection) was not the triggering factor for the GBS. Attempts have been made to demonstrate an association between GBS and other vaccines, if one exists, but unlike the 1976 swine flu vaccine, to date no other vaccine has been convincingly demonstrated to have a causal association with GBS. I’ll provide a few of the more interesting examples of ‘other’ vaccines and GBS:

- Earlier formulations of rabies vaccine, which entailed inoculation of live rabies virus into mature sheep or goat brain and inactivated with phenol, appeared to be associated with a higher-than-expected incidence of ‘neuromuscular adverse events’ thought to be GBS [36,37]. This was presumed to be due to the presence of brain protein in the formulated vaccine, with the possible generation of antibodies cross-reactive to peripheral nerve proteins. Newer formulations of the rabies vaccine, which are derived from chick embryo cells, do not appear to be causally associated with GBS, though brain-derived rabies vaccine continues to be used throughout much of the resource-poor world.
- Early concerns about an association between oral polio vaccine (OPV) and GBS were raised due to a situation in Finland in 1985, at which time 94% of the Finnish population was vaccinated with OPV over a 5-week period between 10 February and 15 March due to an outbreak of wild-type poliovirus. During and shortly after the immunization campaign, several hospitals reported admitting unusually large numbers of suspected GBS patients, with 10 cases admitted during the first quarter and 6 cases during the second quarter of 1985, corresponding to the immunization campaign [38–40]. However,

later studies assessing monthly reports of GBS between 1981 and 1986 suggest that the number of GBS cases started to rise prior to the OPV campaign; since there had been an influenza epidemic between December and April of that year, the authors concluded that the small apparent increase in GBS observed during that time could have been associated with the circulation of wild-type poliovirus or influenza virus in addition to OPV; it was just not possible to say with certainty. No other assessments have supported an association between OPV or injected polio vaccine (IPV) and GBS [38].

- A suspected association between GBS and a formulation of meningococcal polysaccharide diphtheria toxoid conjugate vaccine (Menactra; MCV4) appears to have been a false alarm. The vaccine was licenced in January 2005 for use in the United States for persons aged 11–55; in February 2005 a U.S. immunization advisory committee recommended MCV4 vaccination for 11- 12-year-old children and before high school entry for individuals not previously vaccinated, in addition to the ongoing recommendations for routine vaccination for persons living in college dormitories, army bases, prisons or other crowded living conditions. By October 2005, 5 cases of GBS following MCV4 vaccination had been reported to the Vaccine Adverse Events Reporting System (VAERS), a national passive reporting system that collects reports of potential adverse events following immunizations from both clinicians and the lay public [41]. By September 2006, 17 suspected cases of GBS had been identified among vaccinees aged 11–19 within 6 weeks of vaccination. Based upon these 17 cases, a calculated IRR of 1.78 (95% CI 1.02, 2.85) was found, representing a bit less than 1 additional GBS case per million vaccines [42]. This estimate, however, needed to be interpreted with caution, given the passive nature of VAERS reporting and the imprecise estimated background rate of GBS in adolescents at that time. Subsequent evaluations of GBS risk following MCV4 using controlled studies and other systematic methodologies have failed to substantiate any increased risk of GBS following MCV4 [43].
- Tetanus toxoid vaccine, in the form of tetanus-diphtheria (Td) vaccine, has been associated with one apparent case of challenge/rechallenge in which a 42-year-old male developed GBS following Td vaccine on 3 separate occasions over a 13-year period, raising the possibility of causality in this individual [44]. However, larger assessments of GBS following tetanus toxoid-containing vaccines have failed to substantiate an increased risk [45].

The fundamental fact, as stated earlier, that one can find a case report of nearly any vaccine being followed temporally by onset of GBS does not equate to ‘causality’. Vaccines have truly been one of the miracles of modern medicine. Countless millions of lives have been saved by development and implementation of vaccines, and in many ways, vaccines have become a victim of their own—people are concerned about potential ‘complications’ of vaccines largely because they do not recall the tremendous morbidity and mortality from the very illnesses that vaccines prevent. Any theoretical risk of development of GBS, or any other neurologic illness, for that matter, following vaccines is far outweighed by the benefits of receipt of the vaccine.

During the next 100 years, hopefully, we will gain a better understanding of the incidence, epidemiology and risk factors for GBS in Africa, Asia, the Middle East and other areas of the globe. Continued pharmacovigilance should be conducted to assess for any additional causal associations between vaccines or other immunomodulating products and GBS.

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Appendix: Brighton Collaboration Case Definition, Guillain-Barré Syndrome [27]

Level 1 of Diagnostic Certainty

Presence of:

1. Acute onset of bilateral and relatively symmetric flaccid weakness/paralysis of the limbs with or without involvement of respiratory or cranial nerve innervated muscles
2. Decreased or absent deep tendon reflexes at least in affected limbs
3. Monophasic illness pattern with weakness nadir reached between 12 hours and 28 days, followed by clinical plateau and subsequent improvement or death
4. Electrophysiologic findings consistent with GBS
5. Presence of albuminocytologic dissociation (elevation of CSF protein level above laboratory normal value and CSF total white cell count < 50 cells/mm³)
6. Absence of an alternative diagnosis for weakness

Level 2 of Diagnostic Certainty

Presence of:

1. Acute onset of bilateral and relatively symmetric flaccid weakness/paralysis of the limbs with or without involvement of respiratory or cranial nerve innervated muscles
2. Decreased or absent deep tendon reflexes at least in affected limbs
3. Monophasic illness pattern, with weakness nadir reached between 12 hours and 28 days, followed by clinical plateau and subsequent improvement or death
4. CSF with a total white cell count < 50 cells/mm³ (with or without CSF protein elevation above laboratory normal value) or if CSF not collected or results not available, and electrodiagnostic studies consistent with GBS
5. Absence of an alternative diagnosis for weakness

Level 3 of Diagnostic Certainty (clinical case definition)

Presence of:

1. Acute onset of bilateral and relatively symmetric flaccid weakness/paralysis of the limbs with or without involvement of respiratory or cranial nerve innervated muscles
2. Decreased or absent deep tendon reflexes at least in affected limbs
3. Monophasic illness pattern, with weakness nadir reached between 12 hours and 28 days, followed by clinical plateau and subsequent improvement or death
4. Absence of an alternative diagnosis for weakness

8

A Global View on GBS and a Plea for International Research

Christine Verboon and Bart C. Jacobs

Introduction

Guillain-Barré syndrome (GBS) is a disorder with no boundaries. Reports have been published about patients from all geographical areas, including males and females of all ages. With a current world population of 7.4 billion people and an incidence rate of 1 to 2 per 100,000 per year, the estimated number of persons that yearly develop GBS is about 100,000. However, GBS is also a relatively rare disease for clinicians and researchers and it may be difficult to develop sufficient clinical expertise or to collect sufficient data for research.

The current chapter is a plea that patients, clinicians and researchers would all benefit from international collaboration in their general aim to reduce the worldwide burden of disease. We would like to illustrate this by indicating several topics in research which strongly benefit from international collaboration.

Compare Incidence Rates to Identify Risk Factors for Developing GBS

GBS is probably caused by a combination of host and environmental susceptibility factors which may differ per geographical area. To be able to identify these factors it will be important to conduct large incidence studies that use the same case definitions and study design. The incidence of GBS worldwide has been reported between 0.4 and 3.25 per 100,000 person-years. In one meta-analysis in North American and European countries, the incidence was 0.81–1.89 (median 1.11) per 100,000 person-years [1,2]. Although this range could be considered to be wide, it is even more variable when incidence rates of non-Western countries are considered. For example, the lowest incidence rate of 0.4 per 100,000 persons has been reported in one hospital-based study in Brazil, whereas the highest incidence rate of 3.25 has been reported in children from Bangladesh (<15 years if age) (see [Table 8.1](#)) [3,4,5]. These results are based on a relatively small number of patients and have not been confirmed in larger studies but they may indicate that the exposure to infections as well as host factors may influence the risk of developing GBS.

Another variability regarding the epidemiology of GBS worldwide is whether seasonal fluctuations are observed. Some studies report a peak in the winter, others in the summer and the remaining report no seasonal fluctuations whatsoever. One meta-analysis concluded that in Western countries, the Middle East and Far East, there seems to be a 14% higher incidence of GBS in the wintertime, while in Northern

China, the Indian subcontinent and Latin America there seems to be a predominance of GBS in summer. This difference could be explained by a different type of antecedent event, being upper respiratory tract infections in Western countries and gastroenteritis in the latter [6].

Table 8.1 Incidence of GBS by country

| Study | Incidence rate per 100,000 per year | Age |
|--|-------------------------------------|-----|
| Brazil | 0.4 | All |
| China | 0.44–0.66 | All |
| Tanzania | 0.83 | >12 |
| Western countries (North America and Europe) | 1.11 | All |
| Japan | 1.14 | All |
| Australia | 0.9–1.35 | All |
| Middle East | 1.73–2.11 | All |
| Curaçao | 2.53 | All |
| Bangladesh | 3.25 | <15 |

Many observations regarding the incidence of GBS worldwide have been published but connections and explanations about the reason for the differences are difficult to make, due to different research designs and descriptions (Table 8.1). More international research is therefore needed for improving the understanding of the geographical differences in GBS incidences.

Specific Antecedent Events Triggering GBS

About two-third of patients report symptoms of a respiratory or gastrointestinal tract infection within 3 weeks beforeonset of GBS. A specific infectious cause has been identified in about half of patients, with *Campylobacter jejuni* being the predominant cause. Other infectious agents which have been identified in relation to GBS are cytomegalovirus, Epstein-Barr virus, *Mycoplasma pneumonia*, *Haemophilus influenzae*, influenza A virus and recently hepatitis E virus [7]. The frequencies of these preceding infections, however, highly differ between various geographical areas. Without international collaboration it will be difficult to define if these differences are real or represent differences in study design and techniques used to demonstrate such infections. There are various examples of such comparative and international studies. One example is the collaborative study between Japan and the Netherlands investigating the frequency of preceding infections with *C. jejuni* and 2 serological techniques to demonstrate such infections in patients with GBS [8]. This study indicated that the proportion of patients with preceding *C. jejuni* infections was similar. However, the distribution of anti-ganglioside antibodies (against GM1, GM1b and Ga1Nac-GD1a) actually differed between the countries, indicating that geographical determining factors (either of the host or the triggering infectious agent) play a role in determining the antibody response in individuals [9].

A second example is a collaboration between the United Kingdom and the Netherlands which resulted in the discovery that hepatitis E infection is associated with GBS in 5% of cases, and in 10% of neuralgic amyotrophy [10,11]. The combination of patient cohorts and techniques was crucial to making these discoveries.

Various types of infection are known to precipitate GBS but the type of infections may differ per geographical area. By collecting data and biosamples from patients in various areas using the same

standard protocol and testing these samples in a uniform way it will be possible to determine the differences between countries and the role infections play in the pathogenesis of GBS.

Histopathological Subtype Differences in GBS

Initially GBS was considered to be a primarily demyelinating disorder of the peripheral nerves, in which some patients may develop secondary axonal degeneration. Tom Feasby challenged this concept by describing the first patients fulfilling the diagnostic criteria for GBS and showing initial features of axonal degeneration [12].

In the 1990s, a striking increase in acute flaccid paralysis was observed in children and young adults at rural areas in Northern China. Researchers from the United States visited this region to study together with local researchers this endemic ‘Chinese paralytic syndrome’. They found that the major difference between it and the classic GBS known in that time, was that these patients did not have sensory loss and that a motor axonal polyneuropathy was found on electrophysiological studies [13,14]. They concluded that these patients did have a different form of GBS with predominantly motor signs and an axonal polyneuropathy, the so-called acute motor axonal neuropathy (AMAN).

Later studies have shown that there is a distinct geographical variation in frequency of AMAN. In Europe and North America, AMAN accounts for only 3–17% of GBS cases, whereas this proportion is much higher in Asia and South America (30% to 65%). The opposite applies to the demyelinating subtype, acute inflammatory demyelinating polyneuropathy (AIDP), which is present in 69–90% of Western GBS patients and in only 20–40% of Asian and South American patients [15]. One international collaborative study between Japan and Italy confirmed these findings [16]. The cause of the geographical difference in AIDP and AMAN frequencies is not yet clear. Most probably, it is associated with both internal (host factors) as external factors (infectious agents). As illustrated in this paragraph, international collaboration is crucial to further unravelling this phenomenon.

Electrodiagnostic Criteria for GBS Subtypes

Research on location is not always obligatory. In 2012, Antonino Uncini from Italy and Satoshi Kuwabara from Japan combined their expertise and published a wonderful review on electrodiagnostic criteria for GBS [17]. In this review they give a historical overview of the development of electrodiagnostic criteria for AIDP, AMAN and AMSAN. They also explain very clearly the observations of conduction slowing in AMAN patients without excessive temporal dispersion (reversible conduction failure or conduction blocks). This may result in fallaciously diagnosing AMAN patients with reversible conduction failure as AIDP patients. Despite attempts to include diagnostic criteria to identify this reversible conduction failure, there still was a shift from AIDP to AMAN with serial nerve conduction studies. Uncini and Kuwabara also show that on top of this difficulty, there are numerous terms to describe this phenomenon, making the confusion complete. Moreover, electrophysiological departments frequently use their own standards and protocols. For these reasons, comparing study results from different countries about electrophysiological studies in GBS patients may be difficult and confusing. International collaboration will be essential to developing a worldwide consensus on electrophysiological protocols for examination and criteria for diagnosing the different electrophysiological subtypes.

Differences in Health Care

Too many patients with GBS are living in low-income countries where the majority only receive supportive care at the most. But patients from high-income countries who are treated with intravenous immunoglobulins (IVIg) or plasma-exchange (PE) also show a considerable mortality of about 3% to 7%, and about 10–20% of the patients remain permanently severely disabled [7].

We would like to illustrate the difference in outcome of GBS in low- versus high-income areas by comparing 2 studies. The first study was carried out in the Netherlands in a cohort of 527 patients, of which the majority were treated with either IVIg or PE, with mortality rate of 2.8% [18]. Most patients died from cardiovascular or autonomic complications and death occurred most frequently during the recovery phase.

A second study from Bangladesh, with a prospective study design similar to that of the Dutch study, showed that only one-quarter of the patients were treated with IVIg and that the mortality rate was 14%. Several patients died because of respiratory failure and lack of facilities to offer ventilation at an ICU [19].

These results show the alarming difference in health care and outcome of GBS between Western and developing countries. Hopefully, international collaboration, not only of researchers but also from governments and medical services, can eliminate the global differences in outcome after GBS.

International Collaboration on Treatment Trials

Despite the increase in general health care and number of potentially effective immune modulatory treatments, it is becoming increasingly difficult to conduct international therapeutic studies in GBS. In part this is caused by the more complex regulations for conducting such trials. But GBS is also relatively rare and highly variable in disease course and outcome, making it difficult to demonstrate a general therapeutic effect.

In earlier decades, there were only 2 studies on PE or IVIg with international collaboration: the French Cooperative Group on plasma-exchange in 1987 and 1997 (collaboration of France and Switzerland) [20,21], and the Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group in 1997 (collaboration of United States of America, Canada, United Kingdom, Germany, Belgium, Portugal, Norway, Switzerland, Italy, Israel and Australia) [22].

Currently, there are only 3 *national* randomized controlled trials running in 3 different countries: the Inhibition of complement activation (eculizumab) in Guillain-Barré study (ICA-GBS) in the United Kingdom, the Japanese eculizumab trial for GBS (JET-GBS) in Japan, and the Second IVIg dose in GBS patients with poor prognosis (SID-GBS trial) in the Netherlands.

Ethical committees demand more and more strict regulations on carrying out RCTs, and the problem is that every nation has its own regulations. This makes it difficult to set up international RCTs. An infrastructure and network of dedicated centres for conducting therapeutic studies may strongly support the conduct of therapeutic studies. Less strict rules apply for observational studies and the International GBS Outcome Study (IGOS) is currently running in 18 countries. The main aim of IGOS is to be better able to predict disease course and outcome in individual patients, but it also provides a platform for evaluating different therapeutic strategies internationally. Hopefully, these results could be a stepup for further (international) RCTs to confirm the observational findings.

International Organizations

The GBS-CIDP Foundation International (<http://www.gbs-cidp.org/>) is an international patient organization, currently represented in 43 countries. Their aim is to provide “access to early and accurate diagnosis, appropriate and affordable treatment, and knowledgeable support devices”. The organization consists of a global network of volunteers, healthcare professionals, researchers and industry partners. This organization already plays an important role in international collaboration between clinicians and researchers.

In 2007 the Inflammatory Neuropathy Consortium (INC) was raised by Richard Hughes to support international research in GBS and other neuropathies. This consortium made it possible to initiate the International GBS Outcome Study (IGOS) (www.gbstudies.org). The aim of the prospective longitudinal cohort study is to define all biological and clinical determinants and predictors of the clinical course and outcome of individual patients with GBS. The strength of IGOS is the highly detailed and standardized collections of clinical data and biomaterials in 18 different countries from 6 continents. This way of collection will ensure that the results from different countries are highly comparable. Moreover, by combining forces the IGOS consortium has been able to include currently 1,146 patients with GBS, an unprecedentedly large number of well-defined patients. The consortium of about 100 clinicians and researchers will collaboratively approach many of the most important remaining research questions in GBS, including the ones described above. The collected materials will also be used to compare different techniques for demonstrating preceding infections, anti-neural antibodies and genes potentially involved in the pathogenesis of GBS. Bangladesh is one of the countries participating, coordinated by Dr. Zahir Islam, Badrul Islam and Professor Deen Mohammed, providing the opportunity to compare for the first time data and materials derived from high- and low-income countries. Last but not least the IGOS will provide a unique opportunity to train young clinicians in treating patients with GBS and young researchers in conducting research in GBS. Dedicated clinicians and researchers from all over the world are invited to join the consortium.

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9

The Chinese Paralytic Syndrome—Recollections of 2 Participants

Guy McKhann and Tony W. Ho

Guy's Narrative

First Observations

“I didn't think you still had so much polio.”

I made that statement in November, 1986, while standing in the middle of a paediatric ward of the Beijing Children's Hospital, surrounded by 4- to 6-year-old children, all partially paralyzed, some on respirators. My hosts quickly assured me that this was not polio, it was the Guillain-Barré syndrome (GBS), and they had epidemics every summer. Clearly this was different than my concept of GBS. Thus began a 20-plus-year collaboration between a team of colleagues from Johns Hopkins University, the University of Pennsylvania, and colleagues in Beijing and Shijiazhuang.

I made a longer visit to Beijing in October 1988. At that time I saw about 30 children and had the opportunity to examine 10 thoroughly. I also had the opportunity to discuss this form of GBS with Professor Zhu Fu-Tang, a 90-year-old paediatrician and founder of the Beijing Children's Hospital. Dr Zhu Fu-Tang had been a student of my father's at the Boston Children's Hospital in the 1930s. In discussing the epidemiology, I was also fortunate to be able to speak, at a later date, with Professor Jun-Xiong Mao, the former head of Neurology at the 2nd Teaching Hospital of Hebei Medical University.

Was This a New Disease?

Both of these senior clinicians felt that this was a new condition, first occurring in the 1970s. Prior to that they had seen GBS, primarily in adults, similar to what we have in North America and Europe. At that time, they suddenly began seeing an influx of paediatric patients, so much so that in both hospitals (at least 150 kilometres apart) they would have to take over wards from other services, because their neurology services were overwhelmed. The cases were almost exclusively in the summer, starting in May, and dropping off by October. There were more boys than girls (60/40). The cases came from rural areas, not from the cities. There was no clustering of cases; no multiple cases from a single village; only 1 instance of 2 cases in the same family; and no involvement of exposed medical personnel. The age distribution was strikingly different from 'western GBS', being predominantly children. A prodromal illness was sometimes present, but varied. In view of the subsequent association with *Campylobacter jejuni*, we

asked particularly about gastrointestinal symptoms, but found these occurring in only 15% to 20%.

I decided that we should evaluate this problem further, and that I needed some help. So I put together a team from Hopkins (Drs Jack Griffin, David Cornblath, Tony Ho and myself) and from Penn (initially Dr Art Asbury and then later added Dr Irv Nachamkin—an expert on *Campylobacter jejuni*). We were able to get some funding from the Rockefeller Foundation and were prepared to go when things were disrupted, in June 1989, by the events at Tiananmen Square and other areas. In the meantime, Dr Chun-Yun Li, from Shijiazhuang in Hebei province, came to work with us in Baltimore, primarily with Dr Jack Griffin. Dr Li had seen several hundred cases or more of GBS in China, so it was not entirely clear who was teaching whom. After a year with Dr Griffin, Dr Li returned to Shijiazhuang, where he became head of Neurology at the 2nd Teaching Hospital in Hebei, the site of most of our future work.

In August 1990, we finally got to Dr Li's hospital. The results were outstanding. Dr Li had arranged for 20 patients to be available, all in one large room. The clinical histories were quite stereotyped. Most patients had been previously healthy. The first symptom, particularly in children, was unsteadiness and falling, followed quickly by bilateral, symmetrical leg weakness. The disease then might skip to the cranial nerves, with facial weakness and swallowing difficulties and then breathing difficulties. The last phase would be bilateral weakness of the arms. The duration of onset could be as short as 2 to 3 days, or, rarely, up to a week. There were no sensory complaints or pain as part of the disease process. A tracheostomy or intubation was often performed in part to aid respiratory weakness and also to overcome swallowing difficulties. The patients were afebrile. If CSF was obtained, it showed a few cells (up to 8–10 monocytes) and elevated protein.

In one corner of the large room was our first patient, 'H-1', a young man about 10 years old who was intubated and being ventilated by his father compressing an anaesthesia bag ([Figure 9.1](#)). I hurried through my exam and told Tony, who was from Taiwan and who was acting as our interpreter, to tell them to take the patient upstairs and put him back on his respirator. (I was afraid he might die while we were examining him.) Tony came back a few minutes later and said 'Don't embarrass them—they have run out of respirators due to the number of patients that had come in.' Later we went up to the ward and saw several other patients being kept alive by family members constantly 'bagging' them. This could go on for weeks.

In another part of the room, Dave Cornblath had set up his EMG machine, which he had brought from Hopkins. After about the second patient, he came over and quietly said, 'Boss—I think we have a new disease!' He showed us the EMG tracing, and the EMG clearly pointed towards a motor axonal disease with reduced motor amplitudes, little or no reduction of conduction velocity, and preserved sensory nerve action potentials. This finding was very different from the typical understanding of Guillain-Barré syndrome at that time [1,2]. GBS was thought to be mostly demyelinating affecting, both motor and sensory nerves.

To skip ahead a year (1991), we returned to Shijiazhuang, and particularly asked Dr Li if we could see some of the patients that we had seen the year before. Who should be there waiting for us, but 'H-1' and his father. He had not only survived, but also made a miraculous recovery. He stood up, walked over to us, and shook hands. He was not completely normal. He had some distal atrophy of his hands and feet, and perhaps his tongue. The remainder of his exam was normal. His pattern of recovery was a long, gradual one. He was on respiratory support for at least a month. The physicians at the 2nd Teaching Hospital were, and are, justifiably proud of their low mortality rate. They know if they can get patients through the acute phase, they will gradually recover.



Figure 9.1 A child with AMAN is ventilated by hand by his father

What Is This Disease?

We had a lot of discussion, both among ourselves and with others, about what this disease might be (see [Figure 9.2](#)). *Was it polio?* The patients were afebrile, had symmetrical disease, and had no signs of inflammation in their CSF. Polio seemed untenable. *Was it related to the polio vaccine?* I was contacted by both Albert Sabin (see *The Mexican Connection*, below) and Jonas Salk about this possibility, each pointing to the other's vaccine. For most patients, polio vaccination had occurred months or years prior to the onset of this illness. Further, there was no clustering of cases. A polio vaccine-related illness also seemed untenable. *Was this some toxin or pesticide?* We seriously considered this possibility, but were put off by the sporadic nature of the disease.

The Pathology

The Chinese do very few autopsies. However, Dr Li was able to perform limited autopsies on 12 patients. He removed the lower spinal cord, anterior and posterior roots, peripheral nerve, and muscle. The tissue was fixed for immunocytochemistry and electron microscopy and brought to Hopkins after our visits. At that point, Art Asbury and Jack Griffin took over.

- 3 -
POSSIBILITIES

9/16/92



Figure 9.2 What is the possible relationship between this new disease 'Chinese paralytic syndrome' (CPS) and Guillain-Barré syndrome? This hand-drawn note dates from the original discussions in 1992.

To begin with, it was clear what this was not: an inflammatory demyelinating polyneuropathy (AIDP) as Dr Asbury and colleagues had described in 1969 [3]. Rather, in fatal cases, there was extensive Wallerian-like degeneration of motor axons (thus called acute motor axonal neuropathy [AMAN]), and, in some cases, involvement of both sensory and motor axons (thus called acute sensory-motor axonal neuropathy [AMSAN]). In both instances a prominent feature was the presence of macrophages within the periaxonal space, displacing the axon (Figure 9.3). The movement of macrophages to the axon was defined [4-6]. These clues suggested that the macrophages were attracted to something in the paranodal or nodal regions. Under Jack's leadership, Charlene Macko, Kazim Sheikh and Tony Ho later carried out detailed work on these precious samples and demonstrated the presence of IgG and the complement activation product C3d bound to the axolemma of motor fibres, especially in the nodal axolemma. This is in contrast to the AIDP cases in which the complement activation products were on the outer surface of Schwann cells [7,8] (see Figure 9.3).

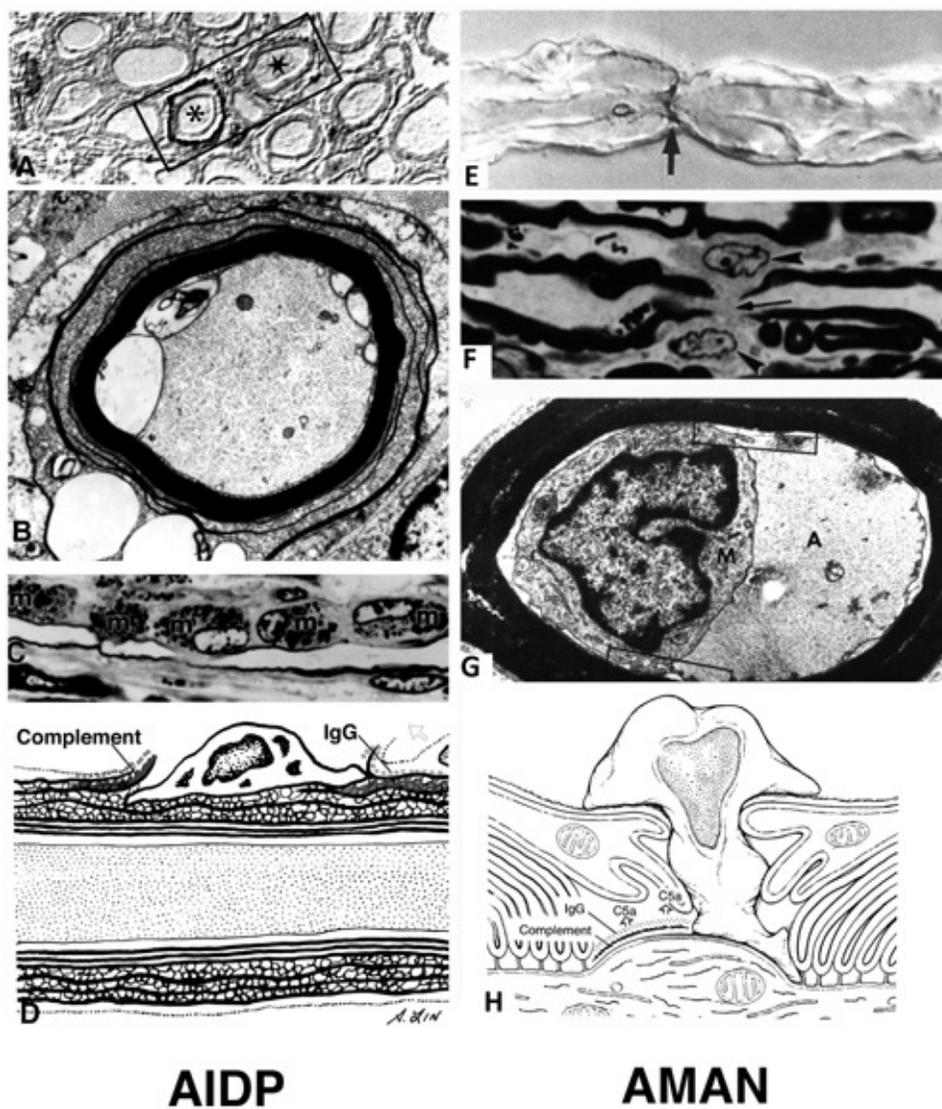


Figure 9.3 (A) Deposition of the complement product C3d on outer surface of two fibres from an AIDP case. (B) Extensive vesiculation of the myelin sheath in the same case. (C) Foamy macrophages associated with a fibre from the same AIDP case. (D) Schematic illustrating antibody, complement and macrophage attack directed against the outer surface of the myelin sheath in AIDP. (E) C3d deposition at the node of Ranvier in ventral root in an AMAN case. (F) Node of Ranvier on ventral root with nodal lengthening and two overlying macrophages in a case of AMAN. (G) A macrophage in the internodal axon beginning to extend processes towards the periaxonal space. (H) Schematic illustrating a macrophage inserting itself at a node of Ranvier where there is antibody and complement deposition. AIDP images (A, B, C) adapted with permission from [7]. AMAN images (F and G) adapted with permission from [5]. Images D, E and H work of McKhann G, originally published in [18].

Tony's Narrative

The Relationship to Chickens and to *Campylobacter jejuni* (*C. jejuni*)

Our first trip was in 1990. As a medical student, it was an exciting trip for me accompanying 4 world-famous neurologists to investigate this new disease. Guy took us to Beijing Children's Hospital and we met Dr Zhu Fu-tang (Figure 9.4). Guy was very interested in his observation that this disease suddenly increased in prevalence in the 1970s. We did not get any real clues on the first day we visited him. During the next day, Guy asked him the same question, 'What happened twenty years ago?' again to no avail. On the third day, Guy asked the same question again! I thought to myself, 'Haven't we asked this question before?' To my surprise, we got some very useful clues. As it turns out, Dr Zhu Fu-tang had been carefully thinking about Guy's question for the past 3 days. He recollected that most of these children were from the countryside and usually appeared after rainfalls. This disease only occurred in children who were older

than 1 year, and many got sick after drinking unboiled water or eating raw eggs. These clues pointed us to possibility that a diarrheal illness was the potential trigger to the disease. After returning from our first trip, given some reported association of *Campylobacter* with GBS in Australia by Kaldor and Speed [9], we decided to follow up on this lead. We first contacted Dr Martin Blaser of Vanderbilt, given his expertise in *Campylobacter* serology. The initial data showed promising results; many of Chinese patients did indeed have elevated anti-*Campylobacter* antibody titres, suggesting antecedent infection [10].

After the 1991 trip, the focus of our research was now on *Campylobacter* and gangliosides. One day, Art came in and said, ‘Guess what, we had the world expert on *Campylobacter* in our backyard all this time!’ We quickly enlisted Dr Irving Nachamkin from Penn to the research team. He told us we needed to culture these bacteria from the patients. Under Irv’s direction, I started spending my post-call days during my internship at Massachusetts General Hospital in the basement bacteriological lab, learning how to grow *Campylobacter* under microaerophilic conditions. This turned out to be a good investment. After my internship, I spent the summer of 1992 at Shijiazhuang seeing these patients, doing nerve conduction studies, and teaching our Chinese colleagues how to culture *Campylobacter*. This is when we found the striking association with chickens. A 14-year-old girl came in with advancing disease. She told us that she was studying in the city. Her mother had called her and asked her to come back to the village to take care of a ‘sick’ chicken. After feeding the chicken with some form of medicine (presumably vitamins), she fell ill and became paralyzed. We sent a team to her village to examine these chickens and took stool cultures. The pictures were striking: several chickens had their heads drooped to one side (Figure 9.5).

The nerve pathology in the chickens showed the typical axonal damage we had seen in humans. In addition, we were able to isolate *Campylobacter jejuni* from the girl and these chickens. We took the natural next step. Dr Li bought some live chickens from the market and fed them *Campylobacter*. To our surprise, in the first batch of 33, all got diarrhoea and half became weak. In the second batch, a quarter developed paralysis. These weak chickens had pathology in their sciatic nerves similar to AMAN patients. An animal model of *Campylobacter*-associated AMAN had been developed [11]. Most interestingly, the chickens were housed next to 6 monkeys that had been in the animal facility for almost 10 years. Four monkeys came down with diarrhoea, followed by paralysis! (Presumably they were able to reach out from their cage and play with the chickens or their stools). The first paralyzed monkey was cultured positive for *C. jejuni*. By the fifth day, he was quite sick. Fearing his imminent demise, he was sacrificed, and the subsequent autopsy material showed essentially no findings except minor changes in the ventral roots (looking back, maybe the paralysis was mainly due to conduction blocks). The second monkey also came down with paralysis but slowly, over 1–2 months. His teased fibres showed extensive axonal damage consistent with AMAN. Examining these monkeys’ nerves showed that they indeed also had motor axonal damage.



Figure 9.4 Top from right to left: Dr Zhu Fu-Tang (centre), Dr Hu-sheng Wu (left), Dr Arthur Asbury, Dr Guy McKhann, Dr Tony Ho, Dr Jack Griffin at Beijing Children Hospital; Bottom: Dr Chun-Yun Li, Dr Arthur Asbury, Dr Jack Griffin in front of New Research Laboratory at 2nd Affiliated Hospital of Hebei Medical University



Figure 9.5 Naturally occurring animal model of GBS in the chicken. The chicken on the left displays a flaccid weakness following *Campylobacter jejuni* enteritis.

We were very encouraged with these results and were eager to establish similar models in the United States. We tried to repeat similar experiments with a number of chicken species at Penn. Art revealed his Kentucky roots, adeptly showing Nachamkin and I how to handle these chickens and how to feed them with *Campylobacter*. We were never successful in establishing the model—but in retrospect, these chickens may not have been *C. jejuni* naïve. If they were colonized with other strains of *C. jejuni*, we may have had difficulty infecting them with the pathological strain of *C. jejuni*.

Ganglioside Epitopes: Connection to *C. jejuni*

Many anti-glycoconjugate antibodies have been described in GBS patients. The strongest association is between the Fisher syndrome and IgG anti-GQ1b. Our initial investigation had focused on anti-GM1 antibody because there were reports of its association with GBS. Later, collaborating with Professor Hugh Willison, we were able to investigate the anti-glycoconjugate profile and to correlate with different subtypes of GBS. What we found was that both AMAN and AIDP patients had elevated anti-glycoconjugate antibody to GM1, GD1b and GA1. However, only the AMAN patients had elevated GD1a [12], suggesting that this may be the pathogenic epitopes for AMAN. Because *C. jejuni*'s liposaccharides shared sugar structures similar to those of gangliosides, the question arose as to whether the antibodies were generated against these epitopes. The investigation turned out to be very fruitful. Irving was able to show that the pathogenic strain of *C. jejuni* (mostly Penner 19) did contain GM1, GD1a-like epitopes, suggesting that 'molecular mimicry' may be the underlying pathogenesis. Further evidence suggested that motor axons were particularly enriched with GD1a epitopes, which may explain the predominant motor axonal damage. Working with Professor Yuki, we have found additional ganglioside epitopes such as GM1b and GalNAc-GD1a [13].

Recovery from Axonal Damage

One of the most important questions was how, if this was true axonal damage, patients could recover. As mentioned above, we were shocked when we saw these completely paralyzed patients who had no or low amplitude distal motor-evoked potentials return a year later walking with only limited residual muscle atrophy. The observation by Jack and Art that the focus of immune attacks is the nodal or paranodal regions suggested that these patients could have reversible nodal blocks without significant axon damage. Nobuhiro Yuki has since further substantiated these observations [14]. Another possible explanation for the rapid reversibility was very distal axonal degeneration. The evidence of this actually came from the United States, not China. We saw a woman with *Campylobacter*-associated AMAN at Hopkins. She improved quickly following plasmapheresis. Motor-point biopsy showed denervated neuromuscular junctions and reduced fibre numbers in intramuscular nerves, showing that distal axonal degeneration could explain the rapid improvement [15].

The Mexican Connection

Who better a person to ask about acute paralysis in children than Dr Albert Sabin? Guy and I made a trip to Washington, DC to visit Albert after we got back from China in 1990. It was an interesting trip. As soon as we sat down, Albert said, 'I know why you are here. I have seen those kids in Beijing. They are

very similar to the patients we saw in Mexico City.’ He handed us a paper from 1969 in which he and Manuel Ramos-Alveraz were the lead authors describing an epidemic of acute flaccid paralysis that they named ‘nuclear neuronopathy and cytoplasmic neuronopathy’, describing the ballooned anterior horn cells they saw [16]. At that time they were testing a live polio vaccine in Mexico when these children developed acute flaccid paralysis. Even though the children had non-inflammatory CSF, both Manuel and Albert were blamed for the outbreak. Albert was forced to leave Mexico and Manuel was ostracized from the Mexico medical community.

We followed up this lead and tracked down Manuel in a little alley in Mexico City. To our surprise, he had kept all the original pathology from these children. We brought these pathology blocks back to Hopkins. With modern techniques, we were able to confirm that these children indeed had similar pathology. While Albert and Manuel had focused on the anterior horn cells, Jack and Art focused attention to the anterior roots which showed similar Wallerian-like degeneration of motor roots. What Albert and Manuel observed was chromatolysis from the motor nerve damage.

We then made several trips to Mexico and discovered that there were still children suffering from acute motor axonal neuropathy, and that AMAN was occurring in the summer, as in China. We were successful in culturing *Campylobacter jejuni* from these children. When these *Campylobacter* were analysed for their genetic linkage, to our surprise, these *Campylobacter* were closely related to the Chinese strains and carried similar ganglioside-like epitopes [17].

Current Status of AMAN in China

In 2012, Tony made another visit to Shijiazhuang. China had completely changed since we were there. They now have a bullet train from Beijing to Shijiazhuang which cut the travel time from 6 hours to about an hour. Dr Li is now a member of the Chinese Academy and has built a state-of-the-art research institute at the 2nd Teaching Hospital. AMAN and AIDP still occur every summer after rainfalls. However, the physicians in Shijiazhuang now have modern and ample respirators, and treat these patients with IVIg.

We are writing this report almost 30 years after we first started looking into a strange form of GBS in China. We would summarize our group’s contributions as follows:

1. Characterization of the clinical form of acute motor axonal neuropathy (AMAN)
2. The delineation of the differences in electrophysiology between AIDP and AMAN
3. The delineation of the distinctive pathology of AMAN
4. The association of AMAN and *Campylobacter jejuni*
5. The development of a chicken model of AMAN
6. The association of ganglioside epitopes with AMAN, and the development of the theory of molecular mimicry as applied to AMAN

We dedicate this report to our all-important colleague, Jack Griffin.

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10

GBS in the Indian Subcontinent: Sitting on the Other Side of the Fence

Ansh Mehta, Zhahir Islam and Govind Chavada

Introduction

Since the launch of the global polio eradication drive by the World Health Organisation in 1988, there has been a significant reduction in the incidence of poliomyelitis worldwide, and, indeed, most Indian subcontinent countries except Pakistan became polio free in 2014. In the pre-eradication era, polio was the major cause of infective acute flaccid paralysis (AFP), so it comes as no surprise that it has remained a major focus of attention for the past 50 years on the Indian subcontinent. Whilst active surveillance for polio still continues, focus has now shifted to other, non-polio virus-related causes of AFP (NP-AFP). There are number of conditions that may lead to NP-AFP, which include toxic, metabolic, infective and inflammatory conditions. Amongst all, Guillain-Barré syndrome is the leading cause of NP-AFP in this region.

In the context of the resource-poor Indian subcontinent, GBS represents a major health care challenge to improving outcomes. GBS continues to remain a clinical diagnosis and therefore clinical differentiation between GBS and other NP-AFP can be difficult. Correct identification of GBS cases help guide the appropriate management of patients, especially when resources are limited and the cost of immunotherapy is a major factor. There is extensive literature available describing GBS incidence rates in the Western world, but similar data from developing countries are lacking. Availability of these data is hugely important, not only for the better understanding of the disease pattern in this region but also for resource allocation and formulation of national health policies. Similarly, from Western literature we know that most patients with GBS make a good recovery but about 20% remain disabled after a year; however, the overall burden of GBS in developing countries is not known, especially when there is no universal availability of immunotherapy and rehabilitation facilities in these countries. Conducting comprehensive epidemiological and sophisticated clinical studies in such a diverse and largely rural subcontinent population poses the greatest challenge in these regions.

Subcontinent countries are perfect examples of the inverse care law, whereby those with the greatest need have the greatest difficulty in accessing health care facilities. For example, in a country like India with a population of 1.3 billion, 70% live in rural areas with a ratio of 3–5 primary care physicians per 100,000 inhabitants. When it comes to specialist neurology care, as of 2013 there were only 1,100 practising neurologists, of which half were based in big cities, which effectively equates to one neurologist per 1 million inhabitants. In comparison, the United Kingdom has 10 neurologists per million. Thus, availability of specialist neurology care is grossly inadequate and the vast majority of GBS cases

are managed by general physicians in peripheral hospitals [1]. Grossly inadequate public health care resources force patients to use private health care facilities, which provide up to 80% of patient care in these countries [2]. In most GBS cases, diagnosis is made clinically and additional tests such as nerve conduction studies and CSF examination are performed only if the patient can afford them and the resources are available. Often the diagnosis is delayed, especially in rural settings where patients have to travel a long distance, the cost of health care is an obstacle and clinical expertise even in regional centres is lacking. Treatment of GBS in these countries largely depends on what patients can afford and whether treatment facilities such as plasma exchange (PE) and mechanical ventilators are available. On an average, PE costs around 1,200 USD, while indigenous IVIg costs around 2,400 USD; therefore PE is frequently preferred over IVIg treatment. Often poorer patients are treated with mechanical ventilation without IVIg or plasmapheresis, especially in government hospitals where plasma exchange is not available. Rehabilitation of GBS patients poses a further challenge due to the inadequate numbers of specialist centres, which in turn contributes to poor outcome.

Due to huge socioeconomic and cultural constraints and various other health care priorities, subcontinent countries are far behind in GBS care and research.

While literature from some countries is limited to case reports and retrospective studies, countries like India and Bangladesh have made considerable progress and the vast majority of publications from this region come from these two countries. The purpose of this 'Top 10' is to highlight the contributions of the region's scientific community, in chronological order. Certainly, this list is not comprehensive, and by no means detracts from the contributions of others, whom we unfortunately have not the space to mention in this brief chapter.

Seth RK. Landry-Guillain-Barré syndrome in children. *Indian Journal of Pediatrics*, 1965

Seth's was probably the first comprehensive case series of GBS from this region [3]. In this article Seth describes 8 paediatric GBS cases. What is striking in this case series is that one GBS patient was wrongly diagnosed and treated as having 'post diphtheritic polyneuropathy' and 3 patients died of respiratory failure due to the unavailability of mechanical ventilators. Interestingly, 6 out of the 8 cases in this series were treated with steroids, which at the time was 'widely acceptable'. This article also highlights the diagnostic and therapeutic challenges faced by developing countries, where other causes of AFP such as poliomyelitis and post-diphtheritic polyneuropathy were a common problem.

Tharakan J, et al. Small volume plasma exchange in Guillain-Barré syndrome: experience in 25 patients. *Journal of the Association of Physicians of India*, 1990

Encouraged by the first-ever case report by Brettle and colleagues [4] and subsequent anecdotal reports showing the efficacy of plasma exchange in GBS, in 1984 Osterman and colleagues conducted the first-ever randomised controlled trial showing the efficacy of PE in GBS patients [5]. Keeping pace with the Western world, Tharakan and colleagues published their experience of 25 GBS patients treated with small-volume plasma exchange (SVPE), with promising results [6]. However, what is surprising is that despite being a cheap alternative to conventional PE, very little effort has been made to validate these results, which otherwise could potentially transform GBS treatment in the developing world, where the

cost of immunotherapy is a major limiting factor.

Gupta SK et al. Acute idiopathic axonal neuropathy (AIAN): a clinical and electrophysiological observation. *Acta Neurologica Scandinavica*, 1994

Controversy surrounding underlying pathological processes in GBS continued to dominate the 1980s and 1990s. GBS was traditionally considered to be a demyelinating disease until Feasby and colleagues challenged this view and published the first report of ‘acute axonal GBS’ in 1986 [7]. McKhann and colleagues later substantiated this view and described a case series of Chinese GBS patients with predominant axonal degeneration as an underlying pathology [8]. Not far behind, within a year, Gupta and colleagues published the first AMAN case series in India, describing clinical and electrophysiological features of 20 axonal GBS patients with poor recovery patterns, reinforcing the idea that axonal GBS should be considered a distinct entity [9].

Kalita J, et al. Neurophysiological criteria in the diagnosis of different clinical types of Guillain-Barré syndrome. *Journal of Neurology, Neurosurgery and Psychiatry*, 2008

Electrophysiological (EP) criteria of GBS has remained a topic of major controversy in the field. Most initial criteria was designed according to the traditional thinking that GBS represents a demyelinating disorder of peripheral nerves; however, when this view was challenged, new criteria were designed to accommodate ‘axonal GBS’. One of the issues regarding these criteria is the ‘EP definition of demyelination’, which is completely arbitrary without any laboratory evidence. Kalita and colleagues conducted a study comparing the diagnostic sensitivity of 6 different criteria and found significant variation in diagnostic sensitivity of these criteria, underscoring the importance of consensus-based EP diagnostic criteria [10].

Gupta A, et al. Guillain-Barré Syndrome: rehabilitation outcome, residual deficits and requirement of lower limb orthosis for locomotion at 1 year follow-up. *Disability and Rehabilitation*, 2010

‘Neurological rehabilitation’, which is an integral part of neurology care in the Western world, remains a luxury in the developing world. In the context of GBS, this interesting report by Gupta and colleagues describes the overall rehabilitation outcomes of 35 GBS patients [11]. The study showed that only 14% of patients diagnosed with GBS were admitted to a rehabilitation unit, which is well below the figure of 40% in some earlier studies. It also showed that while most patients make a very good functional recovery, about 34% of patients required foot orthosis to walk and about 25% of patients continued to complain of neuropathic pain, highlighting the need to develop effective neuro-rehabilitation facilities in low-income countries.

Sendhilkumar R, et al. Effect of pranayama and meditation as an add-on therapy in rehabilitation of patients with Guillain-Barré syndrome—

a randomized control pilot study. *Disability and Rehabilitation, 2013*

Over the past few decades the ancient Indian practice of yoga has gained tremendous attention around the world. Integrated yoga programmes, in addition to traditional physiotherapy, have been shown to be effective in long-term rehabilitation of various rheumatologic disorders. In this fascinating pilot study, the authors conducted a single blind randomised control trial of 22 GBS patients, comparing the efficacy of yoga with conservative treatment, and although the study failed to show any beneficial effect of pranayama and meditation on patients' pain, anxiety and depression level, it showed significant improvement in quality of sleep [12]. Clearly, sleep-related issues are very common in GBS and can affect the overall quality of life; therefore, if these findings are substantiated by further large RCTs, yoga may have a place in GBS rehabilitation.

Islam Z, et al. High incidence of Guillain-Barré syndrome in children, Bangladesh. *Emerging Infectious Diseases, 2011*

This article reinforces the idea that, as the most developing countries are inching closer to achieving the complete eradication of poliomyelitis, GBS is emerging as a major cause of AFP [13]. Not only that, it also highlights an interesting fact that in countries like Bangladesh, GBS rates are at least 2 to 3 times higher than what has been reported in the Americas and Europe, and the burden of this disabling disease could be much higher than we all think. In this study Islam and colleagues systematically analysed the surveillance data on reported AFP cases from Bangladesh and have shown that while Bangladesh has been successful in eradicating poliomyelitis since 2000, non-polio AFP cases are continue to occur, with an incidence rate of 3.25 per 100,000 children less than 15 year of age, and that a great proportion of these non-polio AFP cases are diagnosed as GBS, with crude incidence rates of GBS in children varying from 1.5 to 5 per 100,000 per year in Bangladesh.

Islam Z, et al. Axonal variant of Guillain-Barré syndrome associated with *Campylobacter* infection in Bangladesh. *Neurology, 2010*

This fascinating article by Islam and colleagues portrays a real-time picture of GBS in developing countries like Bangladesh [14]. This prospective case-control study from the Dhaka area showed that most GBS cases were axonal variant. What stands out from this study is the fact that most patients did not receive any specific immunotherapy and about 43% of these patients had poor outcome as defined by an "inability to walk unaided at 6 months" after disease onset. Studies like this clearly show the real gap in availability of treatment and outcome of GBS, between developing and developed nations. The international GBS community needs to make concerted efforts to tackle this potentially disabling disease.

Islam Z, et al. Guillain-Barré syndrome-related *Campylobacter jejuni* in Bangladesh: ganglioside mimicry and cross-reactive antibodies. *PLoS One, 2012*

Many studies on the pathogenesis of GBS report findings that are consistent with the mimicry hypothesis, but none of these studies originated from a developing country. In this study, Islam and colleagues provide evidence in support of the hypothesis that even in the developing world, *C. jejuni* infections induce GBS

in these patients by molecular mimicry and induction of a cross-reactive immune response to nerve gangliosides [15]. The authors found evidence of IgG antibodies to certain strains of *C. jejuni* LOS in GBS patients' sera, following which, they showed that anti-GM1 and GD1a monoclonal antibodies react to *C. jejuni* LOS. Further, they closed the loop by performing a mass spectroscopy analysis showing GM1 and GD1a gangliosides-like structures on certain strains of *C. Jejuni* LOS.

Islam MB, et al. Small volume plasma exchange (SVPE) for Guillain-Barré syndrome (GBS) patients in Bangladesh: a phase 2 clinical trial. 7th Meeting of the Inflammatory Neuropathy Consortium (INC) of the Peripheral Nerve Society; Dusseldorf, Germany, 2014

Access to costly immune therapy such as IVIG and PE for GBS patients remains a major challenge for most low-income developing countries. A full course of intravenous immunoglobulin, for an adult of 60 kg, costs around 12,000–16,000 USD, and a conventional PE for 5 days costs around 4,500 to 5,000 USD, which makes these treatments inaccessible to the majority of patients. Therefore cheap alternatives must be sought. One such attempt is this ongoing combined Dutch-Bangladesh study hoping to show that SVPE has can change the therapeutic landscape in most developing countries [16].

Conclusion

GBS continues to remain a leading cause of AFP in subcontinent countries. While the true incidence of GBS in these countries is not known, there are very good reasons to believe that the incidence is higher than previously thought. Lack of systematic epidemiological and outcome studies, and poor research infrastructure to perform such studies, remain major challenges in this area. The outcome of GBS, which is otherwise thought to be very favourable in the majority of cases in the Western world, may not be so in the developing world due to inaccessibility of highly expensive immunotherapy and lack of rehabilitation facilities.

Concerted efforts by the international GBS community are needed to bridge this gap between the developing and developed world. With recent globalisation, the socio-economic landscape is rapidly changing in this region and with it the availability of health care resources and research infrastructure. Today countries like India and Bangladesh are developing close links with international research centres, a trend we hope will continue and that will contribute further in understanding this disease.

The 100th anniversary of GBS reminds me of a great man, scientist, mathematician and inventor of the first solar cooker of India, my grandfather Chandra Mehta, the person whom I was not fortunate enough to meet as we lost him to GBS before my birth. I am sure there are many like me and who wish to find better treatment for this potentially disabling and life-threatening illness.

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11

Clinical Diversities of Guillain-Barré Syndrome in Korea: A Wondering Stranger's View from the Far East: Is the Dawn Coming to Him?

Jong Kuk Kim

A man seeking the ultimate truth left his home on a long journey. Finally he arrived at Scotland, the land of folktales. Could he find the legendary sword here? He is trying to get answers to questions that have troubled him for many years.

My quest began when I was a young resident in neurology training. I met a college student suffering from acute onset dysarthria and dysphagia. There was no evidence of other neurological symptoms or signs, but he had had an upper respiratory tract infection (URI) in the preceding days. All investigations, including a brain MRI, electrophysiology and blood work, including for acetylcholine receptor antibodies, did not reveal the answer. I could do nothing for that young patient. Although the patient's symptoms improved spontaneously over time, a question still remained. What was the true cause of acute dysphagia?

Time passed and I entered a fellowship training course in the neuromuscular division. I met 3 consecutive cases with diplopia within 2 months. Two of them had preceding URIs. The interesting features were that they all had asymmetric and incomplete bilateral oculomotor palsy with internal ophthalmoplegia, without apparent gait disorders, falling or ataxia. "How should I conceive of and differentiate this kind of case when I meet it? What can I do for them?" I thought. A test for anti-GQ1b antibodies was strongly positive in the acute phase serum in all these patients [1]. If this antibody was not positive or even if we could not perform a study for this antibody, should we interpret this kind of problem as a variant form of autoimmune neuropathy? What if we did not know whether this antibody had a very close relationship with Miller Fisher syndrome (MFS) [2]? If so, MFS could appear with various features other than the classical triad. In addition, the anti-GQ1b antibody syndrome can manifest with various features overlapping those of Guillain-Barré syndrome (GBS) and MFS [3].

After several months, I met another interesting case, a 22-year-old man suffering from acute quadriparesis after several days of diarrhoea. There was apparent evidence of peripheral neuropathy in his nerve conduction study. But in neurological examination his muscle stretch reflex was exacerbated rather than depressed. I thought, "Can I regard this as a typical finding for GBS?" I decided to get immunological information. I asked Professor Susumu Kusunoki, who was the first to find the significance of anti-GQ1b antibody in MFS when the serum was positive for auto-antibody against a rare ganglioside GalNAc-GD1a. I learned that deep-tendon reflex can be preserved or exacerbated in some types of GBS with positive anti-ganglioside antibodies [4]. And I also learned that this hyperreflexia is mostly found in

an acute motor axonal neuropathy subtype of GBS in Asia, including China and Japan [5]. This GalNAc-GD1a ganglioside is located in paranodal axonal membranes and appears to be a target for autoimmune peripheral neuropathy [6]. But the result of electrophysiology in the previous case was curious. Although there was conduction block and abnormal temporal dispersion, including prolonged terminal latencies, motor conduction velocity was absolutely normal. Most of the abnormal findings were limited to motor nerves and parameters of sensory nerve were quite normal. Did these findings represent demyelinating or axonal neuropathy?

Subsequently, I learned that interpreting electrophysiological findings in GBS is complicated by the dynamic changes in these patients. Many neurologists in my home country thought that GBS mainly damaged the peripheral nerve myelin. Finally I found that not all electrophysiological studies will necessarily reveal the correct underlying pathophysiological mechanism. Evidence was accumulating that some of the patients' real problems lay in the peripheral nerve axon, even though in electrophysiological studies they displayed the traditional features of demyelination [7]. In addition, anti-ganglioside antibodies showed apparent differences between GBS in Eastern and Western countries, and they provided the critical, determining pathophysiology [8].

What was the situation in my home country? Does demyelinating GBS predominate there, as it does in Western countries, or is axonal GBS as prevalent in Korea as it is in China and Japan? Until that time, it was a very confusing situation with 2 studies producing completely contradictory results [9,10]. If electrophysiological classifications cannot determine this, it would be better to find the presence of auto-antibodies. So I decided to get some additional help from the expert of a neighbouring country and visited Professor Kusunoki's laboratory, which had sera from many Korean GBS patients. We found very interesting results from the analysis of anti-ganglioside antibodies and clinical information. Various kinds of anti-ganglioside antibodies were positive in more than 50% of Korean GBS patients, and the patients with positive antibodies associated with the axonal subtype were classified as having demyelinating neuropathy according to electrophysiological criteria. But most of those exhibiting demyelinating GBS with positive antibodies showed pure motor presentation with conduction block and normal conduction velocity. This meant that the antibodies could determine the underlying pathophysiology [11]. We found that studying the anti-ganglioside antibodies was essential to understanding the disease.

I wondered how many variant types of GBS existed in my home country. What could we learn by finding anti-ganglioside antibodies from these various types of GBS? In MFS, we identified a delayed facial palsy [12]. Even though it was rare, there was another variant of GBS presenting with facial diplegia without prominent limb weakness. These patients showed minor changes in electrophysiology with tingling hands and negative anti-ganglioside antibodies [13]. In addition, ophthalmoplegia in GBS was more closely associated with anti-GT1a antibodies than was MFS [11]. Finally, there was a group of patients who could not be classified as a representing subtype according to criteria. They apparently had a type of GBS with acute bulbar palsy accompanied by various degrees of ophthalmoplegia or ataxia [14]. Anti-ganglioside antibodies would be the important clue to diagnosis in these unusual variants or subtypes of GBS.



Figure 11.1 Korean neurologists participating in the 2014 Inflammatory Neuropathy Consortium at Düsseldorf

GBS has many faces in my home country. Can we make an accurate diagnosis if we use only limited information during assessment? Recently, there was an interesting article about whether U.S. President Roosevelt suffered from poliomyelitis or GBS. According to this story, he was frequently misdiagnosed as having poliomyelitis, despite a lack of firm evidence. Other evidence apparently showed that his problem was closer to GBS [15]. This taught me that we have to use as much information as possible to classify and understand the variations of GBS. This is very important in Asian countries, including Korea (figure 11.1).

Which criteria should we use for defining GBS? Are there any universal standards? How do antibodies contribute to our diagnostic search? How are they valuable in determining the various forms of GBS encountered in my home country? Have all the important antibodies been found, and if not, where can I explore more? A new level of complexity has emerged in this context: Antigen/antibody reaction is not a two-dimensional picture drawn on a piece of paper but a three-dimensional interaction existing in space, and in a variety of combinations and interactions [16]. So we need a new way to explore this more complex antibody-antigen relationship [17]. New subsets of antibodies to ganglioside-complex may yet shed light on the pathophysiology of this disease, including even demyelinating GBS.

Someday we may have a more complete understanding and explanation of the clinical and pathological heterogeneity of GBS in my home country. For now, I am walking in the highlands in hope of a new dawn.

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CLINICAL

12

The Evolution of the Diagnostic Criteria for GBS

Christiaan Fokke and Bart C. Jacobs

Introduction

The Guillain-Barré syndrome (GBS) consists of a heterogeneous group or spectrum of acute immune-mediated polyradiculoneuropathies. In classical textbook cases, a patient will present with ascending bilateral weakness and sensory disturbances of the legs and arms, in a proportion combined with pain, cranial and autonomic nerve involvement, or respiratory weakness. The rapid development of these symptoms combined with a flaccid paresis and reduced tendon reflexes at neurological examination in absence of other causes will usually lead to the early diagnosis of GBS. In other cases, the diagnosis may be less straightforward. Diagnosis in patients at the borders of the GBS spectrum or with ‘overlap syndromes’ may be more challenging. A continuous spectrum from paraparetic to pharyngeal-cervical-brachial variant is recognized, although international discussion remains whether purely sensory deficits, hyperreflexia or spinal cord involvement should be considered as a part of the GBS spectrum. These diagnostic dilemmas also apply to clinical overlap syndromes with Miller Fisher and Bickerstaff encephalitis. Another diagnostic dilemma may occur in the acute phase when a specific symptom, like pain, predominates or when it is less evident that causes other than GBS should be considered and excluded. Accurate and early diagnosis, however, is essential, considering that patients require treatment and monitoring to prevent life-threatening complications. In addition, proper diagnostic criteria are required to conduct therapeutic trials and epidemiological studies.

From First Case Descriptions to Diagnostic Criteria

Reports of progressive numbness and weakness over a short period exist in medical literature since the early 19th century. In 1859 Jean Baptiste Octave Landry was the first to describe a neurologic condition characterized by ascending motor paralysis with poor prognosis that he referred to as “ascending paralysis” [1]. One hundred years ago, 2 soldiers with acute areflexic paralysis and raised protein levels with normal cell count in cerebrospinal fluid (CSF) were described by Georges Guillain, Jean-Alexander Barré and André Strohl [2]. These remarkable CSF findings set the condition apart from infectious causes of limb paralysis including syphilis, tuberculosis and poliomyelitis. Around 40 years later an unusual variant of acute idiopathic polyneuritis with ophthalmoplegia, ataxia and areflexia was described by Charles Miller Fisher [3] and a combination with hypersomnolence by Edwin Robert Bickerstaff [4].

Table 12.1 Diagnostic criteria for Guillain-Barré syndrome developed by the NINDS (based on the criteria set forth in Asbury and Cornblath [6])

| Features required for GBS diagnosis | | |
|--|---|--|
| a) Progressive motor weakness of more than one limb b) Areflexia or hyporeflexia | | |
| Features supportive of diagnosis | Features casting doubt on diagnosis | Features that rule out diagnosis |
| <ul style="list-style-type: none"> • Progression of weakness in 2–4 weeks • Relative symmetry • Mild sensory symptoms or signs • Cranial nerve involvement • Recovery in 2–4 weeks after progression ceases • Autonomic dysfunction • Absence of fever • Elevated CSF protein • $10 \leq$ leukocytes/mm³ in CSF • Electrodiagnostic features supportive of the diagnosis | <ul style="list-style-type: none"> • Marked, persistent asymmetry of weakness • Persistent bladder or bowel dysfunction • Bladder or bowel dysfunction at onset • More than 50 mononuclear leukocytes/mm³ in CSF • Presence of polymorphonuclear leukocytes in CSF • Sharp sensory level | <ul style="list-style-type: none"> • History of hexacarbon abuse • Porphyria • Recent diphtheria • A purely sensory syndrome • Poliomyelitis, botulism, hysterical paralysis, or toxic neuropathy |

The question about accurate criteria for the diagnosis became apparent after the swine flu vaccination campaign of 1976–1977 when there was a suspected increase in the incidence of GBS after this vaccination. This diagnostic problem led to the introduction of more strictly defined diagnostic criteria for GBS in 1978 by the US National Institute Neurological Disorders and Stroke (NINDS) [5]. The criteria were reaffirmed in 1990 and are still the most widely used for GBS to date, especially in scientific research (Table 12.1) [6]. The key features in these criteria are the presence of symmetrical flaccid weakness and decreased reflexes and the absence of alternative causes.

Additional attempt has been made to classify GBS patients into different subgroups, for example by incorporating nerve conduction studies (NCS) and anti-ganglioside results [7]. The Brighton collaboration developed different levels of diagnostic certainty in order to standardize case definitions with the aim of improving vaccine safety (Table 12.2) [8]. Development of new criteria continued, with detailed descriptions of the different subgroups [9]. An important limitation of most of these criteria for current clinical practice is that the monophasic course becomes evident after a follow-up of days to months. In addition, GBS has an extensive differential diagnosis but it remains unclear to what extent additional tests should be performed in the acute phase to exclude other causes.

Table 12.2 Brighton criteria and case definitions for GBS (based on the criteria set forth in Sejvar et al. [8] and used with permission from Fokke et al. [10]). + = present, - = absent, +/- = present or absent.

| Diagnostic criteria | Level of diagnostic certainty | | | |
|---|-------------------------------|-----|---|-----|
| | 1 | 2* | 3 | 4 |
| Bilateral and flaccid weakness of limbs | + | + | + | +/- |
| Decreased or absent deep tendon reflexes in weak limbs | + | + | + | +/- |
| Monophasic course and time between onset-nadir 12 hours to 28 days | + | + | + | +/- |
| CSF cell count <50/ μ l | + | + | - | +/- |
| CSF protein level > normal value | + | +/- | - | +/- |
| EMG findings consistent with GBS | + | +/- | - | +/- |
| Absence of identified alternative diagnosis for weakness | + | + | + | + |

*If CSF is not collected or results not available, electrophysiologic studies must be consistent with GBS.

Additional Investigations

GBS is still considered to be a syndrome, because there are at present no diagnostic and pathogenic markers available with sufficient sensitivity and specificity. There are, however, several investigations that can be helpful to support the clinical diagnosis.

Cerebrospinal Fluid Examination

The original publication from 1916 suggested that an albuminocytological dissociation in the CSF is a typical finding for GBS. We now recognize that elevated CSF protein levels are found in approximately in only 50% of patients in the first 3 days after onset of weakness [10]. This is usually also the time window in which the patient presents to the emergency department and has therefore diagnostic limitations. More important is the investigation of the number of cells in CSF, especially to exclude other diagnoses. A mild pleiocytosis is found in 15% of patients with GBS and doesn't exclude the diagnosis. According to the Asbury and Cornblath criteria, only CSF cell counts > 50 cells per μ l should cast doubt on the diagnosis of GBS.

Anti-Ganglioside Antibodies

Many different antibodies to single, or combinations of, gangliosides have been identified in the serum from patients in the acute phase of GBS. In specific clinical situations, testing for some of these antibodies may be helpful to finding support for a diagnosis of GBS. Up to now, the most clinically relevant anti-ganglioside tests are for patients suspected of having MFS (anti-GQ1b) or acute motor axonal neuropathy (AMAN) (anti-GM1 and anti-GD1a IgG). The general low frequency of each specific antibody in other patients with GBS results in a low negative predictive value. Moreover, the presence of anti-gangliosides could also occur in other diseases. Another disadvantage of using anti-ganglioside serology for the diagnosis of GBS is that a test result may only occur at a later stage, when important clinical decisions about monitoring and treatment have already been taken. Research into antibodies to gangliosides and other peripheral nerve targets, however, is rapidly progressing, and faster techniques supporting more sensitive and more specific tests may emerge in the near future.

Nerve Conduction Studies

NCS can help to support the clinical diagnosis of GBS and to discriminate between axonal and

demyelinating subtypes of GBS. Unfortunately, there are 2 important diagnostic limitations of NCS in GBS. First, GBS is also highly heterogeneous with respect to NCS findings and various criteria for demyelination and axonal degeneration have been developed. A related problem is that in the acute phase of the disease a considerable proportion of patients with GBS present with abnormalities in NCS but do not fulfil the criteria of one of the GBS subtypes [10]. Unfortunately, there are no criteria to support the diagnosis of GBS in general nor to set GBS apart from other peripheral nerve disorders. Second, GBS is a highly dynamic disorder in which the NCS findings may change significantly over time. The classification of demyelinating and axonal forms of GBS based on the NCS may change during the disease course of an individual patient. The NCS abnormalities in most patients tend to peak around 2 weeks after the onset of weakness, which makes this test unsuitable in the majority of patients in the acute phase.

MRI Scanning

This underappreciated diagnostic test in GBS is pointed out by the fact that the only prospective evaluation of MRI lumbosacral nerve root enhancement was performed 20 years ago. This relatively small study in 24 consecutive GBS patients showed that 18 out of 19 with 'typical' GBS had nerve root enhancement, compared with 2 of 5 with a variant presentation [11].

Interestingly, no relation was found between nerve root enhancement and the timing of the MRI or CSF protein level. Whether this is due to the small number of patients or the possibility that MRI nerve root enhancement is an additional and independent factor is not clear at this point. The importance of nerve root enhancement is supported by the correlation with pain, GBS disability grade and duration of recovery. For patients with a variant GBS it might be more useful to image the clinically relevant area, but it is essential that contrast is administered if the diagnosis is suspected, as noncontrast sequences appear essentially normal. The increasing availability of MRI scans and the ability to see results within a few hours make this a potential important diagnostic test in the acute phase. It has the possibility to show a positive finding suggestive of GBS and to exclude important differential diagnoses.

Future Directions

Now we are celebrating the 100-year anniversary of GBS, although we doubt whether this name will still be in use in the 100 years to come. As soon as we better understand the pathogenesis and heterogeneity of GBS, it may not be required to use a syndrome diagnosis. Instead, GBS may be replaced by more specific sets of diagnostic criteria for an acute monophasic immune-mediated disease. Regarding the future diagnostic criteria for this spectrum of immune-mediated neuropathies, 2 aspects are very relevant.

First, the *diagnostic criteria* should be pragmatic and support the clinician at the emergency department confronted with a patient with rapidly progressive symmetrical limb weakness. These criteria should be a combination of neurological and additional investigations of which results are available on the same day to prevent delay of treatment. If no ideal diagnostic biomarker becomes available, a combination of major and minor criteria would be able to cover the extensive variety of symptoms within this clinical spectrum. Importantly, diagnostic criteria also provide the diagnostic boundary of GBS when a patient doesn't fulfil these criteria and the clinician is encouraged to continue the search for the right diagnosis.

Improvement of these criteria could be achieved by incorporating additional investigations in the future, for example, development of a diagnostic test which screens patient sera for a whole battery of different antibodies. Also, prospective clinical trials should include MRI data of clinical relevant areas

of patients and investigate the relation between nerve root enhancement and onset of symptoms. It is possible that nerve root enhancement on MRI with normal cell count in CSF might be very specific for an 'acute immune mediated neuropathy'. Future investigation should also point out the diagnostic value of biomarkers and ultrasound.

The second should be *classification criteria*, to divide the broad spectrum of GBS patients into homogenous groups. Where the *diagnostic criteria* should look for the common pathway of acute immune neuropathies, the *classification criteria* should point out the differences in this clinical spectrum. These different subgroups are not only formed by similarities regarding signs and symptoms but also by retrospective clinical data, like disease course. Also, different additional tests could be incorporated into these *classification criteria*, for example NCS results or future biomarkers which may take days to process. Currently, the aim of this classification would be mainly for research purposes, as treatment regimens are still similar between all patients with an acute immune mediated neuropathy. However, further investigation into pathological pathways of different subtypes could provide important insight and might result in specific therapies in the future.

Summary and Conclusions

One hundred years since Guillain, Barré and Strohl's publication, the diagnosis of the GBS is still challenging due to a broad clinical spectrum without specified borders and the lack of specific diagnostic tests. Future investigations should provide the diagnostic value of biomarkers, ultrasound and especially MRI with gadolinium. One option is to develop diagnostic and classification criteria for the acute immune mediated neuropathy by combining major and minor criteria consisting of clinical signs, symptoms and results from additional investigations.

Key Outstanding Questions

- What are the clinical boundaries defining the full GBS spectrum, compared to other diseases?
- What are the common pathological pathways in the GBS spectrum and can this knowledge be used for diagnosis?
- What is the optimal and most cost-effective diagnostic workup in individual patients to confirm the GBS diagnosis and exclude other diagnoses?
- What is a better name for this disorder than Guillain-Barré syndrome?

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13

Miller Fisher Syndrome

Atsuro Chiba

Introduction

Miller Fisher syndrome (MFS) has attracted the interest of many neurologists because of its unique combination of core manifestations—ophthalmoplegia, ataxia, and areflexia—that do not initially appear to be easily linked. The pathological mechanisms and the nosology of the syndrome had been discussed for several decades, but the arguments lacked a cornerstone—a disease-specific biomarker. Along with the progression of research on anti-glycolipid antibodies related to Guillain-Barré syndrome (GBS), an IgG antibody against ganglioside GQ1b was discovered in MFS. In this top-10 story, many papers are from Japan, perhaps because Japanese research groups have some advantages in this field: a fairly high incidence of MFS in Japan compared with other countries (27% of all GBS cases including MFS [1]), and a long history of research into nervous system glycolipids originating from professor Tamio Yamakawa's laboratory at the University of Tokyo.

The Origin of the Story

Fisher M. An unusual variant of acute idiopathic polyneuritis (syndrome of ophthalmoplegia, ataxia and areflexia). *The New England Journal of Medicine*, 1956

There is an aphorism in clinical research called the 'three-case rule' in the neurological department of my alma mater: 'Note the first case in your mind, suspect a trend after the second, and believe after the third'. Fisher's penetrating original paper [2] embodies this rule. He had met 2 patients within a few years of each other in the early 1950s, and might have been waiting for the third one, the Case 1 in the 'Case Reports', who was fully studied in Massachusetts General Hospital (MGH) and must have prompted him to this paper finally. Fisher seemed to already have some idea even in the first patient (the Case 3), in whom "although no diagnosis was made, a rise in the cerebrospinal-fluid protein was looked for" because of polyneuropathic symptoms, the numbness of the fingers and areflexia. Albuminocytologic dissociation, however, was not detected in a lumbar tap at the end of 6 weeks. The detailed clinical information on the second patient (the Case 2) came from a hospital file. He saw this patient because of an unrelated matter, and the patient recounted a past history of total ophthalmoplegia. One of the most important points that made him conclude that this syndrome was a variant of GBS, would be the albuminocytological dissociation in the cerebrospinal fluid, and this was eventually detected from the serial spinal tap in the third patient at MGH. The biochemical feature common to GBS might be needed for the conclusion. Interestingly, episodes of an antecedent infection, which is now in the centre of the

pathogenesis of GBS and MFS, were not so emphasized.

This paper established the concept of the syndrome almost by itself. Atypical features obviously attributed to the central nervous system (CNS) were also described, such as changes in arousal level and a preserved Bell's phenomenon in a state of total external ophthalmoplegia, implying the true extent of the disorder. Despite these and other features that could not be easily attributed to peripheral lesions, his reluctant conclusion with respect to the core manifestations was a variant of GBS with "an unusual and unique disturbance of peripheral neurons". This conclusion might be owed to Fisher's career in cerebrovascular disease. Before Fisher, patients with this syndrome could have been thought to have a stroke in the brainstem, but as a leading stroke neurologist, he would not be able to agree with this idea. Every phenomenon has a cause. To answer riddles, we first have to notice their presence.

Disease-Specific Biomarker, IgG Anti-GQ1b Antibody

Chiba A, et al. Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. *Neurology*, 1993

IgG anti-GQ1b antibody in MFS was first reported in 1992 [3]. This antibody was identified in a deductive screening approach. In the context of this research field, where Ilyas and colleagues first reported anti-ganglioside antibodies in GBS in 1988 [4], it would be a matter of time before this discovery, and the antibody was soon reported independently [5,6]. This paper in *Neurology* in 1993 [7] demonstrated a possible relation between the antibody and ophthalmoplegia, one of the core manifestations in MFS, from clinical, immunohistochemical and biochemical aspects. Molecular mimicry theory, the core theory for GBS pathogenesis, consists of 2 parts: (A) antigenic epitopes on the agents of antecedent infections induce antibodies that cross-react with antigens that mimic molecular structures in nervous tissues; and (B) the localization of the tissue antigens defines the clinical features. The first part was demonstrated by the combination of *Campylobacter jejuni*, anti-GM1 antibody and acute motor axonal neuropathy. The story of MFS and GQ1b—a high positive rate of the antibody and consistency between clinical findings and specific antigen distribution—is a good example of the second part. Clinically, IgG anti-GQ1b antibody is tightly associated with acute ophthalmoplegia following infectious episodes: MFS, GBS with ophthalmoplegia, and acute ophthalmoplegia without ataxia, which could be a mild or incomplete form of MFS. Histochemically, GQ1b antigen accumulates specifically in the paranodal region of the 3 cranial nerves (oculomotor, trochlear and abducens). Biochemically, the amount of ganglioside GQ1b in these 3 nerves is significantly higher than in the other cranial nerves or peripheral nervous tissues, except for the optic nerve [8]. However, whether the antibody could physiologically affect nerve conduction was still unknown at this point.

Ataxia and Areflexia

Kuwabara S, et al. Special sensory ataxia in Miller Fisher syndrome detected by postural body sway analysis. *Annals of Neurology*, 1999

MFS must have disclosed a special, previously unknown type of ataxia. Although Fisher described it as cerebellar in type, he also pointed out the absence of 'cerebellar speech' even in severely ataxic cases. In his original description he speculated on an alternate cause, saying that "if the cerebellar system is not to

be incriminated one must postulate that in the syndrome herein discussed, a unique, widespread and selective attack on the sensory neurons underlying postural adjustment must be occurring". An abnormal H-reflex with preserved motor conduction was reported, suggesting an abnormality in 1a afferent fibres from the muscle spindle and a dysfunction in proprioception [9,10]. Kuwabara and colleagues performed comprehensive physiological studies, including analysis of the nature of the posture instability itself by body-sway analysis, and concluded that the condition was a type of sensory ataxia probably caused by the selective involvement of group 1a muscle-spindle afferents [11]. Thus, sensory ataxia with a preserved sense of joint position is what makes ataxia in MFS unique. This can also explain areflexia in MFS in a manner different from that in GBS, in which areflexia could result from a malfunction of 1a efferents.

Liu IX. Immunolocalization of GQ1b and related gangliosides in human extraocular neuromuscular junctions and muscle spindles. *Investigative Ophthalmology and Visual Science*, 2009

The question then arises as to where anti-GQ1b antibodies might bind and exert their pathological effects in human tissue. Considering the consistent combination of the triad in typical MFS, it is simple and natural to suppose that the antibody is related to each of them. Liu and colleagues used immunohistochemistry to localize the epitopes in human tissues, focusing especially on the neuromuscular junction (NMJ) and the muscle spindles [12]. Their study revealed that the GQ1b/GT1a epitope was more richly localized in the NMJs of the extraocular muscles (EOMs) than in those of limb muscles, in which the NMJs were rarely stained. This finding is consistent with the preferential involvement of EOMs and with a study of single-fibre electromyography showing that transmission at neuromuscular junctions in the limbs was usually unaffected in MFS [13]. Liu also detected the GQ1b/GT1a epitope on the surface of individual intrafusal fibres of limb-muscle spindles at the equatorial region, where abundant group 1a sensory terminals wrap around the fibres. This observation supports the involvement of group 1a afferents from the muscle spindles in ataxia and areflexia. This immunohistochemical study also revealed that GD1b and GQ1b/GT1a were localized similarly, but that anti-GD1b antibody is not usually linked to MFS. Thus, other factors would be necessary for anti-GQ1b antibody to exert its pathogenic effect on these regions.

Pathological Effect of Anti-GQ1b Antibody

Halstead SK, et al. Anti-disialoside antibodies kill perisynaptic Schwann cells and damage motor nerve terminals via membrane attack complex in a murine model of neuropathy. *Brain*, 2004

It is always possible that autoantibodies, including anti-ganglioside antibodies, could be epiphenomena or secondary to tissue injury. To rebut this possibility in a clinical setting, detection of the antibodies before onset would be helpful, especially for ruling out secondary responses to injury. Actually, IgG anti-GQ1b antibody was also detected before neurological onset of MFS in patients whose sera were obtained in that period by chance. Although a more effective way is with an animal model, my personal search in many animals, including up to crab-eating macaque, has failed to find an animal that expresses the GQ1b epitope in the ocular motor nerves as it is in humans. Willison's research group has extensively studied the pathological effects of patient sera, their IgG fractions and monoclonal antibodies (mAbs) against GQ1b using an ex-vivo mouse hemidiaphragm preparation [14]. Their series of works has demonstrated:

(A) patients' sera, patients' IgG and the mAbs cause neuromuscular block; (B) the blocking effect was complement-dependent and α -Latrotoxin-like; (C) the disialylgalactose epitope common to GQ1b is expressed in the NMJs and perisynaptic Schwann cells; (D) mAbs destroyed presynaptic structure—both nerve terminals and perisynaptic Schwann cells, with deposition of a membrane-attack complex; and (E) passive transfer of mAbs also caused morphological change in NMJs. These findings could not be simply expanded to the human disease, but the GQ1b-antigen was also detected in the NMJ of the human extraocular muscles [12]. Neuromuscular transmission has been studied in MFS patients using single fibre electromyography, but the results are contradictory. While Kuwabara and colleagues did not detect transmission defects in limb muscles [13], Lo and colleagues detected those in the orbicularis oculi [15]. This disagreement might result from the different muscles that were examined.

Clinical Features and Related Conditions

Mori M, et al. *Neurology*, 2001

Mori and colleagues conducted a retrospective clinical analysis of 50 consecutive patients with typical MFS in Japan [16]. This remains the largest case series from a single centre so far and has drawn the clinical features of the syndrome representatively. [Table 13.1](#) shows the specifications for the disease described in their paper. Mori and colleagues also reported the effect of IVIg and plasmapheresis in MFS patients, showing that these therapies only provided limited benefits [17].

Yuki N, et al. An immunologic abnormality common to Bickerstaff's brain stem encephalitis and Fisher's syndrome. *Journal of Neurological Science*, 1993

An ongoing debate is the nosological position of MFS: is it a variant of GBS or a kind of brainstem encephalitis—the so-called Bickerstaff brainstem encephalitis (BBE)? There have been 2 fundamental limitations contributing to this debate. One was that pathophysiological homogeneity of the cohorts could not be determined without knowing the biomarkers involved in pathogenesis, and the other was that, as shown in Fisher's original paper, MFS itself could manifest some CNS symptoms. When the anti-GQ1b antibody was discovered, it appeared to be an attractive biomarker that could be used to answer the debate. Yuki detected the antibody in all 3 patients diagnosed as BBE and revealed the common immunological features in MFS and BBE [18]. His research group then increased the number of patients to 53 BBE and 466 MFS patients, and, several years later, compared clinical, serological, radiological and physiological features [19]. Interesting observations in this study regarded the loss of soleus H-reflexes and the detection of 1-Hz power-spectrum peak on the postural body-sway analysis, which both suggest dysfunction in 1a fibres. These were found in about 70% of both BBE and MFS cases. While some patients with MFS do indeed show CNS signs, the majority of those diagnosed as BBE have peripheral features. It would be natural to suppose that BBE, which has a common immunopathological biomarker with MFS, is an atypical form of MFS with prominent CNS signs.

Koga M, et al. Nationwide survey of patients in Japan with Bickerstaff brainstem encephalitis: epidemiological and clinical characteristics. *Journal of Neurology, Neurosurgery and Psychiatry*, 2012

To have an effective discussion about the relationship between MFS and BBE, we have to know the

makeup of the groups in question. A Japanese nationwide survey on BBE has given a suggestion regarding this point [20]. It revealed that BBE patients with atypical neurological findings or without IgG anti-GQ1b antibodies had features different from those with typical neurological findings with the antibodies. Atypical symptoms were linked to longer duration from onset to nadir, slower recovery, higher frequency of brain MRI abnormality, and increased protein concentration and marked pleocytosis in CSF. This suggests that BBE is heterogeneous in terms of the pathomechanism. BBE patients with IgG anti-GQ1b antibodies could be the true variant of MFS, and BBE without the antibody may be the true ‘-itis’ in the brainstem.

What controls whether MFS manifests with or without CNS signs? One candidate is a serum factor in BBE patients that increases the permeability of the blood-brain barrier and is accompanied by autocrine secretion of matrix metalloproteinase-9 [21]. Research in this direction might provide new methods for treatment other than those related to antibodies, especially in severe cases with profound CNS manifestations.

Table 13.2 is my personal arrangement of MFS-related conditions. They are divided into 3 categories: typical MFS (the prototype of these conditions); MFS-minus, in which ataxia or ophthalmoplegia is absent throughout the entire clinical course (acute ophthalmoplegia and acute ataxia, respectively); and MFS-plus, in which motor weakness in the spinal nerve areas or CNS symptoms are present (GBS with ophthalmoplegia and BBE, respectively).

Table 13.1 Epidemiological and clinical features, and natural prognosis of Miller Fisher syndrome (modified with permission from Mori et al., 2001 [16]). Values in parentheses are ranges, and values before the parentheses are medians. a: number of patients examined; b: Yr = years, Dy = days, Mo = months; c: period between antecedent infection and neurological onset; d: these include dysesthesia in the limb, blepharoptosis and photophobia; e: gait ability in the Hughes functional grading scale; f: symptoms are not explained by or dissociated from peripheral disability; g: observation in patients without any immunomodulatory therapies; h: Op = ophthalmoplegia, Ax = ataxia, DTR = hyporeflexia

| | n ^a | | unit ^b | | n | | unit |
|--|----------------|------------|-------------------|--|-------------|--|------|
| Number of MFS/% of whole GBS | 201 | 25 | % | Other neurological symptoms | 50 | | |
| Age of onset | 50 | 40 (13–78) | Yr | Internal ophthalmoplegia | 42 | | % |
| Male:female ratio | 50 | 2:1 | | Blepharoptosis | 58 | | % |
| Antecedent symptom | 50 | | | Facial palsy | 32 | | % |
| Respiratory | | 76 | % | Bulbar palsy | 26 | | % |
| Gastrointestinal | | 4 | % | Dysesthesia | 24 | | % |
| Fever | | 2 | % | Decrease in superficial sensation | 20 | | % |
| None | | 18 | % | Decrease in deep sensation | 18 | | % |
| Immunological incubation period ^c | | 8 (1–30) | Dy | Motor weakness (MRC grade 4) | 20 | | % |
| IgG anti-GQ1b antibody | 36 | 89 | % | Micturition disturbance | 16 | | % |
| Symptom at neurological onset | 50 | | | Supranuclear feature of ophthalmoplegia ^f | 27 | | |
| Diplopia | | 78 | % | Preserved Bell's phenomenon | 7 | | % |
| Ataxia | | 46 | % | Preserved convergence | 4 | | % |
| Both diplopia and ataxia | | 34 | % | Gaze horizontal nystagmus | 7 | | % |
| Others ^d | | 2–14 | % | Internuclear ophthalmoplegia | 4 | | % |
| Interval from onset to nadir | 50 | 6 (2–21) | Dy | Natural recovery course ^g | 28 | | |
| Severity at nadir | 50 | | | Follow-up period | 4 (1–185) | | Mo |
| Complete ophthalmoplegia | | 30 | % | Order of improvement: Op-Ax-DTR ^h | Almost all | | |
| Ataxia ^e : FS4 | | 14 | % | Period: onset-beginning of recovery | | | |
| FS3 | | 16 | % | Ophthalmoplegia | 12 (3–14) | | Dy |
| FS2 | | 45 | % | Ataxia | 15 (3–46) | | Dy |
| FS1 | | 14 | % | Period: onset-disappearance | | | |
| | | | | Ophthalmoplegia | 88 (29–165) | | Dy |
| | | | | Ataxia | 23 (8–271) | | Dy |
| | | | | Nonrecovery from hyporeflexia | About 2/3 | | |

Table 13.2 Miller Fisher syndrome-related conditions.

| | Popular diagnostic term | Plus or Minus feature |
|-----------|--|---|
| MFS-plus | Bickerstaff encephalitis Guillain-Barré syndrome with ophthalmoplegia | + CNS signs * +Motor weakness in limbs |
| Prototype | Miller Fisher syndrome (MFS) | |
| MFS-minus | Acute ophthalmoplegia (rare subtypes) Acute isolated ptosis Acute isolated internal ophthalmoplegia Acute ataxia | –ataxia –ophthalmoplegia |

* Alteration in consciousness and/or corticospinal tract sign

Antecedent Infections and Antibody Production

Koga M, et al. Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry. *Neurology*, 2005

One patient in Fisher's original paper developed the syndrome following pneumonia, which probably resulted from *Haemophilus influenzae*. This short but faithful description attracted the attention of researchers to this gram-negative organism in the respiratory tract, especially after the historical success of molecular mimicry theory in the case of *Campylobacter jejuni*. Koga and colleagues performed comprehensive studies on antecedent infection in MFS and found serologically significant associations between the disease and both *H. influenzae* and *C. jejuni* [22]. *C. jejuni* strains isolated from MFS were significantly associated with the GQ1b epitope when detected using antibodies, and biochemical analyses identified a possible GT1a-mimicking tri-sialosyl oligosaccharide structure in the lipooligosaccharides (LOSs). As for *H. influenzae*, epitopes recognized with anti-GQ1b antibody were also detected in the LOSs extracted from clinical isolates of MFS patients. Finally, in an *H. influenzae* strain isolated from an MFS patient, Houlston and co-workers biochemically identified a novel disialosyl galactose structure that is common to the nonreducing terminals of GQ1b and GT1a [23]. Although antecedent infectious agents have not been identified in the majority of MFS patients, the molecular mimicry theory could also be applicable in MFS related to both *C. jejuni* and *H. influenzae*.

Heikema P, et al. Siglec-7 specifically recognizes *Campylobacter jejuni* strains associated with oculomotor weakness in Guillain-Barré syndrome and Miller Fisher syndrome. *Clinical Microbiology and Infection*, 2013

Although tetra-sialosyl oligosaccharide structures in LOS that exactly mimic GQ1b have not been identified, immunization of animals with the GT1a-mimicking LOS from *C. jejuni* has been shown to induce antibodies that react with both GQ1b and GT1a [24]. In the human disease, those mimicking molecules could induce anti-GQ1b/GT1a antibodies. A group of I-type lectins primarily expressed on hematopoietic cells—sialic acid-binding Ig-like lectins, or 'siglecs'—are candidate molecules for recognizing sialylated oligosaccharide antigen. Fifteen siglecs have been identified in humans, and each member is unique in terms of expressed cell and ligand specificity. Among them, siglec-7 strongly binds to GT1a-mimic-bearing LOS from *C. jejuni*, and binding to disialo-oligosaccharide, especially the α 2–8 linkage, causes its conformation to shift dramatically [25,26]. In a study using clinical material, Heikema

and colleagues showed that binding between siglec-7 and *C. jejuni* LOS (clinical isolates from GBS and MFS patients) was significantly higher in the patient group with anti-GQ1b [27]. This finding would give one molecular part linking between the antigens on the pathogens and the antibody in the patients. Siglec-7 expressed on monocyte-macrophages could bind to *C. jejuni* with disialylated LOS, process it, give an antigen-presentation, and induce production of the antibody against terminal α 2–8 disialylated residues. The next question must be whether polymorphisms of siglec-7 can affect anti-GQ1b antibody production and MFS, as has been reported in other disorders [28].

Key Outstanding Questions

1. What are the pathogens of antecedent infections other than *H. influenzae* and *C. jejuni*?
2. How do the pathogens induce the antibody production?
3. What are the immunological pathogenic factors in sero-negative cases, in whom anti-GQ1b/GT1a antibodies are not detected?
4. What is the difference in clinical phenotype, especially between MFS and the MFS-plus (BBE or GBS with ophthalmoplegia)?

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14

Axonal Guillain-Barré Syndrome

Thomas E. Feasby

Introduction

Guillain-Barré syndrome (GBS) was thought of as the classic demyelinating disease of the peripheral nervous system (PNS). I was drawn to it by my interest in demyelination and in particular by my interest in the physiology of demyelination. I had investigated the nature of nerve conduction in regenerating nerve fibres in the laboratory of Professor Tom Sears at Queen Square, UK and observed the transition from continuous to saltatory conduction in these fibres as they became myelinated. I published work on conduction block in demyelinating GBS with my colleague Bill Brown. With my colleagues, I had investigated passive transfer models of demyelination using both rabbit anti-galactocerebroside antibodies and rat T cells. I was thus startled when I came upon a patient with GBS who didn't fit the model of demyelination but rather turned out to have an axonal form of GBS. Publication of this work was controversial and began a 10-year debate about this new concept which ultimately resulted in its validation.

Background

The pathology of GBS has been discerned over the years through the lens of the microscope and through electrophysiological recordings of responses to nerve stimulation. The former has the advantage of direct observation but the limitation of being a snapshot in time. The latter allows repeated observations but, limited by its indirect nature, requires inferences.

The first major pathological study of GBS, by Haymaker and Kernohan [1], examined the central nervous system and the nerve roots, but not the peripheral nerves, in 50 autopsied cases. They described oedema in the nerve roots and adjacent spinal nerves and infiltration of lymphocytes and phagocytes, which they thought might be secondary. They saw demyelination and what they thought was secondary axonal degeneration. In their last case, of a patient who died after 46 days, the axonal degeneration was very severe so that “by Bodian activated silver method, not a single normal axis cylinder was encountered”. Was this perhaps a case of ‘axonal’ GBS?

Twenty years later, Asbury, Arnason and Adams [2] described autopsy results in 19 GBS cases. Their most important observation was the lymphocytic infiltration, sometimes very extensive, seen in all cases, including those at early stages of their illness. They observed demyelination of varying degrees in all cases as well as axonal injury/degeneration in most. They noted that “axonal interruption and consequent Wallerian degeneration was observed frequently, most commonly and to a more marked degree in cases with the more intense inflammatory changes”. Like Haymaker and Kernohan, they considered axonal

degeneration to be secondary, but in their case, secondary to the inflammation.

Waksman and Adams [3] described experimental allergic neuritis (EAN), the first experimental model for GBS which they induced by immunization of rabbits with sciatic nerve tissue plus adjuvants. This model mimicked human GBS and was analogous to experimental allergic encephalomyelitis (EAE), the experimental model of multiple sclerosis. They observed intense lymphocyte and mononuclear cell inflammation, widespread demyelination and lesser degrees of axonal degeneration, again presumably secondary.

Many electrophysiological studies of GBS, by McLeod [4] and others, have detected typical signs of demyelination, including slowed conduction velocities, temporal dispersion of the compound muscle action potential evoked by nerve stimulation and delayed 'F' responses. In 1984, Bill Brown and I [5] described the significance of widespread conduction block secondary to demyelination as the major cause of acute weakness in most GBS patients. We also pointed out the common occurrence of axonal degeneration and noted its role in the residual weakness and limited recovery in some patients. At that point we were unaware of the phenomenon of axonal degeneration occurring in the absence of demyelination.

Axonal Guillain-Barré Syndrome

In 1985, I cared for a 64-year-old woman who, 2 weeks after a bout of diarrhoea, had a very rapid onset of severe GBS so that she was quadriplegic with marked cranial nerve abnormalities and respiratory failure within 36 hours. She had minor sensory findings. Electrophysiological studies done by Bill Brown showed inexcitable motor nerves, a very unusual finding. She showed no improvement, developed autonomic instability and died at day 28 after a cardiac arrest.

Joe Gilbert and I carried out an autopsy within hours of her death, on a Sunday, sampling the peripheral nervous system very extensively. We found widespread severe axonal degeneration on light microscopy from the nerve roots to the periphery, unaccompanied by evidence of demyelination or lymphocytic inflammation. This was confirmed by teased fibre studies and electron microscopy, where we also found evidence of unmyelinated fibre loss. My colleagues and I cared for 4 more very acute GBS patients who also had inexcitable motor nerves. Three of these patients had very slow and incomplete recoveries. We had much internal discussion about the meaning of these observations. I well remember debate with Charlie Bolton, who was concerned that the one case with reasonable recovery, his patient, didn't fit and might have had AIDS. Fortunately, we kept that case in the series and Charlie remained a co-author.

We were convinced that we had seen something new, and we submitted an abstract describing our findings for presentation at the American Academy of Neurology meeting in 1986 entitled 'Inexcitable Motor Nerves in GBS'. Apparently, the selection committee was not excitable either and they rejected our submission. In the meantime, *Brain*, being more receptive, published our description of these cases in 1986 with the title of 'An acute axonal form of Guillain-Barré polyneuropathy' [6]. This publication led to almost a decade of controversy over whether our results were valid.

In 1987, the American Academy of Neurology accepted our more excitingly retitled abstract, 'Severe acute axonal degeneration in Guillain-Barré syndrome', for presentation at the annual meeting in New York [7]. It was a potentially daunting experience, as I presented the paper in a large, very full room, at a session chaired by Arthur Asbury and Barry Arnason, 2 giants of the GBS and neuroimmunology world. When I finished my presentation, the aisles filled with questioners queuing at the microphones to engage me in debate. But before they had their chance, the 2 chairmen challenged me on our findings and

interpretations. The main points of criticism were that such severe axonal degeneration must have been secondary to demyelination and inflammation, and that since our autopsied patient died after 28 days, those findings could have subsided and hence we missed them. I defended our position, saying that if the axonal degeneration we observed had been secondary, the inflammation and demyelination must have been extreme and widespread and we couldn't possibly have missed it. We were quite confident that we had sampled the peripheral nervous system thoroughly. I enjoyed this little joust with our critics and it was made more poignant for me to have my 15-year-old son in the audience to see his dad on the hot seat.

My colleague, Angelika Hahn, and I turned to the model of experimental allergic neuritis in the Lewis rat to respond to this criticism. In a 1988 paper, we showed that EAN became more severe as the dose of antigen was increased and that the amount of axonal degeneration correlated with the amount of inflammation and demyelination [8]. This supported our contention that, if the severe axonal degeneration in our case had been secondary, we could not have missed inflammation and demyelination because it would have been equally severe.

Important support for the concept of an axonal form of GBS came from 2 cases reported by Yuki and colleagues in 1990 [9]. Both cases had severe acute motor neuropathy with markedly reduced compound muscle action potentials, evidence of denervation on EMG and poor recovery suggestive of axonal loss. They made the notable observation that both patients had a preceding diarrhoeal illness, had serological evidence of a preceding *C. jejuni* infection, and had positive titres of anti-GM1 antibodies. Subsequent work by their group and others established the principle of molecular mimicry in these cases and that the anti-GM1 antibodies attached to the nodal axolemma and triggered complement-mediated destruction facilitated by macrophages.

Nevertheless, the controversy continued, with major figures in peripheral nerve research expressing scepticism of our work. Two books on GBS failed to endorse us, although Richard Hughes was supportive in his 1990 book *Guillain-Barré Syndrome* [10]. Two years later, P.K. Thomas said that GBS was “no longer a simple concept” and did support the notion of an axonal form of the disease [11]. Cros and Triggs in 1993 then argued that we failed to find evidence of demyelination and lymphocytic inflammation in our autopsied case because the lesions might have been focal and because of “incomplete sampling” [12]. They also said it “was possible that a fulminant but short-lived inflammatory-demyelinating process was no longer evident at autopsy” after 28 days. In a commentary on this and other aspects of the dispute, Peter Dyck in an article entitled “Is there an axonal variety of GBS?” weighed the evidence and suggested that there likely were 2 or more ‘varieties’ of GBS and suggested the collection of further cases to delineate these types [13].

In 1993, my colleagues and I published a further study of 4 patients with acute severe GBS with inexcitable motor nerves [14]. All had nerve biopsies. Three patients showed pathological evidence of acute motor sensory axonal neuropathy (AMSAN), including one studied by both biopsy and autopsy. The autopsy findings were similar to our first case, described in 1986. The fourth patient had nerve biopsies at days 15 and 75. The first biopsy showed marked inflammatory demyelination and the second showed an almost total lack of myelinated axons, indicating severe secondary axonal degeneration. This showed that axonal degeneration in GBS can occur by 2 different mechanisms. It also showed that inexcitable motor nerves may be caused by severe acute demyelination.

Meanwhile, Guy McKhann, Jack Griffin, David Cornblath and colleagues in 1993 described a series of cases of apparent GBS occurring in northern China in children which were predominantly axonal in nature but seemed to have a good prognosis [15]. I visited Shijiazhuang in China in 1995 with David Cornblath and saw a ward filled with these young patients, many of them being ventilated by their parents by hand. Remarkably, the outcome was very good for most. This new form of GBS was different from

what we described but offered strong support that GBS was not a uniform entity.

The most important support for our thesis came from the same Johns Hopkins group, led by Jack Griffin [16,17]. In a series of 14 patients who died with GBS in Hebei province China, 4 had pathology consistent with acute axonal motor sensory neuropathy (AMSAN) [16,17], similar to what we had described. The findings were of extensive axonal degeneration involving nerve roots and nerves, without significant lymphocytic infiltration or demyelination. Furthermore, activated macrophages were found in the periaxonal space in perinodal regions. Two cases had preceding infection with *C. jejuni*. Of note, the index case from our 1986 paper had diarrhoea of unknown cause 2 weeks before the onset of GBS, perhaps *C. jejuni*. Other cases in the Griffin series showed evidence of axonal involvement restricted to motor fibres, hence the term acute motor axonal neuropathy (AMAN).

Conclusion

It turns out that GBS really is a syndrome, composed of several subtypes that share clinical features but also differ in important ways and almost certainly differ in pathogenesis. What has been a very healthy debate began in earnest over the issue of ‘axonal GBS’. A PubMed search on axonal GBS in November 2015 yielded 805 articles, all published in 1986 or later. We now have a much deeper understanding of the basis of axonal GBS and the other subtypes, but there is much more to learn. The practical value of this information is that, ultimately, we should be able to identify patients by their subtype early in the disease course, have good predictors of prognosis and provide a precision medicine approach to treatment.

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15

The Clinical Semiology of GBS: *Formes Frustes* and What They Tell Us

Allan Ropper

I located and reviewed the original notes and papers that I used to assemble the two brief articles on variants of GBS published in 1986 and 1994 [1,2]. These stimulated several recollected anecdotes. In 1980 [3] I had reported at the American Academy of Neurology meeting four of the earliest cases of GBS treated with plasma exchange. It was the subsequent plasma exchange trial and the prospect of a useful treatment for GBS that flooded large university centres with these cases and allowed a concentration of clinical material. Until then steroids were used but with little confidence and a small clinical trial had failed to demonstrate efficacy. I was at the right place at the right time because of our well-organized plasma exchange unit. I noticed these cases among a series of what became about 100 patients over 5 years that I cared for in our neuro-ICU and a subsequent consecutive prospective series that I accumulated up to 1991 after the trial. The latter became the basis of a monograph with Eelco Wijdicks and Bradley Truax [4].

Just as I had begun to consider writing up the first group of variant cases in 1985, I ran into Dr John (Jack) Griffin of Johns Hopkins as he was eating breakfast at the American Academy of Neurology meeting. He said, “You know, there are odd cases of GBS that we are seeing in the plasma exchange trial.” Just in the weeks before I had formulated the pharyngeal-cervical-brachial variant in my mind and was thinking how to present these cases in a paper. As Jack was an open and generous person, I told him I was working on the problem and had a paper in mind. I had the impression later that he graciously backed off on writing his own.

In writing the chapter on the history of the syndrome in monograph with Truax and Wijdicks I was sensitized to the failure by Guillain, Barré and Strohl to acknowledge earlier papers on the disease, including the famous one by Landry in 1859. In looking at the previously published papers in my folder it was clear that others had come across similar variants to the ones I was seeing and described them in different ways. In particular, the paper by Munsat and Barnes [5] was prominent. That paper made me aware that there are few truly original clinical ideas in neurology; most are the reframing of older notions with greater clarity so they can be used as handles by clinicians.

I had an opportunity to discuss the variants with C.M. Fisher, himself the revealer of the most famed variant. He was encouraging and suggested that I contrast them with alternative diagnoses such as myasthenia gravis. He had earlier in my career suggested that I keep a series of small notebooks on interesting cases, and also on major errors I had made over the years. The former was the repository for notes on the clinical variants and the latter has become the basis of a series of lectures on neurological errors that we published and that I often give as a visiting professor. But the most useful aspect of Fisher’s

career example was to collect cases of a similar type until a pattern of the core features emerged. This is how he advanced the study of cerebrovascular disease—for example lacunar strokes—and articulated not only the diagnostic features that became essential to clinical practice but variations on each theme, and this is what I intended to do with the initially heterogeneous variants of GBS that I was accumulating in the ICU.

Table 15.1 Regional and Functional Variants of Guillain-Barré Syndrome and Their Approximate Frequency in My Experience in Adult Patients

| Regional variant | Approximate frequency in adult patients |
|---|---|
| Brachio-cervical-facial (usually with ptosis) | 1% |
| Pure leg weakness | <1% |
| Bilateral VIth with distal paresthesias | 1% |
| Bilateral VIIth with paresthesias | > 1% |
| Lumbar polyradiculoneuritis (painful, not Lyme) | 2% |
| Pure ophthalmoplegia | 1% |
| Combinations, esp. Fisher and BCF | 2% |
| Functional Variants | |
| Fisher syndrome | 5% |
| Ataxia alone | 2% |
| Pure motor | 2% |
| Pure sensory | <1% |
| Pandysautonomia | <1% |
| Axonal GBS | 6–9% |

Of the variants described in the two aforementioned papers and summarized in [Table 15.1](#), the most interesting was the pharyngeal-cervical-brachial weakness that resembled botulism, diphtheria and myasthenia gravis. Several of these patients had been referred to the ICU from community hospitals with specifically one of these alternative diagnoses on the admission sheet and accompanying notes, making it clear to me that an exposition of their connection to GBS might be useful. I recall struggling clinically at the bedside with the first patient, a 19-year-old woman with blurred vision and the inability to raise her arms, who was thought by our senior clinicians (including Fisher!) to have botulism because of ophthalmoparesis and ptosis. Things went as far as to have a deltoid muscle biopsy done, but deep tendon reflexes were absent in the arms and she did not have iridoplegia. The idea that Bayes theorem might applied at the bedside had not yet permeated clinical work. The second variant, paraparesis that resembled a cauda equina or spinal cord lesion, was memorable because my first patient, age 64, began to have leg weakness and radicular pain while she was bowling, an activity she undertook avidly even at her age. Rather than the usual complaint in GBS of inability to climb stairs, her legs buckled as she tried to straighten up from releasing the ball. A third variant was certainly alluded to in earlier papers by others and was mainly to point out that ptosis could be quite severe in GBS without ophthalmoplegia, thereby simulating myasthenia. The same ptosis was evident in the pharyngeal-cervical-brachial variant. Finally, in the first paper on GBS variants, I described the acute severe midline back pain in the thoracic region that preceded or accompanied rare cases of GBS (*'coup du poignard'*). I subsequently saw this in three other patients who had been on orthopaedic services for days or weeks until it became clear their more serious problem was quadriparesis, but it also resulted in one of the worst missed diagnoses in my career, an example of overconfidence that is detailed below.

Using the model of Fisher syndrome and its ostensible relationship to GBS, I looked for commonalities between the variant syndromes and conventional polyneuritis. The criteria I established as a working theory to determine if a syndrome was part of the GBS spectrum were (A) the clinical syndrome began with unusual features that compelled another diagnosis such as myasthenia but eventually evolved to typical GBS; (B) the variant syndrome was known to occur as a component of fully developed GBS, for example ophthalmoplegia; (C) electrophysiologic tests showed conduction block, proximal block, absent F-waves, lost sensory potentials or other features typical of GBS, but these might be present only in one region of the body; (D) the CSF protein was elevated in a pattern and temporal course that were consistent with GBS; (E) the illness was acute or subacute and monophasic, with recovery that accorded with the typical course of GBS; and of course, (F) other causes of the syndrome, particularly myasthenia gravis, botulism, diphtheria and myelitis, were excluded with as much precision as possible. After seeing one or two examples of a variant, it became possible to pick them out of the larger group of GBS cases, to point out the differences to residents and other faculty, and thereby to redirect treatment.

Moreover, regarding the clinical utility of knowing these variants, they should be viewed from the perspective of Bayes' theorem. In the patients I see in a teaching hospital in Boston, GBS is so much more common than diphtheria and botulism that anything appearing to simulate these rarer diseases clinically is still likely to be a GBS variant. This is even true when there is pupillary paralysis and dry mouth. Myasthenia gravis only superficially imitates the cranial and the pharyngeal-cervical-brachial variant of GBS, but the distinction may become difficult when there is ptosis, as there often is.

These variants have taught me several things about GBS. First, every disease process has to start somewhere in the body. It is conceivable that there is inflammation in every nerve during acute GBS, but the concentration of this activity in a few regions gives rise to special topographic syndromes. Since some regional variants remain persistent, pure and profound, there may be special epitopes, which are distributed regionally in the peripheral nervous system, making the system immunologically far more complex than anticipated. Alternatively, the blood-nerve barrier may be opened up in some places more than others, and this allows the inflammatory response to concentrate in one region. In a casual article, I offered the observation that most people with cranial nerve and Fisher variants awakened with their first symptom, whereas more typical GBS came on during the active daytime hours. It is unlikely but interesting to think that the body position prior to the onset of an immune reaction could have an influence on areas of blood-nerve disruption. This is probably overly simplistic.

It comes as little surprise therefore that Hugh Willison and colleagues [6] and more profusely in publications from Yuki's lab, as summarized in their Medical Progress article [7], have found that auto-antibodies to certain gangliosides are disproportionately associated with certain variants but few are specific. The relationship between anti-GQ1-b antibodies and ophthalmoplegia and Fisher syndrome has been consistent, but others such as GT1a are perhaps less so. Except for GQ1b, few of these have made it into clinical practice.

Second, familiarity with the variants created a risk of the misuse of the availability heuristic [8]. I referred above to one glaring example of a patient with spinal epidural abscess that I initially mistook for GBS because of quadriparesis and *coup du poignard* between his scapulae. He was areflexic and was sent to our ICU for IVIg treatments, having had a lumbar MRI that was normal. I examined him in the middle of the night with my residents and he was in extreme pain in the back and in distal extremities. He'd been given considerable narcotics and was intubated for respiratory failure. I was able to wake him up enough to get him to mouth words, close his eyes and purse his lips, and I felt all of these movements were weak and that therefore the picture was consistent with GBS. An EMG the next morning showed multiple inexcitable nerves as well as absent late responses and our electrophysiologist gave a diagnosis

of GBS. We ordered the IVIg and went about our morning rounds. Later in the day, I asked to see the lumbar MRI since the patient was febrile and intermittently hypotensive. At the very upper edge of the image was a white shadow. It quickly became clear that this was the lower edge of a massive epidural abscess. I had put together the interscapular pain, with quadriparesis, areflexia, respiratory failure and a too easily confirmatory EMG, all as GBS. We made the diagnosis about 14 hours after our initial assessment and the patient went to the operating room but his spinal cord was already necrotic. At autopsy, there was a massive staphylococcal epidural abscess extending from the upper cervical through the upper lumbar cord that I recounted in an entire chapter of my book for the public on neurological problems, *Reaching Down the Rabbit Hole* [9].

This points out the problem of having too much experience with unusual clinical processes and not having the scepticism and simplicity of a medical student. It is reminiscent of the story of a middle-aged gentleman who was admitted to the hospital with severe back pain and progressive wasting. He was examined with every conceivable test and seen by the most skilled clinicians in the hospital. No diagnosis could be made and he died. At autopsy, there was an angiosarcoma of the spleen. When the chief of medicine later reviewed the chart, he noticed that a medical student had made the correct diagnosis! He called the student to his office to congratulate him and asked how he, a beginning student, could have made this diagnosis when every senior clinician missed it. The student responded, “What else causes back pain?” The GBS variants, therefore, have made me both a better and a worse clinician.

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16

My Top 10 GBS: Pit Stops in a Sandstorm

Thirugnanam Umapathi

Pit Stops in a Sandstorm

*Strong gusts.
Sands whipped
Eyes fail.
Spirits sag
Directions lost,
Weary of,
Weary from,
Pitfalls.*

*Pit stops!
To repose,
Re-Pose.
Convene, confer.
Invigorate and
Animate.
Ideational oases*

*I wonder ...
The destination's form*

1 Rag Dolls, No More

*Rows of weakened children.
Flaccid rag dolls.
Iron-clad lungs
Keeping afloat,
From near certain suffocation,
And an inevitable life of poliottic paralysis.
Oh, but ...
One child recovers!*

In 1859 Octave Landry published 'Note sur la paralysie ascendante aiguë' [1], in which he described with clarion precision 5 personal cases and 5 from the literature of acute ascending paralysis:

The main problem is usually a motor disorder characterized by a gradual diminution of muscular strength with flaccid limbs

and without contractures, convulsions or reflex movements of any kind....

One does not observe any symptoms referable to the nervous system.... The intellectual faculties are preserved until the end....

The weakness spread rapidly from the lower to the upper parts of the body with a universal tendency to become generalized....

The progression can be more or less rapid. It was eight days in one and fifteen days in another case.... When the paralysis reaches its maximum intensity the danger of asphyxia is always imminent.... In two cases, death occurred at this stage ... when the paralysis recedes it demonstrates the reverse of the phenomenon, which signaled its development.

...

On the day before dying of asphyxia the patient was lying quietly on his back and there was hollowing of the abdomen during inspiration and outward movement during expiration.

In 1916 Guillain, Barré and Strohl described 2 soldiers with an acute paralytic illness that was unlike poliomyelitis [2]. Besides weakness and slight sensory loss, they emphasized the loss of deep tendon reflexes. They also directed attention to the increased spinal fluid protein with normal cell count using the technique of lumbar puncture developed by Heinrich Quincke in 1891. The 2 patients had CSF protein of 2.5g/dl and 0.85g/dl, respectively, with normal cell counts. Both patients started improving about a month after the onset of illness.

2 To the Curious Incident of the Dog in the Night-time

Gregory (Scotland Yard detective): "Is there any other point to which you would wish to draw my attention?"

Holmes: "To the curious incident of the dog in the night-time."

Gregory: "The dog did nothing in the night-time."

Holmes: "That was the curious incident."

"Silver Blaze", Sir Arthur Conan Doyle, 1892

Eyes riveted lock-still.

Gait inebriated.

Tendons insensate.

"This is acute and follows an infection.

Note the paralyzed eyes.

And it all fades in 3 weeks.

What is missing?" ...

"The cells!"

"Unmask the culprit GBS"

Said Charles Miller (Holmes) Fisher

"There flees the criminal's accomplice, GQ1b! Catch him!" shouted Chiba (Watson).

Miller Fisher described 3 patients with acute ophthalmoplegia, ataxia and absent tendon reflexes that occurred acutely following an antecedent infection in 1956 [3]. These patients had minimal or no weakness. All 3 recovered spontaneously. One out of the 2 patients who had spinal fluid analysis had cyto-albuminogic dissociation. Fisher hypothesized a common etiopathological link between this disorder and Guillain-Barré syndrome (GBS). Chiba and colleagues subsequently showed the close association between Miller Fisher syndrome and anti-GQ1b antibody [4].

3 Once upon a Time in China

*Doctor McKhann visits,
Perchance the summer epidemic,
Of acute flaccid weakness.
His accessory Jack
Muses at this bizarre cyclical GBS.
Amidst excited shouts, "Axonal changes!"
Of Cornblath in the EMG room.
Jack demonstrates indeed,
Macrophages slip-sliding,
Below plump Schwann cells,
Devouring sweet-fatty axolemma.*

*The conspiracy of the corkscrew villain,
Campylobacter jejuni,
Exposed!*

Drs McKhann and Griffin led a team to China and delineated the AMAN subtype GBS in the early 1990s [5]. Eighty-eight of the 90 patients they saw had a pattern of disease characterized by reduced compound muscle action potentials with normal velocity and sensory-nerve action potentials on nerve-conduction studies. Two-thirds of the patients had serological evidence of recent *C. jejuni* infection (compared to 16% of village controls). Likewise a greater proportion of patients had IgG anti-GM1 antibodies [6]. In 6 axonal GBS cases that were autopsied, the pathological findings were Wallerian degeneration with macrophages within the periaxonal space that were surrounding or displacing the axons that had an intact myelin sheath. The pathological changes were subtle in some of the patients that died from severe paralysis, implying that functional abnormalities in the axolemma such as conduction block might be responsible for the paralysis [7,8]. This could also explain the relatively rapid recovery of some patients; the median time to regain the ability to walk 5 metres with assistance was 31 days in the AMAN patients, not dissimilar to those who had the demyelinating AIDP subtype [9].

4 Morpheus

*Morphs a plenty,
Forms, and in geography,
Blends and overlaps.
In core-essence: GBS.
Antecedence,
Nadir in weeks,
Proteinaceous-acellular spinal fluid.
And
Recuperating convalescence.*

Following the description of the Miller Fisher syndrome, other regional variants of GBS are being continuously recognized. Pharyngeal-cervical-brachial weakness [10], acute ataxic neuropathy [11], paraparetic GBS [12], facial diplegia [10], acute ophthalmoplegia [13], polyneuritis cranialis [14], acute oropharyngeal palsy [15], acute ptosis [12] and acute mydriasis [16] are some of the sub-types of GBS that have been described. They are usefully named after their cardinal features. The diagnosis of these regional variants can be challenging. The close association of some of these conditions with raised anti-ganglioside antibodies, such as anti GQ1b or GT1a antibodies, can aid in the diagnosis.

Tendons Frisky!

*Suppressed and absent.
That's how you are.
You do sometimes appear intact.
Occasionally florid,
And even flamboyant.
A trickster,
In the malady of axons.*

In 1984, Pryor and colleagues reported a patient who developed GBS after a *C. jejuni* infection with remarkably intact deep tendon reflexes [17]. In the series of AMAN patients from China, hyperreflexia was noted during the early phase of recovery in some patients [5]. Kuwabara and colleagues corroborated this observation in 13% of 54 consecutive GBS patients. Most had AMAN and had raised anti-GM1 antibodies [18]. Subsequently Yuki and colleagues demonstrated that up to 10% of GBS patients can have normal or exaggerated deep tendon reflexes during the entire course of illness [19].

5 Double Agent

*Boundaries apparently regimented
Walls, border-post, customs
Man and material separated.
Yet GBS, a resident of the PNS,
Spotted dallying in CNS.
Somnolence,
Babinski,
Bells,
SIADH,
Are guises he uses
In his forays*

Bickerstaff and Cloake described 3 cases of ophthalmoplegia associated with ataxia and clouding of consciousness in 1951 [20]. They drew attention to the similarities with GBS, even though there were unequivocal signs suggestive of ‘mesencephalitis and rhombencephalitis’. This report preceded Miller Fisher’s report on the ophthalmoplegia-ataxia-areflexia triad of the now-classic Miller Fisher syndrome. Remarkably, one of the 3 patients described by Miller Fisher did have drowsiness. Yuki and colleagues wrote the conclusive chapter in this saga in 1997, proving the spectral relationship between GBS and Bickerstaff [21]. They described the presence of anti-GQ1b antibody in Bickerstaff encephalitis, an antibody that by then had been well associated with MFS. This serendipitous discovery was made when they were attempting to distinguish a single case of apparent Miller Fisher syndrome that lapsed into a coma from Bickerstaff encephalitis.

6 Mimics

*O hell! What have we here?
A carrion Death, within whose empty eye
There is a written scroll! I'll read the writing.*

*All that weakens rapidly is not within the GBS fold^a;
Often have you heard that told:
Many a patient's life hath souled^a.
But my outside to behold:
GBS diagnoses do mimics enfold^a.
Had you been as wise as bold,
Young in limbs, in judgement old
Your answer had not been inscroll'd
Fare you well, your suit is cold.*

Murphy and colleagues described a patient that presented with acute flaccid weakness of his limbs associated with depressed reflexes that evolved over 3 weeks [22]. These features were consistent with a diagnosis of GBS. However, the presence of unilateral wrist drop, Horner's syndrome, normal cranial nerve examination, confusion, normal CSF and MR changes of central pontine myelinolysis were atypical for GBS. It became clear then that the patient had been drinking large amounts of beer and spirits for more than 15 years, and had stopped eating anything else for a week before admission. This was after an apparent upper respiratory infection (which further confused the clinical picture). Nerve conduction study showed an axonal sensorimotor polyneuropathy. The patient improved with high-dose thiamine and other vitamin supplementation. Although the diagnostic serum pyruvate and red cell transketolase were not tested (as patient had already received high doses of vitamins) the patient most likely had dry beriberi neuropathy that mimicked GBS.

*Bacchus beckons
High and higher.
Ceres grounds,
Cajoling to eat her grains and bread.
The call of Bacchus drowns her out.
Downed in spirits.
Hallucin-ens many,
Is that you darling, Mnemosyne?
Don't leave me.
I can't chase after you.
For I am losing my hands and feet*

Other pertinent GBS mimics are acute myelopathy, periodic paralysis, HIV seroconversion, hereditary neuropathy with liability to pressure palsy, treatment-related neuropathy of diabetes (previously termed 'insulin neuritis'), acute intermittent porphyria, primary meningeal lymphoma/carcinomatous meningitis, acute cryoglobulinemic neuropathy, Lambert-Eaton myasthenic syndrome and acute sensory neuronopathy.

7 The Long Campaign

*There is a plaque in me
It is everywhere,
Suffocating me with its Fab arms
Crushing me with the weight of its Fc pillars
Rid me of this enemy, please!
Battle cry! Bugle sounds!*

No! The steroid-sword is ineffective!

Warns Commander Hughes.

We need another weapon,

Lest the war is lost.

Voices let by Jack

Rally the charge.

Exchange the milieu!

Clean aliquots,

5 volumes 10 days,

Calls out the command.

Patients walk!

The Dutch's kinship of the Sea.

With their prescience

They say flood and wash away,

With pools of antibodies.

Patients walk!

The battle is won, but

The war ravages on.

Let's attack on both fronts.

Exchange and flood,

Cajole the French.

But patients walk the same.

The enemy complements the attack,

MAC is their key weapon.

Masked chimera commandos,

Half human half rodent,

Sneak in to liquidate MAC.

Can they shift the balance of the war?

Hughes and co-workers demonstrated unequivocally the ineffectiveness of corticosteroids, used in isolation, for the treatment of GBS [23]. Subsequently 2 seminal studies have established the role of plasma exchange [24] and intravenous immunoglobulin [25] in hastening the recovery of GBS patients, while another showed the lack of additional benefit of the combination [26].

Another promising therapeutic strategy is the inhibition of complement activity. Eculizumab is a humanized monoclonal antibody that blocks the formation of human C5a and C5b-9 and consequently interferes with action of membrane attack complex (MAC). This ultimately inhibits antibody-induced, complement-mediated neuropathic damage of GBS [27].

8 A Haiku in a Kabuki

Sialated ganglioside,

NOT LPS armoured C-jejuni

Halt antibodies!

The Penner 19 serotype (PEN 19) is an organism frequently isolated from GBS patients and rarely found in *C. jejuni* enteritis without GBS. In a case of AMAN that occurred after a bout of diarrhoea, Yuki and co-workers demonstrated elevated levels of anti-GM1 antibody and a 4-fold increase in anti-*C. jejuni*

antibody [28]. *C. jejuni* was cultured from a stool sample of this patient and identified as the Penner 19 serotype. The lipopolysaccharide capsule (LPS) of the isolated *C. jejuni* was purified and analysed. It showed binding activity of cholera toxin, which specifically recognizes the GM1-oligosaccharide. Analysis using gas-liquid chromatography-mass spectrometry showed that the purified LPS contained Gal, GalNAc and NeuAc. It was also shown that this oligosaccharide structure protrudes from the LPS core and its configuration is identical to the terminal tetrasaccharide of the GM1 ganglioside. This was the first study that demonstrated the feasibility of molecular mimicry between membrane component of nerve and an infectious agent, *C. jejuni*, that precedes GBS.

Kabuki (歌舞伎) dance

Sing (歌), dance (舞), skill (伎) mask villain

Seduced to fratricide!

9 Axonal Blocks

As Schwann cells wither away

Currents leak,

Signals bleak

Distal-nodes, indifferent, inexcited,

Wires silent ...

The celebrated demyelinating blocks.

Gangliosides attract trouble;

Antibodies to nodes and paranodes.

Squatting on sodium channel abodes.

Node-conduction fails,

Wires silent ...

The overlooked axonal blocks.

Capasso and colleagues described 2 patients with an acute motor neuropathy, intact reflexes, multiple conduction block and quick spontaneous recovery [29]. These patients had high titres of anti-GD1a and GM1 antibodies, and one had recently had a *C. jejuni* infection. Both recovered within a few weeks. This illness, acute motor conduction block neuropathy (AMCBN), is believed to be a subtype of GBS-AMAN. It is intriguing that it appears to be an acute equivalent of MMN, analogous at least nosologically to an AIDP-CIDP relationship. A subsequent report by Kokubun and colleagues demonstrated unequivocally the presence of conduction block in axonal GBS [30].

10 Man's Best Friend

In health and sickness you are my ally

What did we do to deserve your love and loyalty.

Wolves you may have been,

Threatening my father's sheep.

Tools you have become; and my buddy.

Count the ways that you aid me.

By yet another act of serendipitous sacrifice,

A helping paw to wipe away this scourge.

GBS too,

Your service leaves untouched.

In 1967 Cummings and Haas published the clinical features of 9 and the pathological findings of 5 dogs of various breeds with coonhound paralysis [31]. This is an acute paralytic illness of dogs now referred to as acute canine paralysis (ACP). The authors' attempt to draw parallels with GBS occurring in humans deserve to be quoted *ad verbatim*:

In both entities weakness begins acutely in the lower extremities and later ascends to involve the upper extremities and the cranial nerves. The illness reaches its peak within several days, at which time flaccid weakness is extreme and symmetrical, and some degree of respiratory embarrassment is present. Areflexia accompanies this loss of power. Sensory loss is mild and involves the acral parts of the extremities. Bladder and bowel function are unimpaired. Pyrexia due to the disease *per se* is absent. Recovery is slow, but good return of power is achieved within several months.

Distal paraesthesiae, a symptom undeterminable in the dogs, usher in the human illness in about half the cases.

Certain variations are common to both. The initial site of weakness may be the upper extremity.

Although recovery is typical, death may occur, generally due to respiratory failure

On the other hand, the degree of weakness may be quite mild, hardly preventing ambulation. Transient impairment of bladder and bowel function is not uncommon.

A difference between the canine and human syndromes is the lack of cerebrospinal fluid protein elevation in the dogs [although only 2 dogs had lumbar puncture].

Remarkably, the authors' description of the nerve conduction studies in these animals was consistent with an axonal neuropathy. Recently, Rupp and colleagues demonstrated the presence of anti-GM2 ganglioside antibodies in 14 out of 25 ACP dogs [32]. Only one control dog (out of 19 with non-neurological illness, and 15 with epilepsy) had anti-glycolipid antibodies. The authors also demonstrated GM2 localization in the abaxonal Schwann cell membrane of canine sciatic nerve. This raises the exciting possibility of ACP as a naturally occurring animal model for GBS.

Sand Gets into the Eye

*The bricked homes remain,
Walls pocked, tiles loose, leaking pipes.
Such was the vigour of the sandstorm.
But at least the houses stand,
Whilst many tents lie broken
Irreparably damaged.*

*The storm has shifted the sand into dunes.
I climb on one of them.
Standing on its shoulder,
The sand, as dunes,
Lets me see far and wide
The same sand, as storm,
A while ago,
Hampered my vision.*

*I re-start the trek
With enthused spirits.
Sand gets in my eye.
Reminder of my earlier tryst.
Blink! Blink!*

Gaze unwavering,
Fixed on the horizon, where
The clear blue sky embraces
Arms out-stretched in an arc of
Sensuous caress,
Conjugating with perfect geometric fit,
The curves of the hot naked desert sand.

A coital embrace
Consummation imminent.

Dedicated to my teachers (in chronological order), Drs S.C. Loong, Jack Griffin, Richard Hughes and Nobuhiro Yuki.

Acknowledgments to Dr Shermyn Neo for critique of prose, and the rest.

a Modified with implicit permission from William Shakespeare.

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17

My Top 10 Clinical Pearls in GBS

Kenneth C. Gorson

Many neurological disorders metaphorically resemble good movies, plays and books. Allowing for new characters (symptoms and findings), plot twists (a multitude of confounding and complicating factors that affect the clinical course), and occasionally surprising outcomes (unanticipated full recovery or death), the neurological story weaves a fascinating narrative with a beginning, middle and end. GBS is no different. The beginning of the GBS ‘movie’ is the myriad of possible clinical presentations and missed opportunities for accurate diagnosis; the middle represents the variable evolution of new symptoms and findings, and confounding medical complications in the hospital; and the end of the story can be viewed as the residual and often disabling features during recovery and plateau. Each segment poses unique challenges. Below are a few salient clinical pearls I have accumulated over the years.

The Opening Scene

Pain and Paresthesias in the Emergency Department

Missing GBS in the emergency department (ED) is common. The patient suddenly experiences acral paresthesias or numbness, and often back and radicular leg pain. He is understandably anxious and may lead a clinician to a diagnosis of panic disorder due to anchor bias, further compounded by the potential for gender bias when the afflicted individual is an otherwise healthy young woman. A cursory neurological examination is deemed ‘nonfocal’, and the patient is dismissed with a diagnosis of anxiety, hyperventilation syndrome, ‘neurasthenia’, or no diagnosis at all, and discharged home. Seldom is a neurological consultation considered in those without overt motor deficits. The patient returns (often to a different ED) days to a week later with progressive generalized weakness and ventilatory failure, and the diagnosis of GBS is established. McGillicuddy and colleagues have suggested that a majority of GBS cases are misdiagnosed at the first ED evaluation and delay may lead to a worse outcome [1]. *At one time the notion of ‘ascending paralysis’ as a hallmark of GBS may have been a useful oversimplification for teaching purposes, but the concept has outlived its utility.*

Fully one-third of patients have generalized weakness of the arms and legs at onset, and 15% have weakness spreading in a descending pattern. This does not include those patients with the Miller Fisher syndrome or pure sensory variants. Sadly, the view that ‘GBS equals ascending paralysis’ is steadfastly fixed in the minds of many clinicians to the exclusion of the other diagnostic features of GBS. For example, symptom onset is always acute, as the patient usually comments he has never experienced anything ‘quite like this before’, and although paresthesias and numbness may fluctuate, the symptoms are constant (not episodic), generally symmetrical, and, at least initially, distal to the wrists and ankles. The

discrepancy between prominent sensory symptoms and little or no objective sensory loss is a hallmark of early GBS. In contrast to panic disorder with hyperventilation, facial paresthesias are uncommon in GBS. In my experience, abnormal deep tendon reflexes are too often overlooked. In those without a pre-existing neuropathic disorder, generalized hyporeflexia, or isolated, symmetrically absent Achilles reflexes, is not normal in an otherwise healthy individual with acute neuropathic symptoms; GBS always should be considered.

Nothing above the Neck

Not infrequently a patient with rapidly progressive, flaccid, areflexic quadriplegia and sensory loss is transferred with 'GBS'. Neurology trainees sometimes come to premature diagnostic closure in such cases and initiate IVIg therapy. Although ophthalmoparesis, facial and oropharyngeal weakness, or ventilatory compromise are not requisite findings in severely affected GBS patients, *I always reconsider the clinical diagnosis when there are no cranial nerve findings in quadriplegic patients*. Acute flaccid paralysis and areflexia also may be seen in spinal shock below the level of the lesion. Acute transverse myelitis is the primary concern and my diagnostic rule always has been to obtain a gadolinium-enhanced MRI of the cervical and thoracic spinal cord. In addition to the absence of cranial nerve findings, clinical features that favour an acute myelopathy include complaints of rapidly progressive sensory loss in the legs extending proximally to the trunk, and early and prominent urinary retention or incontinence which is often overlooked, as virtually all patients now have urinary catheters placed upon ICU admission. A bladder ultrasound performed before catheter placement which demonstrates high post-void residuals is a useful clue. In acute thoracic transverse myelitis, there will be leg paralysis with bladder and bowel involvement and complete sparing of the upper limbs. In such cases excruciating upper- or mid-thoracic pain is common (*le coup de poignard*) and rare in GBS. Careful sensory examination invariably shows a spinal sensory level, and in high cervical lesions this may not be evident unless sensory testing extends to the clavicle; pinprick testing also should be performed just lateral to the spinous processes to avoid confusion with escutcheon sensory loss on the anterior trunk, which may be present in GBS and acute ganglionopathies.

What Does Not Belong

Virtually all major reviews of GBS provide a regurgitation of a long list of diseases that mimic the disorder. This usually falls into two basic categories: the first are the acute neuropathic disorders that share the rapid progression of a paralytic peripheral neuropathy, but are so strikingly rare as to be discussed almost exclusively for pedagogic purposes and then promptly dismissed. Such entities include diphtheria, arsenic and thallium intoxication, acute intermittent porphyria, etc. My mentors have undoubtedly encountered a case or two of these oddities during their half-century careers, but I personally have never seen a case of diphtheric demyelinating polyneuropathy, or for that matter arsenic or thallium poisoning. Additional examples are provided in [Table 17.1](#). These exceptionally rare conditions have less in common with classical and even atypical forms of GBS due to the panoply of non-neurological features, and erudite educators talk more about them than actually see them.

Table 17.1 Selected examples of 'mimics' in the differential diagnosis of Guillain-Barré syndrome (GBS)

| Disorder | Clinical features inconsistent with GBS |
|---|--|
| Non-poliovirus poliomyelitis (e.g., West Nile virus) | Flu symptoms, fever, myalgias, stiff neck, asymmetric painful limb paralysis, no sensory features, CSF pleocytosis |
| Diphtheria | Fever, sore throat, cervical adenopathy, early paralysis of the palate, delayed limb weakness |
| Arsenic poisoning | Acute gastroenteritis with nausea, vomiting, diarrhoea, desquamating skin rash, prominent neuropathic pain, mental status changes |
| Thallium poisoning | Acute painful neuropathy, alopecia, gastrointestinal symptoms, altered mentation |
| Porphyria | Odd sensory patterns ('bathing trunk' and 'arm band' sensory loss), abdominal pain, mental status changes with prominent psychiatric features, triggered by medications, seizures, possible family history, axonal loss pattern on NCS |
| Botulism | May occur in outbreaks, prominent gastrointestinal symptoms, early oculobulbar findings, very early respiratory failure, disproportionate to limb weakness, presynaptic neuromuscular transmission abnormalities on repetitive stimulation |
| Acute myasthenia gravis | Pure motor, prominent oculobulbar features, fluctuating weakness, pathological fatigability, post-synaptic neuromuscular transmission abnormalities on repetitive stimulation |
| Acute fulminant myopathies (polymyositis, acute necrotic myopathy, critical illness myopathy) | Muscle pain, no sensory features, elevated creatine kinase levels, myopathic EMG |
| Basilar artery stroke | Altered cognition, central eye signs, bilateral asymmetric hemiparesis, hyperreflexia, limb ataxia |

The second category of disorders discussed in the context of GBS is non-neuropathic diseases causing acute, rapidly progressive weakness. Conditions other than transverse myelitis include basilar artery thrombosis, myasthenia gravis, botulism, tick paralysis, periodic paralysis, acute fulminant myopathies, etc. (see [Table 17.1](#)). These conditions should be considered when accompanied by the appropriate associated symptoms or clinical findings, but the typical GBS patient usually does not require routine evaluation to exclude these disorders. Two of my mentors, Louis Caplan, MD, and Allan Ropper, MD, frequently espoused 'Fisher's rules' on teaching rounds, reflecting the clinical genius of their mentor, C. Miller Fisher. Rule 5 has suited me well in assessing patients with GBS: "In arriving at a clinical diagnosis, think of the 5 most common findings (historical, physical findings, or laboratory) found in the disorder—if at least 3 are not present, the diagnosis is likely to be wrong" [2]. I believe the converse is also true: If there are too many features that do not belong, consider an alternative disorder. For example, the observations of a fever at onset of symptoms, prominent gastrointestinal or other organ dysfunction, psychiatric or cognitive impairment, seizures, skin or hair changes, central nervous system findings, multifocality or prominent asymmetry, pain disproportionate to sensory findings, CSF pleocytosis (> 50 white blood cells), and electrodiagnostic findings indicative of a primary axonopathy (allowing for axonal forms of GBS), neuromuscular junction disorder, or myopathy all warrant reconsideration of a

The Plot Twists

How Electrodiagnostic Studies and Spinal Fluid Analysis Help and Hurt

The value of electrodiagnostic and cerebrospinal fluid (CSF) studies are covered in greater depth elsewhere in this volume, such that only a few comments are provided. Both tests are crucial objective measures that establish the diagnosis in the majority of GBS patients. The anticipated findings are acquired demyelination on nerve conduction studies (NCS) and cyto-albuminological dissociation on CSF analysis, and yet these studies also are helpful when neither is present or other unexpected abnormalities are found that point to an alternative diagnosis. For example, in paraparetic patients where the only NCS abnormalities are prolonged or absent late responses limited to the lower extremities, other conditions that affect the nerve roots or motor neurons must be considered, such as neoplastic cell infiltration, sarcoidosis, Lyme disease, and rarely, acute infectious polyradiculitis due to herpes zoster or simplex, HIV and West Nile virus infection. In such scenarios there is usually a CSF pleocytosis in addition to an elevated protein level. Conversely, in a validation study of the Brighton criteria for GBS, Fokke and co-workers [3] observed that no GBS patient had a pleocytosis greater than 50 cells/ μl , but approximately 15% had a 5 to 50 cells/ μl ; therefore, although other conditions should be thoroughly investigated, a moderate pleocytosis does not exclude GBS in the appropriate clinical context. Similarly, finding an elevated CSF protein concentration is dependent on the timing of the lumbar puncture. Approximately one-third of otherwise classic GBS patients have a normal CSF protein level during the first week of symptoms, *such that a normal CSF analysis does not exclude GBS*.

There are NCS findings that mimic GBS, and atypical cases of GBS may lack classical demyelinating abnormalities. Multiple conduction blocks in named motor nerve territories correlate with clinical weakness in GBS, but also may be an early finding in acute mononeuritis multiplex due to systemic vasculitis. When the EMG shows normal sensory potentials and low or absent motor amplitudes consistent with a motor axonopathy, the acute motor axonal neuropathy (AMAN) variant of GBS is likely, but West Nile virus infection and tick paralysis (especially in children in appropriate geographic areas) also should be considered. Acute ataxic and pure sensory variants of GBS usually demonstrate absent or reduced sensory potentials in the limbs without demyelinating findings, and sometimes are confused with acute ganglionopathies due to paraneoplastic neuropathy or Sjögren's syndrome. Fokke and colleagues have shown that *40% of GBS patients may not fulfil diagnostic criteria for demyelination on the initial study*, and approximately 1% of patients have a normal NCS [3]. Sometimes this occurs because too few nerves were sampled, and Albers and associates have suggested that more extensive testing detects more demyelinating abnormalities [4]. In others, electrophysiological abnormalities evolve as the condition advances and diagnostic criteria are fulfilled upon follow-up study.

When to Intubate?

GBS patients are at high risk for developing medical complications during the course of their illness, most commonly pneumonia. As weakness advances, it sometimes can be difficult to determine the exact cause of progressive respiratory decline and the indication for intubation. In those with developing pneumonia or sepsis, there is overt dyspnoea with tachycardia, findings on pulmonary examination, chest X-ray infiltrate and hypoxia on arterial blood gas monitoring. Intubation in these patients sometimes may

be avoided with proper antibiotic therapy and supplemental oxygen.

In contrast, those with progressive ventilatory failure due to GBS have tachycardia without early dyspnoea, their lung fields are clear, and chest X-ray and arterial blood gases are normal. *The utility of sequential measurement of respiratory mechanics (vital capacity, maximal inspiratory force) cannot be over-emphasized.* Estimated normal vital capacity varies as a function of age, gender and height, but a reasonable approximation is 60 cc/kg. With progressive weakness of the diaphragmatic and intercostal muscles there is a steady decline of the vital capacity, and at 20 cc/kg the patient may look reasonably well but should be electively intubated to reduce the risk of aspiration, prevent rapidly progressive ventilatory failure with retention of carbon dioxide, and prevent hypoxia eventually leading to a respiratory arrest. This is generally the reverse sequence of events that occurs in most patients with *parenchymal* lung disease. However, there are useful clinical clues that predict *ventilatory* failure. Lawn and colleagues have shown that there seems to be a correlation between rapid progression of GBS (e.g., quadriplegia evolving over a few days), progressive weakness of the neck flexors on bedside testing, bifacial or tongue weakness, bulbar impairment, and impending ventilatory failure requiring intubation [5].

When in doubt, intubate. Intubate early, in a controlled situation, preferably during the day, when the staff anaesthesiologist may leisurely stroll to the ICU and the anxiety of the patient, family and medical staff are manageable. I have heard of innumerable cases of intubation disasters: The patient initially looks fine on a floor bed, respiratory mechanics and other clinical signs are not followed properly, and hours later the next 'neuro check' finds the patient cyanotic and not breathing. A code is called, followed by crash intubation (usually by a less skilful anaesthesiologist-in-training) and the requisite ventilatory associated pneumonia follows over the next 48 hours. This scenario invariably occurs in the middle of the night on a weekend, but on occasion I have seen it during the day if the medical caregivers are not attentive.

Dysautonomia from GBS, and Attention to Medical Management

Dysautonomia is common in GBS patients, particularly resting tachycardia that remains invariant despite blood pressure fluctuations. These features tend to occur early during an ICU stay and are concordant with rapidly progressive ventilatory failure and quadriplegia. However, in a non-ventilated patient who has been in the ICU for many days or weeks, with a sudden onset of otherwise unexplained tachycardia with respiratory failure requiring intubation, or with worsening tachycardia and respiratory distress in an intubated patient, pulmonary embolus is the leading consideration. A knee-jerk attribution to 'worsening dysautonomia' or 'ventilatory failure due to late progressive GBS' should be avoided until other medical explanations have been excluded. *It is the assiduous attention to the details of medical management in the ICU that leads to better outcomes in GBS.*

New Onset Paralysis in ICU Patients

Over the years I have had frequent consultations for paralyzed patients in ICU, to 'rule out GBS'. These patients were critically ill and ventilated with multi-organ failure. Most were heavily sedated and received paralytic agents to facilitate ventilation. These cases looked like severe GBS; there was severe, generalized, flaccid symmetrical weakness of the limbs with areflexia and sensory loss. Bolton and colleagues clarified our thinking with their seminal work defining critical illness polyneuropathy (CIP) and distinguished this condition from GBS acquired in the ICU [6]. In contrast to GBS, these patients are

neurologically intact upon ICU admission and weakness develops later in the hospital course. The NCS show a primary axonopathy without demyelinating features and the spinal fluid protein concentration is normal. Another related paralytic syndrome, critical illness myopathy, has been associated with the use of corticosteroids and neuromuscular blocking agents. Unlike most myopathies which have a predilection for proximal muscles, distal and proximal muscles may be affected equally, and occasionally distal weakness predominates. Preserved sensation helps to differentiate critical illness myopathy from polyneuropathy, but both can occur simultaneously and confound the clinical evaluation. Most critical care physicians are now aware of these neuromuscular syndromes and are therefore less apt to confuse them with GBS. One should be mindful that genuine GBS very rarely develops in the ICU, such as after a severe viral infection or surgery.

Does CIP contribute to the secondary axonal loss that occurs in severely affected GBS patients in the ICU? In my experience this does not appear to be the case. Ho and co-workers at our facility found that of those GBS patients in the ICU who had follow-up NCS, 68% developed an axonal pattern on the second study, and although their prognosis was less favourable than those with a persistent demyelinating pattern (greater weakness, disability, and prolonged hospital stay), there was no relationship between an axonal pattern and frequency of sepsis, multi-organ failure or Apache II scores [7].

Recovery and Relapse

The characteristic course of GBS is progression to nadir over 4 weeks, then a stable plateau phase for days, weeks or months, followed by a recovery phase for weeks to years. During the plateau or early recovery phase, approximately 10% of patients relapse with recurrent GBS symptoms and findings in otherwise medically stable patients. This is termed a treatment-related fluctuation (TRF), and most respond to retreatment with IVIg or plasma exchange and resume an otherwise uncomplicated recovery. TRFs should be distinguished from transient worsening triggered by a medical complication, e.g., pneumonia, urinary tract or other infection, bowel obstruction, pulmonary embolus, etc. *In such circumstances treatment should be directed at the medical problem, not more immunotherapy.* These patients recover once the medical issue is resolved.

There are occasional patients who have multiple relapses in the weeks that follow nadir without explanation. The first and second relapses might be attributed to TRFs, but by the third relapse, it becomes clear something else is happening. These patients typically respond to repeated courses of IVIg or plasma exchange, but recovery is not sustained. Eventually it is apparent, *but only in retrospect*, that these patients have a variant of chronic inflammatory demyelinating polyneuropathy (CIDP) manifest as acute relapsing GBS. Their course is punctuated by acute, rapidly progressive relapses of recurrent GBS (in contrast to most CIDP patients that progress slowly over months). This CIDP pattern has been termed acute-CIDP (A-CIDP) by Ruts and associates [8]. Our Dutch colleagues have found that it occurs in approximately 5–16% of patients presenting as GBS. This variant can be distinguished from those with GBS and TRFs by the presence of 3 or more relapses, or a relapse that occurs after 8 weeks. A-CIDP patients are less severely affected, have less frequent cranial nerve findings and respiratory compromise, and have slower nerve conduction velocities that are more characteristic of CIDP. Unfortunately, none of these features are useful contemporaneously to predict which GBS patient with a TRF has A-CIDP. In my GBS patients useful clinical clues associated with A-CIDP include *the development of any new neurological findings after 4 weeks*—for example, new weakness in muscles previously unaffected, new sensory abnormalities, or the onset of cranial nerve involvement not evident at nadir. Once the diagnosis of A-CIDP has been established, the patient should be treated according to published treatment protocols

for CIDP. It also should be remembered that in contrast to typical CIDP, these patients relapse quickly and may decompensate from normal to bedbound within days.

The Final Act

Late Fluctuations

Hickam's dictum ("Patients can have as many diseases as they damn well please") also applies to recovered GBS patients. Such individuals are understandably anxious that any trivial change in their residual symptoms represents an impending recurrence. However, fluctuations of paresthesias and numbness in otherwise stable, recovered patients are more likely due to unrelated health issues, such as dehydration, infections, sleep deprivation, or stress, and promptly resolve when the inciting factors have been properly addressed. In contrast, patients are entitled to accumulate other neurological disorders simulating their original GBS symptoms, such as a superimposed diabetic or B12 deficiency-related polyneuropathy or cervical spondylotic myelopathy. These secondary conditions usually evolve slowly and are clarified with careful clinical assessment and judicious testing. *One should be mindful not to routinely attribute new, progressive neurological symptoms to a remote history of GBS.* Lastly, Kuitwaard and colleagues have observed that there are the unlucky few, as many as 6%, who have recurrent GBS [9]. Recurrences range from 2 to 7 attacks over years or decades, with clinical features similar to the first presentation and usually triggered by an infection. Repeated attacks of GBS tend to occur in younger individuals and those with the Miller Fisher variant, and are usually milder with a shorter recovery compared to the first episode. The cause for recurrences remains unknown but there must be genetic and immunological factors unique to those individuals.

The Forgotten Patient

I have become aware of the enormous problem of a lack of long-term follow-up for GBS patients after hospital discharge due to my involvement with the International GBS Outcomes Study (IGOS). This may be related to the nature of medical care in the United States, where patients are often transferred great distances to medical centres with expertise in the care of patients with GBS and which precludes subsequent outpatient follow-up at the treating facility. However, my personal observations suggest it also may be a phenomenon of 'the forgotten patient' once the patient has been stabilized and transferred elsewhere. *Many have persistent symptoms, even if they have made a 'full recovery' by our limited metrics.*

Pain is common in the recovery phase, characterized as neuropathic burning or lancinating pains in a length-dependent distribution. Persistent fatigue causes substantial disability. Afflicted individuals may have normal strength but have great trouble completing routine activities of daily living. Severely affected patients have fixed motor deficits (hand weakness, foot drop) that may benefit from orthotics, assistive devices, and continued physical or occupational therapy. Many have depression and anxiety that have not been treated after hospital discharge. Virtually all patients have questions about the nature of the disease and their prognosis for further recovery. Support groups such as the GBS/CIDP Foundation International are tremendously helpful. GBS patients may continue to improve slowly years after onset. *It is these patients who benefit most from follow-up outpatient evaluations that provide practical treatment interventions, thoughtful guidance, support and hope.*

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18

The Spectrum of GBS and CIDP

Krista Kuitwaard

Introduction

Guillain-Barré syndrome (GBS) is an acute polyneuropathy that reaches its maximum severity (by definition) within 4 weeks, whereas the maximum severity in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is reached after at least 8 weeks. Differentiating between GBS and CIDP can be difficult as it relies on arbitrary clinical criteria and this separation may be mainly nosological. Preceding infections, involvement of cranial nerves or weakness of respiratory muscles are more often encountered in GBS than in CIDP, but they can occur in both. Although GBS is generally a monophasic disorder, treatment-related fluctuations (TRFs) and recurrences occur. CIDP usually runs a progressive or relapsing course but may be monophasic, resembling GBS and requiring only a single course of treatment. Additionally, CIDP patients with an acute or subacute onset, resembling GBS, exist.

GBS and CIDP in the Same Patients

A 46-year-old man developed weakness as well as numbness in his extremities over the course of one year. He responded well to IV immunoglobulins (IVIg) once every 2 weeks. Several attempts to reduce the dose resulted in an immediate deterioration. After 6 years the IVIg could be stopped and he remained in remission. Twelve years later, after an episode of diarrhoea, he developed a tetraparesis and facial palsy within 48 hours. He was treated with IVIg and required artificial ventilation for 2 months. He had a near-complete recovery and no new episodes occurred.

Kuitwaard K, et al. Individual patients who experienced both Guillain-Barré syndrome and CIDP. *The Journal of the Peripheral Nervous System*, 2009

Four separate patients are described as having separate episodes of both GBS and CIDP [1]. All fulfilled the clinical and diagnostic criteria for both GBS and CIDP [2–3]. One patient had CIDP and years later developed a monophasic episode of GBS with respiratory failure. Two patients had GBS and later developed CIDP, and 1 patient was described as having had recurrent GBS with TRFs that evolved into CIDP. Although such case series are rare, this one suggests that GBS and CIDP may constitute a continuum or that there are common host factors that influence susceptibility to these disorders.

Recurrent GBS

A 42-year-old woman developed progressive weakness and sensory disturbances in less than 4 weeks, and complained of

severe pain. Symptoms were maximal within 4 weeks. Seven years later she had similar symptoms after a bout of flu, that developed in less than 2 weeks. Sixteen years thereafter she had another episode after a flu-like infection that developed in 1 week. Five years after the previous episode she developed progressive tetraparesis over a few hours after a bout of diarrhoea, for which she was treated with IVIg. She needed artificial ventilation and had autonomic dysfunction complicated by an asystole. She was successfully resuscitated and eventually discharged to a rehabilitation centre.

Wijdicks EF, Ropper AH. Acute relapsing Guillain-Barré syndrome after long asymptomatic intervals. *Archives of Neurology*, 1990

Five patients that recovered from an initial episode of GBS were described, who had an acute relapse 4 to 36 years later [4]. The clinical features (such as respiratory insufficiency and facial weakness), return of their reflexes and long asymptomatic intervals distinguished their illnesses from CIDP. The patients all had similar antecedent infectious diseases as well as similar symptoms each time. Asbury [5] speculated that residual inflammation may remain between episodes, accounting for the similar symptoms during every episode, although the long asymptomatic intervals make this hypothesis less likely. This case series suggests that some patients have an immunological susceptibility that makes them prone to develop GBS.

Grand'Maison F, et al. Recurrent Guillain-Barré syndrome. Clinical and laboratory features. *Brain*, 1992

The features of recurrent GBS were defined and their clinical characteristics were compared with those described for chronic relapsing polyneuropathy [6]. Recurrent GBS was defined as 2 or more episodes of acute idiopathic demyelinating polyneuropathy with an onset < 8 weeks (fulfilling the criteria for the diagnosis of GBS [2]) followed by a complete or near-complete recovery. Because the authors found a progressive phase of < 4 weeks too restrictive, it is likely that some of these patients had CIDP with a subacute onset. Over a time period when 270 monophasic GBS patients were admitted, 12 recurrent GBS patients with a total of 32 episodes (1–6 recurrences) were identified. The mean age at onset of the initial episode was 28.9 years and the mean interval between episodes 9.7 years. In 67% of patients an infection was noted preceding the first episode. All patients were asymptomatic or had mild symptoms between episodes. CSF protein level was normal or only mildly elevated when obtained within 1 week of onset of the recurrence or in the recovery phase. In all patients the protein level was elevated when CSF was obtained more than 1 week after onset of symptoms during a symptomatic phase. One of these 12 patients had several pregnancy or post-partum related triggers. In patients with the Miller Fisher variant, the presence of ophthalmoparesis and ataxia was constant from episode to episode, although the nature of the preceding infection or trigger tended to differ. The time to reach maximum deficit (nadir), the disability at nadir and the time between recurrences varied considerably and unpredictably between episodes. It was mentioned that it remains unclear whether recurrent GBS and chronic relapsing polyneuropathy represent different conditions.

Treatment-Related Fluctuations in GBS

A twenty-year-old man complained of muscle aches after a flu infection. Two days later he had tingling in his limbs. At the general practitioner's office he fell off the examination couch and could not get up by himself. At hospital admission he had tetraparesis and areflexia. CSF showed a normal protein level. He was treated with IVIg and muscle strength improved quickly thereafter. Just a few days later he developed a bilateral facial palsy and muscle strength decreased, and he was successfully re-treated with another IVIg course.

Ropper AH, et al. Limited relapse in Guillain-Barré syndrome after plasma exchange. *Archives of Neurology*, 1988

Although relapses in patients who improved after plasma exchange had been reported earlier, the first detailed description of 10 GBS patients with a relapse after plasma exchange was from the Boston group [7]. Ten out of 94 GBS patients (10%) showed mild to moderate worsening after an initial improvement following plasma exchange. Eight of these patients were treated with another course of plasma exchange, which was followed by clinical improvement. Although the authors mention that this improvement could have been the natural course, the beneficial effects of re-treatment were likely. During follow-up none of these patients developed CIDP. It was hypothesised that early start and cessation from treatment may lead to continued production of a pathogenic factor. This paper showed that about 10% of GBS patients can have an early relapse after successful treatment, currently defined as treatment-related fluctuations (TRFs).

Kleyweg RP, van der Meché FG. Treatment related fluctuations in Guillain-Barré syndrome after high-dose immunoglobulins or plasma-exchange. *Journal of Neurology, Neurosurgery & Psychiatry*, 1991

As TRFs were described in GBS patients treated with steroids it seemed plausible that these fluctuations would also occur after treatment with IVIg. This paper showed that these TRFs were not treatment specific [8]. A TRF was defined as

1. Improvement in functional score (GBS disability scale) of ≥ 1 grade or improvement >5 points on the MRC sum score within 4 weeks, followed by a decrease in the MRC >5 points or a worsening of ≥ 1 grade in functional score or:
2. Stabilization of the clinical course for more than 1 week followed by a worsening in MRC sum score of >5 points or at least 1 grade in functional score.

In the Dutch GBS trial, high-dose IVIg was compared with plasma exchange. Fourteen out of 147 GBS patients showed a TRF (10%), a percentage similar to what has been found by Ropper [7]. Of these 14 patients, 6 were treated with plasma exchange and 8 with IVIg. Six received another treatment course and 4 received multiple courses. All patients that were re-treated showed an improvement or stabilization after treatment. Four patients were not re-treated for no specific reason and still showed a spontaneous improvement after the relapse, indicating that not all TRF patients need repeated treatment. Although not formally investigated, re-treatment of TRF patients is generally recommended [9]. The hypothesis is that the pathogenic process, suppressed by treatment, is still active or reactivated after treatment. An explanation for the fact that not all patients show TRFs might be that some are treated earlier in the disease course when they are still in the active disease phase. Another explanation could be that some patients have a longer active disease course or more prolonged immune attack than others, requiring a higher dose or longer treatment period.

Acute and Subacute CIDP

A 52-year-old woman developed sensory disturbances after a flu infection. Two days later she was unable to walk. Maximum disability was reached in 6 days. In the period thereafter she had several exacerbations needing IVIg treatment, and was treated subsequently with IVIg once every month for the next 7 years.

Hughes R, et al. Subacute idiopathic demyelinating polyradiculoneuropathy. *Archives of Neurology*, 1992

Seven cases with a subacute idiopathic demyelinating polyradiculoneuropathy (SIDP) were described as having a monophasic episode of progressive weakness over the course of 4–8 weeks [10]. Three patients reported a respiratory infection before onset of neurological symptoms. None of these patients had autonomic dysfunction or required artificial ventilation. All patients clearly responded to prednisone or showed a spontaneous recovery. Six out of 7 patients had an increased CSF protein level. Patients had a predominantly motor polyradiculoneuropathy of both proximal as well as distal muscles and were relatively mildly affected. In the spectrum of GBS and CIDP, SIDP occupies a middle position.

McCombe PA, et al. Chronic inflammatory demyelinating polyradiculoneuropathy. *Brain*, 1987

In this report the clinical features, course and electrophysiology is described from 92 CIDP patients [11]. Sixty patients (65%) had a relapsing course, whereas 32 patients (35%) showed a progressive or monophasic course. CIDP patients with a relapsing course had a significant earlier age of onset. The disability of the relapsing patients was similar to that of the non-relapsing ones. Twenty-nine patients (32%) reported an illness or vaccination in the 6 weeks prior to onset, which is considerably lower than in GBS, where two-thirds reported such a trigger. They found a rapid rate of onset in 15 patients (16%). These patients reached their nadir within 4 weeks. However, they were distinguished from GBS by their subsequent relapsing or progressive course. The authors excluded recurrent GBS patients.

Molín J, et al. Acute clinical onset chronic inflammatory demyelinating polyneuropathy in a dog. *Muscle & Nerve*, 2011

A 5-year-old Rottweiler dog was evaluated for an acute-onset tetraparesis and hyporeflexia developing over the course of 1 week [12]. Electrophysiology showed clear evidence of demyelination. Electromyography showed fibrillations as well as positive sharp waves in all muscles. The clinical findings were fully compatible with acute canine idiopathic polyneuropathy (ACIP). Euthanasia was performed because of rapid deterioration. On postmortem examination, severe enlargement and demyelination of the cervical nerve roots was seen. Pathology showed hypertrophic changes with formation of onion bulbs, and hypomyelinated fibres indicating chronic de- and remyelination. Furthermore, other chronic signs were found. The owner of the dog did not recall any previous episodes and the diagnosis of acute-CIDP (A-CIDP) was made. This report illustrates that differentiating acute from chronic forms of inflammatory polyneuropathy on clinical characteristics can be difficult not only in humans but also in animals. Differentiating between the two is important because corticosteroids are not effective in the acute form either in humans or in animals.

Differentiation between A-CIDP and GBS with or without TRFs

A 33-year-old man developed progressive weakness and paraesthesia in his limbs over the course of 1 week. Three days later he was unable to walk. He improved after IVIg, but 9 days later he developed facial palsy and ophthalmoplegia and was again treated with IVIg. He became tetraplegic and needed artificial ventilation. Initially it was thought he had TRFs, but after 5 exacerbations, all rapidly responding to IVIg, a diagnosis of acute-onset CIDP (A-CIDP) was made.

Ruts L, et al. Distinguishing acute-onset CIDP from fluctuating Guillain-Barré syndrome: a prospective study. *Neurology*, 2010

Distinguishing between GBS-TRF and A-CIDP early in the course of the disease can be important because prognosis and treatment strategy can be different. Out of 164 GBS or MFS patients enrolled in a prospective study, 16 patients had 1 or more TRFs (10%) [13]. The definition of a TRF or exacerbation was similar to the one used by Kleyweg and colleagues [8]. Eight patients initially diagnosed as GBS appeared to have A-CIDP (5%). The first TRF always occurred within 8 weeks from onset of weakness, and most TRFs occurred within 4 weeks. None of the GBS patients had more than 2 TRFs. Patients with GBS-TRFs were more severely affected compared to A-CIDP patients at all time points.

Patients having A-CIDP were less likely to have cranial nerve deficits than GBS-TRF patients (13% vs. 69%, $p = 0.03$). A-CIDP patients more often showed CIDP-like electrophysiologic abnormalities and none of the A-CIDP patients needed artificial ventilation. GBS patients with TRFs were more severely affected and more often had sensory disturbances compared to GBS patients without a TRF. The CSF protein level did not differ between A-CIDP and GBS-TRF patients. Most A-CIDP patients did not fulfil the electrophysiological criteria for CIDP [14]. Signs of axonal damage are rare in A-CIDP but can be seen in more than half of GBS-TRF patients. Patients without cranial nerve dysfunction that remain able to walk independently and that show CIDP-like abnormalities at electrodiagnostic studies are more likely to have A-CIDP. This paper showed that the diagnosis of A-CIDP should be considered when a 'GBS' patient deteriorates beyond 8 weeks from onset or when an exacerbation occurs 3 times or more.

Dionne A, et al. Clinical and electrophysiological parameters distinguishing acute-onset chronic inflammatory demyelinating polyneuropathy from acute inflammatory demyelinating polyneuropathy. *Muscle & Nerve*, 2010

Clinical and electrodiagnostic records were reviewed to look for early predictors to distinguish A-CIDP from GBS. Electrodiagnostic studies were included when these had been performed within 4 weeks of onset. The authors compared 15 A-CIDP patients with 30 GBS patients [15]. Although sensory symptoms were reported equally in both groups, A-CIDP patients more often had prominent sensory signs. Sensory ataxia was more likely in A-CIDP patients as compared to GBS patients (53% vs. 3%, $P < 0.001$). Marked impairment of vibration sense as well as abnormal pinprick sensation were both statistically significantly more likely in A-CIDP patients. Autonomic disturbances, facial weakness and the need for artificial ventilation are unlikely in A-CIDP. A sural-sparing pattern in electrophysiology was not helpful in differentiating between A-CIDP and GBS. Although none of clinical parameters are pathognomonic for either GBS or A-CIDP, when prominent sensory signs are found early one should be aware of A-CIDP, and close follow-up is needed to check whether long-term treatment is required.

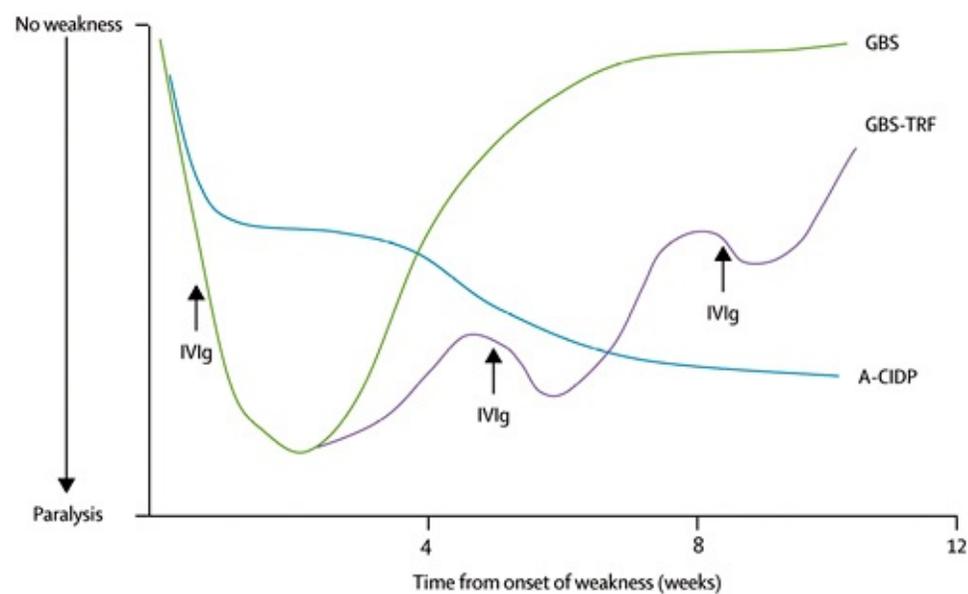


Figure 18.1 The spectrum of GBS and CIDP. Reproduced with permission from Elsevier.

IVIg = treatment with a course of IVIg (2 g/kg bodyweight) over 2–5 days

TRF = treatment-related fluctuation; A-CIDP = acute onset CIDP

Conclusions

Most patients clearly fit either the diagnostic criteria for GBS or CIDP, but some individual patients may have separate episodes of both GBS and CIDP. CIDP can have an acute or subacute onset, and some GBS patients have recurrent episodes of GBS or experience TRFs (Figure 18.1). On the other hand there are CIDP patients with a single monophasic course requiring only one IVIg course. These cases indicate that they can be considered as part of the whole spectrum of inflammatory demyelinating polyneuropathies instead of separate entities. The fact that some patients may have recurrences of GBS or both GBS and CIDP also suggests that some individuals are more susceptible to developing these inflammatory demyelinating disorders. Distinguishing between GBS-TRF and A-CIDP can be important from a treatment perspective, because once it is clear that a patient does not have GBS, but A-CIDP, regular IVIg treatment or a switch to steroid treatment has to be considered. We personally know A-CIDP patients who were initially diagnosed as GBS-TRF and treated with IVIg without an effect who showed a spectacular improvement after steroids when the diagnosis of A-CIDP was made.

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19

Guillain-Barré Syndrome in Children

Joyce Roodbol, Marie-Claire Y. de Wit and Bart C. Jacobs

First Presentation

In 1924, 8 years after the name-giving publication of Guillain, Barré and Strohl, a study of the first child with the clinical presentation of GBS was published by Mettel, a paediatrician from Michigan (USA) [1]. In the following years more cases of children with GBS were reported, most notably in 1937 by Hecht, a paediatrician from Baltimore [2]. Hecht described 7 children with a flaccid paralysis attributed to “acute infective polyneuritis” in detail [2], of which 6 children showed a clinical course typical for GBS. Hecht concluded that this illness is often preceded by a respiratory tract infection and that the overall prognosis in children is good without permanent paralysis, although some children may develop respiratory failure or die in the acute stage of disease.

Incidence

The reported incidence of GBS in children is variable, and may differ between age categories and countries of residence. Some of these studies are based on the WHO surveillance of poliomyelitis in children <15 years old. Probably one of the best estimates of the incidence rate of GBS in children in a Western country is the surveillance made in Finland based on nationwide discharge data from 1980 until 1986. The reported mean annual incidence rate was 0.38 per 100,000 children under 15 years old (95% confidence interval 0.25–0.56 per 100,000 per year, mean population at risk during this period 1.02 million). Remarkably, in a similar study conducted of children from Bangladesh under 15 years old the incidence ranged between 1.5 and 2.5 per 100,000 children [3]. This much higher frequency of GBS in Bangladesh may be related to the higher rate of exposure to infections compared to the rate in high-income countries. Most reports indicate that the frequency of GBS in children is lower than that in adults, and that the frequency may vary between high- and low-income countries. This difference may be related to the rate of infections between these countries.

Clinical Presentation

Probably the best prospectively collected cohort of paediatric GBS comes from the Korinthenberg group in Freiburg, Germany [4]. They included all children with GBS from the age of independent walking to 18 years, from 3 German-speaking countries during a 40-month period and described them in detail. A total of 95 children fulfilled the diagnostic criteria of GBS (53 boys, 42 girls), and the age ranged from 12

months to 16.5 years (median 6.2 years). Disease incidence peaked during the cold months (October–April). The most frequent first-presenting symptoms were unsteadiness of gait (45%), neuropathic pain (34%) and inability to walk (24%). At diagnosis, all patients showed symmetrical weakness and hypo- or areflexia; 27% presented with cranial nerve dysfunction and 33% with autonomic dysfunction. At nadir, 40% were still able to walk independently, 22% could walk with support, 38% were bed-bound, 20% showed signs of respiratory failure and 4 had to be intubated. In 30% pain was rated as severe or very severe. In 80% of all children the CSF findings were typical for GBS. In most children, CSF protein was found to be significantly elevated during the first days of the disease.

This Korinthenberg study describes the clinical presentation of GBS in children in detail, highlighting the most common complaints and the prognosis, and advises on the necessary care. This advice includes closely monitoring autonomic involvement and emphasizes that neuropathic pain is a frequent and early diagnostic feature, occurring in one-third of these patients. The study also describes minor differences between GBS in children and adults which should be considered when treating a child with GBS.

Natural History

The clinical course of GBS varies between countries, partly due to the availability of treatment. Most high-income countries treat adult and children with GBS with IVIg or plasma exchange and have the opportunity to provide ICU care and mechanical ventilation if required. In low-income countries specific treatments and mechanical ventilation may be available only for a minority of cases. Before the time of plasma exchange and IVIg, the natural history of Guillain-Barré syndrome in adults and children was documented in a Dutch cohort of 68 patients admitted between 1975 and 1987 [5] with GBS according to the generally accepted criteria [6]. Of these patients, 18 were under 15 years old. Time from onset of weakness until reaching nadir was similar in children and adults. There was also no difference between children and adult patients in disease severity, need for mechanical ventilation, and median duration of mechanical ventilation and duration of hospitalization. At a 2-year follow-up, 3 children (17%) had not made a complete recovery. Of these 3 children 2 children died from cardiac arrest, 1 in the acute phase, 1 early in the plateau phase. In both cases death had been preceded by severe fluctuations in heart rate and blood pressure. One child had a GBS disability score of 2. Four adults made an incomplete recovery after 2 years (8%). This study showed a very similar disease progression in both adult and paediatric patients, but the groups were small. In later published studies a trend towards a milder disease course and better outcome in children has been described.

Preceding Infections

The cohort study conducted by the Korinthenberg group showed that preceding events were reported in 82% (78 children) of patients [8]. The predominant antecedent event was a respiratory tract infection preceding GBS in 37% (33) of all children, followed by gastrointestinal infections in 15% (14) of children. The infectious agents responsible for these infections in children are unknown. In some cases the preceding infection may be caused by *Campylobacter jejuni*, but the frequency seems to be lower than in adult patients, where *C. jejuni* is the predominant cause of preceding infection. Multiple other types of preceding infections have been reported in children with GBS, most frequently with coxsackievirus, followed by *Chlamydomphila pneumoniae*, cytomegalovirus and *Mycoplasma pneumoniae*. In general, these studies were conducted only in small and potentially biased cohorts of patients.

Problems with Diagnosing GBS in Children

The diagnosis of GBS is still largely based on the findings in the history and neurologic exam, since there are no specific biomarkers and the nerve conduction studies and cerebrospinal fluid examination in the early stage of GBS may be normal. The history and neurological exam, however, may be problematic in children, especially in those of the preschool age (<6 years old). We performed a retrospective cohort study focusing on the clinical presentation and the delay in the diagnosis in preschool children and older children [9]. In this study of 55 children, 23 (42%) were under the age of 6 years old. At the first contact of the patients with a doctor, in the preschool group 15 patients (68%) were misdiagnosed initially, while in the older group, 6 patients (21%) had another initial diagnosis. The delay in diagnosis, indicated by the number of days between the moment a patient was first seen by a physician and the moment the patient was diagnosed with GBS is presented in [Figure 19.1](#). The doctors' delay in the preschool group was significantly longer than for the older group [median 3 (IQR 0–8) vs. 0 (IQR 0) days]. The most common other initial diagnoses were meningitis and coxitis, mostly because of the presence of severe pain as a prominent presenting symptom frequently leading to misdiagnosis. This shows that for preschool children presenting with subacute pain in the legs and difficulty walking the diagnosis GBS should be considered even though other diagnosis are probably more common.

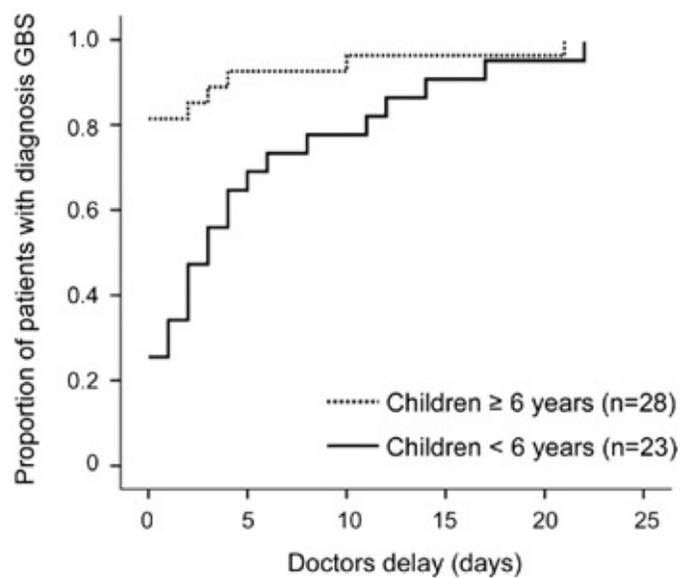


Figure 19.1 Kaplan-Meier curve showing time to diagnosis separated by age groups. Reproduced with permission from [9].

The Role of Additional Testing in Diagnosing Childhood GBS

GBS is a diagnosis made based on clinical presentation and neurological examination. The diagnosis can be supported with CSF and nerve conduction studies. More recently there has been interest in the possible role MRI can have in diagnosing GBS. Smith and colleagues looked at the role of gadolinium-enhanced MRI of the spine in diagnosing GBS in children and comparing it with cerebrospinal fluid analysis and nerve conduction studies [10]. Twenty-five children with the mean age of 4 years and 8 months, diagnosed with GBS between 1997 and 2007, were retrospectively reviewed. Gadolinium-enhanced MRI was performed in 8 children at a mean of 8 days (range 2–16 days) from symptom onset. In 7 (88%) children there was nerve root enhancement present and all had abnormal CSF findings. In 24 children NCS was performed and in 21 children (88%) it was consistent with a demyelinating polyneuropathy. In

20 children a lumbar puncture was performed and in 16 children (80%) cyto-albuminological dissociation was present. CSF protein concentration was normal in 3 children, at a mean of 3 days from onset. One child had supportive NCS when the gadolinium-enhanced spinal MRI was normal. Two children displayed positive nerve-root enhancement when NCS was normal, although F waves were not performed.

This article concludes that GBS is often diagnosed correctly but that the consequences of missing other potential diagnoses (cord compression, transverse myelitis) can be catastrophic. They believe that NCS is the gold standard for confirming the diagnosis of GBS in children, but that gadolinium-enhanced MRI provides a valuable addition to NCS in the evaluation of children with suspected GBS, especially when the presentation is atypical and if the child is presenting to a peripheral centre where paediatric neurological and paediatric neurophysiological expertise is unavailable. After the MRI a lumbar puncture can be performed safely to exclude other infectious diagnosis. A disadvantage of MRI to support the diagnosis of GBS is that young children often need to be sedated, a procedure that cannot always be provided in the acute setting.

Pain

The delay in diagnosis and type of initial diagnosis indicates that pain may be a frequent and predominant presenting feature of GBS in children. Several studies on the occurrence of pain in children with GBS have been published. In a retrospective case study of 29 children (16 boys and 13 girls) with GBS younger than 6 years old published by Nguyen and colleagues, pain was evident in 23 patients (79%) on admission and often the most prominent symptom [11]. This led to a misdiagnosis in 20 patients (69%). The prominent pain syndrome was bilateral deep lower limb pain, exacerbated by straight leg raising (radicular pain). Moreover, 11 patients (38%) with leg pain on examination were also found to have neck stiffness. Also headache (24%) was often described. Most children were treated with acetaminophen (38%); sometimes this was insufficient and additional pain medication was prescribed. Very few studies have reported on the treatment of pain in children and an evidence-based and standardized protocol for daily practice is still lacking.

Autonomic Dysfunction

Autonomic dysfunction, largely of cardiovascular problems, in paediatric GBS patients has been carefully documented. Watson and colleagues focused on hypertension as well as bladder dysfunction in relation to muscle weakness in children with GBS [12]. Twenty-seven patients with GBS presenting between 2002 and 2012 were retrospectively reviewed. Fifty-two percent of the patients (14) developed autonomic dysfunction. On average, autonomic dysfunction had its onset at the same time the disease activity reached its plateau phase. Twenty-six percent (7) of the patients already had bladder or bowel sphincter disturbance at the time of presentation. An additional 3 patients developed urinary retention during the course of the disease. The development of urinary retention correlated significantly with weakness of all 4 limbs and severity of upper-limb weakness. Hypertension was the most common manifestation of autonomic dysfunction and was seen in 12 (44%) patients. Resolution or control of the hypertension was statistically significantly correlated with the length of the hospital stay. Nine (33%) patients required anti-hypertensive treatment. The number of anti-hypertensive medications required correlated significantly with the extent of muscle weakness in both upper and lower limbs. There was no correlation between the

presence of persistent hypertension and the severity of neuropathic pain or the number of medicines used to control paresthesia.

This article confirmed previously presented data that 50% of children with GBS may suffer from autonomic involvement. But this article describes in greater detail the time of occurrence of autonomic dysfunction during the disease course. The relation between neuropathic pain and hypertension is always considered in patients with severe neuropathic pain, but this article shows that this is not always correct. Possibly the occurrence of hypertension is underestimated in children with GBS.

Treatment

There are only a few randomized trials performed regarding treatment of GBS in children. The trial of El-Bayoumi and colleagues compared PE with IVIg in mechanically ventilated children with GBS. They concluded that children with GBS requiring mechanical ventilation respond favourably to both IVIg and PE [7]. A trial looking at the treatment effect of different regimens of IVIg and the time to start treatment has been conducted by Korinthenberg and colleagues [13]. In their multicentre study, 63 hospitals participated and 95 children were included over a period of 40 months. They were divided into 2 groups: the early and late treatment study. The children still able to walk unaided (early treatment study) were randomized for 1g/kg IVIg over 2 days versus no immune treatment. The children who were unable to walk unaided (late treatment study) were randomized for 2g/kg IVIg over 2 days versus 2g/kg IVIg over 5 days. Treatment was to be initiated as soon as possible after the randomization. Twenty-one children were included in the early treatment study and 51 children in the late treatment study. The study concluded that treatment with IVIg before loss of unaided walking ability did not give rise to a less severe course, but recovery occurred somewhat faster. For treatment after loss of unaided walking, there was no significant difference in the effectiveness of 2g/kg IVIg administered over 2 days versus 5 days, but early relapses occurred more frequently after the shorter treatment regimen. Before this study, children were treated the same as adults without the knowledge whether or not this was also effective in children. This study indicated that IVIg was also effective in children and that the currently used method of starting with treatment only if the patient is unable to walk unaided is also sufficient in children. An important remaining question, however, is whether the dosage regimen used in adults and tested in this paper is optimal in children of all ages, or whether the amount of IVIg should be adjusted based on body composition of the growing child.

The Future

In the last few years a rise in the number of publications regarding childhood GBS has been seen, especially from low-income countries. For further reading we recommend a review of paediatric GBS published in 2013 by M. Ryan that describes the variable clinical and neurophysiologic subtypes of paediatric GBS, including the increased use of MRI in diagnosing GBS and the various aspects of the natural history and treatment response that are still poorly understood [14]. Several important aspects of paediatric need to be further investigated, including the following topics:

- While the overall prognosis of children is good, the mental impact of GBS on a child should also be considered during follow-up. There is no guideline available at present on how children with GBS can be best supported.

- Most outcome measures were developed for adult patients with GBS, and there is a need to develop such outcome measures for children with GBS.
- There are no models to predict the chance of respiratory failure or long-term outcome in individual children with GBS.
- The optimal plasma exchange and IVIg treatment regimens are unknown for children with GBS. Children are currently treated with the same regimens evaluated in adult patients, but these regimens are likely less than optimal in children with GBS.
- Pain is a major problem for children with GBS but there is very little information available on how children can best be treated.

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20

The Differential Diagnosis of GBS

Robert D.M. Hadden

Introduction

When a previously healthy person develops a mysterious illness and collapses, many suspicions are aroused. Who better to investigate than James Bond, the fictional British (Scottish) secret agent 007? As I attended the same school as Bond, I suggest the Bond film titles may help to classify and enliven the long list of diagnostic possibilities. The following is my personal selection of the most common or interesting conditions which may be confused with Guillain-Barré syndrome (GBS), although it is not meant to be comprehensive.

Diagnosis can be difficult, particularly in the early stages. Even in formal, randomised clinical trials of GBS where one would expect a greater degree of certainty, around 1% of enrolled patients turn out to have a diagnosis other than GBS or acute-onset chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). My informal survey of 1,192 patients in 6 published trials and series showed 14 (1.2%) were later diagnosed as another condition, such as myelitis, botulism, vasculitis or spinal disc herniation [1,2,3].

Casino Royale

Wakerley BR, Yuki N. Mimics and chameleons in Guillain-Barré and Miller Fisher syndromes. *Practical Neurology*, 2015

The answer to ‘why did it happen to me?’ is usually just bad luck. The roulette wheel of life may provoke a wide range of different conditions. Bond is familiar with disguise and deceit, and Wakerley and Yuki’s article [4] is a clear and practical clinical guide to conditions that may mimic GBS and its variants, and ‘chameleons’ in which GBS mimics other conditions. The same authors also recently proposed a new diagnostic classification for the generalised and incomplete variants of GBS and (Miller) Fisher syndrome (MFS) [5]. The distinction between GBS and acute-onset CIDP is covered in another chapter.

The Man with the Golden Gun: Reflexes and Basic Clinical Skills

Yuki N, et al. Guillain-Barré syndrome associated with normal or exaggerated tendon reflexes. *The Journal of Neurology*, 2012

Although Bond is supplied with the latest technical gadgets by Q, he mainly relies on his quick reflexes

and one core item of equipment to back up his personal skills. Advances in ultrasound [6], contrast-enhanced MRI of nerve roots [7] and other supportive tests have not substantially changed the fact that diagnosis of GBS is primarily based on clinical history and examination by the 'neurologist with the black tendon hammer'. The iPhone's inbuilt accelerometer and gyroscope have been used to record electronically and analyse the time characteristics of the tendon reflex [8].

Unfortunately even the reflexes are diagnostically unreliable: 23 (11%) of 213 Japanese patients had normal or brisk reflexes throughout the entire disease course [9]. These patients were more likely to have acute motor axonal neuropathy (AMAN), milder disease and anti-ganglioside GM1, GM1b, GD1a, or GalNAc-GD1a antibodies. In a European population, as expected from the lower prevalence of AMAN, this proportion was lower: 9% of patients had normal reflexes in weak limbs at onset, of which 2% had persistently normal reflexes [3].

Skyfall: Spinal Syndromes

Cakir E, et al. Clinical course of spontaneous spinal epidural haematoma mimicking Guillain-Barré syndrome in a child: a case report and literature review. *Developmental Medicine & Child Neurology*, 2004

Falls are a common cause of traumatic spinal injury, especially in the elderly where a minor fall (from a lesser height than the sky) may exacerbate pre-existing subclinical spondylotic cervical myelopathy. Acute cord injury may cause reduced reflexes, and the time course of transverse myelitis is identical to GBS [10]. Cauda equina compression may simulate paraparetic GBS, and radicular pain is common in GBS. The textbook distinguishing factors (sphincter disturbance, sensory level, perineal sensory disturbance) may be hard to identify in milder cases.

Quantum of Solace: Pain

Ruts L, et al. Pain in Guillain-Barré syndrome: a long-term follow-up study. *Neurology*, 2010

Solace (meaning comfort or consolation in a time of great distress or sadness) is in short supply for the sufferer of GBS. The Dutch GBS Study Group described how very common pain is in GBS [11]. In a cohort of 156 patients, 36% had pain before the onset of weakness, 66% had pain within the first month after onset and 38% still had pain after one year. Pain at the time of onset was often severe, typically in the extremities, especially radicular pain, painful paraesthesiae and muscle pain. Neuropathic pain medication may give a small quantum of solace.

However a patient who is weak and in pain may not have GBS, but instead pain-related functional weakness. A non-neural cause of back pain is a common cause of apparent weakness in the lower limbs and gait disturbance, theoretically distinguishable by the preserved reflexes, except when the patient is unable to relax. The knowledge that they don't have a serious neural disease may be little solace.

Dry Martini, Shaken Not Stirred: Metabolic Causes

Wöhrle JC, et al. Alcohol-related acute axonal polyneuropathy: a differential diagnosis of Guillain-Barré syndrome. *Archives of Neurology*, 1998

Chronic alcoholism often causes axonal neuropathy, of which occasional cases may have acute onset flaccid tetra-paresis within 2 weeks [12]. Typically these patients are very heavy drinkers and have lost substantial weight due to poor nutrition and likely thiamine deficiency. CSF protein is typically normal. Malnutrition neuropathy may alternatively be due to bariatric surgery or hyperemesis of pregnancy. The clue is the history of severe weight loss and often liver abnormalities. I am not aware of any randomised trials comparing the toxicity of cocktails shaken versus stirred.

Hypokalaemia with acute flaccid weakness may be due to various underlying causes leading to a final common pathway of myopathy [13]. Apart from familial hypokalaemic periodic paralysis, causes include thyrotoxicosis, prolonged vomiting or diarrhoea, renal tubular acidosis, hyperaldosteronism, and liquorice or barium toxicity. Many other metabolic disturbances may cause acute weakness: severe hypophosphataemia or hypermagnesaemia may cause acute neuropathy; diabetic lumbosacral radiculoplexus neuropathy causes weight loss, malaise and pain; and of course the bedbound patient with sepsis or an acute general medical condition is weak, albeit not in the conventional neural sense.

Porphyria should be suspected when a GBS-like syndrome is accompanied by encephalopathy (confusion and sometimes seizures) and abdominal pain. There may be proximal asymmetric weakness and numbness due to polyradiculopathy or neuronopathy [14]. Screening for urine porphobilinogen identified a remarkably high 12 patients (11%) with previously undiagnosed acute porphyria in a Russian cohort of 108 patients with acute polyneuropathy or encephalopathy, together with pain (in back or abdomen) and/or dysautonomia, though another 11 (10%) were false positives due to liver dysfunction [15].

The Spy Who Loved Me: Infections

Brannagan TH 3rd, Zhou Y. HIV-associated Guillain-Barré syndrome. *The Journal of the Neurological Sciences*, 2003

Bond's sexual promiscuity increases his risk of infection. HIV-associated GBS may occur at any stage of infection from seroconversion to AIDS, with CD4 counts ranging from 55 to 800/ μl in a series of 10 patients [16]. Cerebrospinal fluid (CSF) leukocyte count may be normal or mildly raised (0–17 leukocytes/ μl). Immunosuppressed patients may develop myelo-radiculo-neuropathy due to opportunistic infection with various herpes viruses.

Several neurotropic viruses may cause acute pure motor asymmetric flaccid paralysis due to myelitis affecting the anterior horn cells (anterior poliomyelitis syndromes). These include West Nile virus [17], enterovirus 71 or coxsackievirus, as well as the near-eliminated poliomyelitis virus. There is typically a prodromal febrile meningitis illness with myalgia or diarrhoea, with CSF pleocytosis, and MRI may show necrotising myelopathy. Other viral myelitis syndromes include herpes simplex and rabies. Lyme borreliosis may cause a meningoradiculitis with facial palsy.

Although CSF pleocytosis is one of the usual pointers towards an infectious cause, CSF pleocytosis of up to 230 leucocytes/ μl (sometimes polymorphonuclear) has been reported in idiopathic GBS, especially early or in severe fulminant cases [18].

Licence to Kill: Toxins

McCormack J, McKinney W. Thallium poisoning in group assassination attempt. *The Postgraduate Medical Journal*, 1983

Thallium may be considered the perfect poison due to being tasteless, odourless and colourless. It has been used in many notorious cases of murder, by Saddam Hussein against dissidents and in the Bond film *Spectre*. Thallium poisoning causes acute progressive sensory-motor polyneuropathy with prominent painful paraesthesiae, as well as gastrointestinal disturbance, ophthalmoplegia, and cerebellar and extrapyramidal features, but is often not considered until the development of alopecia 2 or 3 weeks later [19]. Prussian blue and haemodialysis may be beneficial. Arsenic toxicity may cause acute demyelinating neuropathy with diarrhoea and vomiting, confirmed by urine analysis [20].

Organophosphate poisoning has occurred due to high-dose exposure to agricultural insecticide crop sprays (especially tri-*ortho*-cresylphosphate), antiparasitic sheep dip, or historical contamination of food products. Two terrorist attacks with sarin in Japan in the 1990s killed 19 and injured 6,000 people [21]. Acute poisoning causes an acute muscarinic syndrome with diarrhoea, salivation and miosis. Weakness due to neuromuscular blockade (and encephalopathy) occurs after hours to days, and typically recovers in a week or two. A few weeks after initial exposure and recovery, some patients develop subacute 'organophosphate-induced delayed polyneuropathy', a distal predominantly motor axonal neuropathy with ataxia [22]. Chronic low-dose exposure to organophosphates (at doses insufficient to cause acute symptoms) does not cause neuropathy.

Pharmaceutical drugs occasionally cause acute severe axonal neuropathy, including nitrofurantoin, colchicine, chloroquine and chemotherapy drugs (vincristine, taxols, platinum compounds and bortezomib). Acute demyelinating neuropathy is sometimes caused by suramin or gold.

During his many travels abroad, Bond may have encountered various animal and fish toxins that can mimic AMAN. Tick bite paralysis (not to be confused with Lyme borreliosis) causes rapidly worsening tetra-paresis in endemic regions (United States and Australia) due to a sodium channel toxin which recovers rapidly on removal of the tick [23]. Ingested neurotoxins after eating pufferfish (tetrodotoxin) or bivalve shellfish (saxitoxin) may cause vomiting and paralysis.

Golden Eye CU: Critical Illness Polyneuropathy

Zhou C, et al. Critical illness polyneuropathy and myopathy: a systematic review. *Neural Regeneration Research*, 2014

In patients on an intensive care unit (ICU) who are too weak to be weaned from the ventilator, it is sometimes uncertain whether they have GBS or critical illness polyneuropathy (CIP) [24]. The neural examination is similar, though CIP is distally predominant and only rarely affects cranial nerves.

Usually the distinction is obvious from the history: a diagnosis of GBS is supported by onset of weakness before ICU admission, or recovery of any preceding infection before the onset of weakness. CIP affects over a third of severely ill patients in ICU, especially those with acute respiratory distress syndrome, sepsis, or systemic inflammatory response syndrome, and almost 100% of those with multi-organ failure. Additional risk factors for CIP include hyperglycaemia and gram negative bacteraemia. The pathogenesis of CIP is likely a combination of microcirculatory abnormality, metabolic derangements, reversible channelopathy, and bioenergetic dysfunction. Many patients also have critical illness myopathy, which is more common following corticosteroids and usually gives a raised creatine kinase. Prolonged use of neuromuscular blocking agents (especially vecuronium bromide) also may lead to persistent weakness. Where the distinction is difficult, GBS is supported by (usually) demyelinating neurophysiology or raised CSF protein, whereas CIP has axonal neurophysiology and normal CSF protein.

You Only Live Twice

Zhang L, et al. Guillain-Barré syndrome after transplantation. *Leukemia & Lymphoma*, 2008

After death, many of our organs can live on through transplantation. Demyelinating neuropathies resembling GBS or CIDP may occur in 0.3–0.7% of patients following solid organ or bone marrow transplant [25], or indeed following chemotherapy or biological drugs affecting the immune system. The time delay from transplant to GBS onset is typically a few months, but may range from days to years. The pathogenesis is unclear but may be related to an immune reconstitution syndrome, graft-versus-host disease or opportunistic infection. Such neuropathies usually respond well to intravenous immunoglobulin or plasma exchange.

For Your Eyes Only: Cranial Nerve Syndromes

Cherington M. Clinical spectrum of botulism. *Muscle & Nerve*, 1998

Variants of GBS predominantly affecting the extraocular muscles and cranial nerves have an alternative differential diagnosis. The following may mimic MFS, Bickerstaff's brainstem encephalitis or the pharyngo-cervico-brachial variant of GBS.

Botulism is an acute, toxic, pure motor and autonomic syndrome, with ophthalmoplegia, ptosis, dilated pupils and faciobulbar weakness, and later descending limb weakness, preceded by diarrhoea and vomiting if the source is foodborne [26]. Diagnosis is by neurophysiological demonstration of presynaptic block or culture of the organism. Treatment is by antitoxin. Myasthenia gravis may also cause acute ophthalmoplegia.

Wernicke's encephalopathy is characterised by acute confusion, ataxia and nystagmus or ophthalmoparesis, due to thiamine deficiency, typically occurring in alcoholics or following gastric surgery or chronic vomiting. Diagnosis may be confirmed by typical MRI changes in the medial thalami, mamillary bodies, midbrain and sometimes cerebellum.

Diphtheria causes bulbar-onset demyelinating neuropathy [27]. A few weeks after pharyngitis (typically with a visible grey pseudomembrane), patients may develop bulbar palsy with facial numbness and often respiratory failure. This may be improving before limb weakness and numbness worsens slowly over weeks. Neuropathy is due to an exotoxin not direct infection, but antitoxin is beneficial only within the first few days. Diagnosis is by throat swab and CSF leukocyte count is sometimes raised.

Die Another Day

Rigamonti A, et al. Guillain-Barré syndrome mimicking brain death. *Journal of the Peripheral Nervous System*, 2009

The ultimate differential diagnosis is between life and death. The British television hospital drama series *Holby City* once asked my advice on how to fake the brainstem reflexes in an actor playing brain-dead on intensive care, which we arranged using plastic ice cubes and a misplaced 'endotracheal' tube. Fortunately this doesn't happen in real life ... or does it? Occasional cases of severe GBS mimic brain death by losing all motor function including brainstem reflexes, resulting in 'locked-in syndrome' [28].

Dr No? Conclusion

Although the amateur diagnostician may rely on Google or myriad tests, the correct diagnosis is still most likely to be reached by a well-read neurologist [29] with enough time to take a history.

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21

GBS Reflections: What GBS Patients Have Taught Me Over My Career: 10 Lessons from Experience!

John Winer

Introduction

I have spent 25 years working as a consultant neurologist in a busy regional centre in England. I have researched GBS and looked after many sick patients with the disease. I first became interested in GBS while working with Richard Hughes and attending his district neurology clinics in Ashford as a medical registrar. Subsequently we worked together on an epidemiological and immunological study in the south of England. This was a sort of mini IGOS, but we spent almost all of our time looking for conventional protein antibodies since anti-ganglioside antibodies were only just being recognised in neuropathies. Timing was never my best point! We were able to show that *Campylobacter* was clearly linked to some patients with GBS.

No matter how well trained you are there are many things that patients can teach you. Sometimes these lessons can be painful, sometimes thought provoking but always helpful. This contribution tries to condense my experience into 10 learning points. Not everyone will agree and this represents a personal reflection so please read with a health warning!

Lesson 1: Predicting Outcome

Winer JB, et al. A prospective study of acute idiopathic neuropathy. *Journal of Neurology, Neurosurgery & Psychiatry*, 1988; van Koningsveld R, et al. A clinical prognostic scoring system for Guillain-Barré syndrome. *The Lancet Neurology*, 2007

I wrote a thesis on how to predict outcome in GBS [1], but after a few years of becoming a consultant I realised that this information was of limited use in managing patients. I can see that for clinical trials it is useful to identify groups that respond poorly to current therapies [2]. There are also some patients that need to know their statistical chance of making a full recovery. Many others need encouragement to improve with physiotherapy and do not need their hope for recovery dashed. Our models for recovery have wide error bars and this is a concept difficult for patients to grasp. I have seen patients with severe deficit following *C. jejuni* with rapid onset of weakness and worrying electrics who have made a full recovery despite everything. These are probably the ones with reversible conduction block and early electrical studies often miss them. Perhaps they are in a minority but for those individual patients that is irrelevant. There are some patients that react to being told they are in a poor prognostic group by vowing

to prove their medical attendants wrong but I suspect that more are discouraged. When we have new treatments to offer this group things may be different.

Lesson 2: Treatment-Related Fluctuations

Visser LH, et al. Risk factors for treatment related clinical fluctuations in Guillain-Barré syndrome. *Journal of Neurology, Neurosurgery & Psychiatry*, 1998; Ruts L, et al. Distinguishing acute-onset CIDP from Guillain-Barré syndrome with treatment related fluctuations. *Neurology*, 2005

Studies on the effectiveness of both plasma exchange and IVIg have shown that they both reduce the time taken to improve in functional grades as well as time in an intensive care unit and other outcomes. Of course this does not mean that all patients that are treated will stop deteriorating and start to improve. In fact, quite a few patients do show some deterioration after treatment [3,4] and still end up having a shorter disease course than might have occurred if they had not received treatment. We are just not comfortable if any patients continue to get worse after we have treated them. Hopefully we will eventually know more about the benefit of repeating treatment in these patients and we all await these studies with expectation. For the moment we have to balance the side effects of a second course IVIg or PE against an unproven benefit. Neurologists are divided on what to do, and I favour holding off retreatment unless the deterioration is significant. Perhaps less difficult is the patient who has a treatment-related fluctuation 6 weeks after IVIg who seems to recover spontaneously after a couple of weeks.

Lesson 3: Recognising GBS Common Mistakes

Hughes RA, *Guillain-Barré Syndrome (Clinical Medicine and the Nervous System)*. Springer-Verlag. London, 1990; Wakerley BR and Yuki N. Mimics and chameleons in Guillain-Barré and Miller Fisher syndromes. *Practical Neurology*, 2015

I would consider myself an expert on GBS, but I have made lots of mistakes in making the diagnosis over the years. One of the first patients I studied for my MD was an inpatient with a rapidly progressive flaccid areflexic quadriparesis who turned out to have neuromyelitis optica at autopsy. The patient did develop optic atrophy some weeks into the illness but otherwise looked just like GBS. All trials of GBS have included the occasional patients with MS presenting as an apparently lower motor neurone disorder that only later shows on scan or clinically as a CNS disorder. In the emergency department I have been caught out with acute polymyositis and hypokalemia, although both became clear with later blood tests. Brain stem strokes can mimic Miller Fisher or vice versa. An infamous case on the ITU of my district hospital just before I started as a consultant was thought to be brain stem dead with no brain stem reflexes and an internal ophthalmoplegia but turned out to have normal alpha rhythm on an EEG! I have seen porphyria mimic GBS with a subacute presentation. The distinction of GBS with acute onset CIDP is always difficult but can be helped by an obsessive history from both patient and relative [5,6].

Lessons 4 and 5: Recognising Incipient Respiratory Failure and the Dangers of Oxygen

Chevrolet JC and Deleamont P. Repeated vital capacity measurements as predictive

parameters for mechanical ventilation need and weaning success in the Guillain-Barré syndrome. *American Review of Respiratory Disease*, 1991; Nunn JF. *Applied Respiratory Physiology*. Oxford: Butterworth-Heinemann, 1987

Twenty-five percent of GBS patients need respiratory support, and recognising which patients need transfer to the ITU is critically important [7,8]. In our unit we try to monitor all patients who are still deteriorating with a full vital capacity (FVC) but others use alternative measures of respiratory reserve such as sniff pressures. Getting the message across to our juniors that peak-flow measurements can be unreliable is hard, and it often just takes experience to see when a patient is struggling for breath when speaking. Strangely, some patients with GBS have very low vital capacities but do not appear to be short of breath or in apparent distress. I have always wondered if this was because of their autonomic neuropathy masking some of the lung receptors that normally create dyspnoea. Not being comfortable lying flat is always a worrying sign. If a GBS patient tells you they are short of breath even when they do not look like it I always take it very seriously. Our juniors are tempted to give such patient oxygen but we have made it a rule that oxygen cannot be prescribed for a neuromuscular patient without consultant approval. If the problem is a ventilation issue with healthy lungs, oxygen will not influence the CO₂ levels, and although it might increase alveolar oxygen a little it stands a chance of making the hypercapnia worse. At the very least its prescription is a distraction from getting the patient into ITU as an emergency.

Lesson 6: Late Complications

Darweesh SK, et al. Health-related quality of life in Guillain-Barré syndrome patients: a systematic review. *Journal of the Peripheral Nervous System*, 2014

Neurologists are very involved in the acute side of GBS but our skills and experience are less suited to late complications of the disease [9]. Severe muscle atrophy can lead to contractures, persistent hypoventilation, pain and weakness. A colleague with a good knowledge of rehabilitation techniques and access to innovative aids and supports can transform the life of a patient with residual deficit. Hopefully, the move from intensive physiotherapy to rehabilitation and support will be seamless, but I have seen cases where the patient feels abandoned when the active treatment phase of the disease is over and this transition is poor. Pain is a common problem in GBS, both in the acute phase and during rehabilitation. This has received less attention in the literature over the years and we have all developed our own strategies for trying to help. I favour asking the advice of a pain expert, especially later on in the course of the disease.

Lesson 7: Autonomic Problems and Bradycardia in the ITU

Winer JB and Hughes RA. Identification of patients at risk of arrhythmia in the Guillain-Barré syndrome. *Quartely Journal of Medicine*, 1988

I remember a patient from the early 1980s with a severe neuropathy who stayed for 6 months in ITU before making it back on to the ward. She had a severe autonomic neuropathy. She died from cardiac arrest without warning on the anniversary of her first symptoms of GBS. While I was a research registrar I saw 2 patients in ITU with autonomic problems who developed a resistant sudden cardiac arrest after tracheal suction. There were comments in both of these cases about mild bradycardia on tracheal suction

24 hours before their terminal event, so I always take any sign of bradycardia on suction very seriously. I discuss such patients with my cardiology colleagues and err on the side of prophylactic pacing to try to prevent intractable asystole [10].

Lesson 8: Anaesthetists and Hope!

One of the nice things about being a clinical neurologist is seeing patients progress over a period of time. The feedback you get from seeing patients with severe GBS get better is rewarding and reminds one never to give up hope. ITU doctors never usually see patients when they are well, but I make a point of asking my GBS patients to visit ITU when they have recovered. This gives ITU doctors an opportunity to see how much better recovery can occur in patients with profound deficit and encourages them to still have hope for the future. Otherwise, there is a tendency for doctors to give up on patients who still have a chance to go on and lead useful lives. The role of a neurologist is often to champion the interests of patients when it comes to rationing expensive and in-demand ITU resources!

Lesson 9: Improvement over Years

I have been a member of the GBS support group in the UK (now known as GAIN [11]) for many years and have attended countless AGMs of the group. Although we traditionally consider that nerve regrowth and repair largely stops after about 18 months and up to 2 years, patients have told me that they have noticed continued improvement for many years after the acute illness. Mostly this is in sensory perception but it is clear that this translates into useful improvement in quality of life. I am now very cautious about ever saying that no further improvement will occur in the deficit from GBS.

Lesson 10: The Unselfishness of Patients

GBS is a rare disease and I would understand if any patient just wanted to forget about the illness after recovery. Surprisingly, many are still happy to contribute to teaching about the disease. They want doctors to recognise the illness more promptly and above all not give up on them if recovery is slow. In my experience they have been delighted to contribute to research even when it is invasive and unpleasant. Support group members will come and talk to patients in the acute phase of the disease and give them some hope for the future. They fund considerable research into GBS and work hard to raise awareness about the disease. My gratitude and admiration goes out to all of them.

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ANTECEDENT EVENTS AND SUSCEPTIBILITY

22

The Spectrum of Preceding Infections in GBS— Beyond *Campylobacter*

Patrick M. Meyer Sauter, Annemarie M.C. van Rossum and Bart C. Jacobs

Introduction

Guillain-Barré syndrome (GBS) is considered the prototype of a post-infectious immune-mediated disorder. One of the first descriptions of the association between an infectious trigger and GBS dates back to 1957, when Campbell reported that 60% of polyneuritis cases had preceding respiratory symptoms like cough, sore throat and fever, and about 10% to 20% had recent diarrhoea [1]. At this time, GBS was believed to be caused by a virus infection of unknown aetiology, and was linked to an illness in cats ('feline enteritis').

To date, it is well known that about two-thirds of GBS patients experience respiratory or gastrointestinal symptoms days to weeks before the onset of neurologic symptoms [2]. A specific pathogen can be identified in about half of patients with GBS (**Figure 22.1**). *Campylobacter jejuni* accounts for around one-third of these infections [3,4].

Over the last 10 years, evidence on the role of preceding infections and cross-reactive anti-glycolipid antibodies in the immunopathology of GBS has been accumulating. Most notably, there is now convincing evidence that *C. jejuni* is a potential trigger of GBS and is associated with the acute motor axonal neuropathy (AMAN) subtype and IgG antibodies against GM1, GM1b, GD1a or GalNAc-GD1a [5]. Much less is known about the role of preceding infections and the pathogenesis in the patients with GBS who did not have a preceding *C. jejuni* infection.

Many other identified pathogens have also been reported in association with GBS, including *Mycoplasma pneumoniae*, *Haemophilus influenzae*, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and influenza virus [3]. There are even more pathogens reported in single GBS case reports or series. The involvement of these pathogens in GBS is less clear and has not been determined in controlled larger studies. Here, we focus on the spectrum of these preceding infections in GBS beyond *Campylobacter* (**Table 22.1**).

Detecting a Pathogen in GBS: What Does It Mean?

The demonstration of a causative pathogen in GBS is rather complex. First, there is usually a delay of days to weeks between the infection and the diagnosis of GBS. The detection rate of a pathogen by culture and/or polymerase chain reaction (PCR) at GBS onset is low [2]. It is possible that the pathogen has already been cleared at the infectious site by the immune reaction that may have also caused GBS.

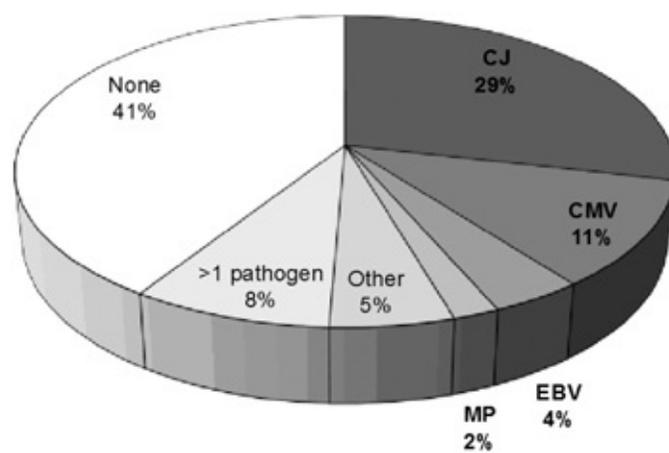


Figure 22.1 Incidence of positive infection serology in 154 adult GBS patients.

Abbreviations: CJ = *Campylobacter jejuni*; CMV = cytomegalovirus; EBV = Epstein-Barr virus; MP = *Mycoplasma pneumoniae*. Other pathogens were *Haemophilus influenzae*, influenza virus A/B, parainfluenza virus, adenovirus, herpes simplex virus and varicella-zoster virus. Most of GBS patients with > 1 pathogen detected had positive EBV and/or CMV serology in addition to other pathogens. Adapted from Jacobs et al. [3]; rates vary to those in the original publication because only single positive infection serologies are indicated for CJ, CMV, EBV and MP.

Second, demonstrating the presence of a pathogen in a GBS patient does not necessarily implicate that there is an infection that triggered an immune response to the nerves. Many pathogens, apart from *C. jejuni*, persist or are carried in healthy asymptomatic individuals without any symptoms. Multiple, coexisting bacterial and viral pathogens can be detected in respiratory samples of healthy asymptomatic individuals, particularly children [6]. This asymptomatic carriage of pathogens may also elicit immune responses [6,7]. It is unclear whether these immune responses reflect unrecognized damage by the pathogen to the host. Thus, a positive, single-sample serological result, either IgM or IgG, may simply reflect one or more previous encounters with the pathogen and are not necessarily related to GBS [8]. This also applies to human herpes viruses that persist in the host and where the seroprevalence in adults is considerably high. Caution should be taken in the interpretation of these diagnostic test results, also because some serological assays may lack sensitivity and specificity.

Third, GBS is a highly heterogeneous disorder with respect to the type of preceding infection, and some infections may have triggered the onset of disease only in a small minority of the cases.

Fourth, some patients may have already received intravenous immunoglobulins (IVIg) before blood samples were obtained to investigate infection serology, and such serology may then be false positive.

Infectious Diagnosis in GBS: What Is the ‘Gold Standard’?

In GBS, it may be of lesser importance to know whether or not the detected pathogen is the cause of the infection. It is all about the presence of cross-reactive antibodies, induced by either infection or carriage of a pathogen, that are associated with GBS.

What is more important is to know whether the encounter with a pathogen leading to the production of (cross-reactive) antibodies was a recent event. This may be achieved by using paired patient sera in order to detect seroconversion and/or a ≥ 4 -fold increase in antibody titres, which still serves as ‘gold standard’ for many infectious diseases [9].

Interestingly, we recently established the aetiological diagnosis in 2 GBS patients by the detection of a specific intrathecal antibody synthesis against *M. pneumoniae* [10]. The detection of a specific intrathecal antibody response is an established diagnostic method to prove an intrathecal infection with *M.*

pneumoniae, CMV, EBV, influenza viruses, etc. in encephalitis patients [9]. An intrathecal antibody synthesis can be established either by calculation of an antibody index or through parallel immunoblotting of simultaneously collected cerebrospinal fluid (CSF) and serum samples [11,12]. The autoimmune response in GBS results in nerve inflammation at peripheral nerves and nerve roots, which are surrounded by CSF. The detection of a specific intrathecal antibody response may therefore be useful as an additional diagnostic tool to further establish the infectious diagnosis.

Infectious Association or Cause?

When an infectious diagnosis can be made, how sure are we that there is a causal relationship between the identified pathogen and GBS?

Despite the association between many infections and GBS, the overall risk of developing GBS is very small. For example, only one in 1,000–5,000 patients with *C. jejuni* enteritis will develop GBS in the subsequent 2 months [13], a fact which certainly also applies to other infectious pathogens, where the acute disease is much more frequent than this severe post-infectious complication.

One of the critical steps in GBS pathogenesis is the generation of antibodies that cross-react with specific gangliosides. It has been shown for *C. jejuni* that such cross-reactive antibodies are not produced during uncomplicated *C. jejuni* enteritis [5]. The variety in types of preceding infection is related to the diversity in clinical presentation [14], outcome [15], and specificity of antibodies to glycolipids [3]. Therefore, the detection of a distinct anti-glycolipid antibody type could give an indication on the preceding infectious agent that triggered the antibody response through microbial mimic of the target glycolipid antigen [16]. It is not excluded that for other GBS patients cross-reactive T-cells or very different mechanisms than cross-reaction may play a role in the pathogenesis.

Specific Infections

An overview of reported infectious triggers associated with GBS is shown in [Table 22.1](#).

Table 22.1 The spectrum of preceding infections in GBS (beyond *Campylobacter jejuni*)

| Pathogen | Main disease spectrum (apart from GBS) | Detection rate (%) | | | Anti-glycolipid antibodies | | | Microbial mimic structure |
|--|--|---|--|------------------------------------|---|---|--|--|
| | | GBS | HC | NC | GBS | Controls | | |
| Bacteria | | | | | | | | |
| <i>Mycoplasma pneumoniae</i> | Upper respiratory tract infections, atypical pneumonia, extrapulmonary manifestations | [3] [†] : 5% [20] [†] : 15% [22] [†] : 21% [21] [†] : 3% | 0% 1%* 14% – | 1%* 2%* 14% – | <i>M. pneumoniae</i> +: [28] [†] :anti-GalC 73% | <i>M. pneumoniae</i> +: [28] [†] :anti-GalC 58% [34] [†] :anti-GalC 18% | <i>M. pneumoniae</i> -: [28] [†] :anti-GalC 0%* [34] [†] :anti-GalC 0% | GalC -like [27]; immunisation with GalC caused demyelinating neuropathy in rabbits [30] |
| <i>Haemophilus influenzae</i> | Upper respiratory tract infections, otitis media, epiglottitis, pneumonia, bacteraemia, meningitis, arthritis, osteomyelitis, cellulitis | [36] [†] : 9% [37] [†] : 7% FS: 11% [3] [†] : 1% | 0% 6% 6%* – | 8% 7% 7%* – | <i>H. influenzae</i> +: [36] [†] :anti-GM1 100% anti-GQ1b 25% [37] [†] :anti-GQ1b and anti-GT1a 86% (GBS + FS) | – | – | GM1 -like [38]; GT1a/GQ1b -like [36] |
| <i>Salmonella enterica</i> species | Typhoid fever (<i>S. typhi</i>), paratyphoid fever (<i>S. paratyphi</i>) | [43] [†] : 0% [44] [†] : 1% | – – | – – | <i>S. Paratyphi A</i> +: [48] [†] :anti-GQ1b ¹ | – | – | – |
| Viruses | | | | | | | | |
| Cytomegalovirus (CMV) HHV-5 (β-herpes virus) | Congenital and neonatal infections, mononucleosis-like disease | [3] [†] : 13% [20] [†] : 6% [52] [†] : 8% FS: 6% [21] [†] : 12% [53] [†] : 15% [54] [†] : 12% [55] [†] : 8% | 0%* 1% 0%* 0% – – – – | 2%* 2% – – – – – | CMV +: [56] [†] :anti-GM2 ⁵ 22% [52] [†] :anti-GM2 ⁵ 100% [21] [†] :anti-GM2 ⁵ 42% [3] [†] :anti-GM2 25% | CMV +: [56] [†] :anti-GM2 ⁵ 2%* [52] [†] :anti-GM2 ⁵ 60% [21] [†] :anti-GM2 ⁵ 47% – | CMV -: [56] [†] :anti-GM2 ⁵ 0%* [52] [†] :anti-GM2 ⁵ 0%* [21] [†] :anti-GM2 ⁵ 0% – | GM2 -like, expressed by CMV-infected fibroblasts [57] |
| Epstein-Barr virus (EBV) HHV-4 (γ-herpes virus) | Infectious mononucleosis | [3] [†] : 10% [20] [†] : 4% [21] [†] : 1% [55] [†] : 2% | 0%* 0% – – | 1%* 0% – – | No antibodies against known glycolipids detected [3, 21] | – | – | – |
| Herpes simplex virus (HSV)-1/2 HHV-1/2 (α-herpes virus) | Herpes labialis (HSV-1), herpes genitalis (HSV-2) | [3] [†] : 1% | – | – | – | – | – | – |
| Varicella-zoster virus (VZV) HHV-3 | Chickenpox (primary infection), herpes zoster (HZ; reactivation) | [3] [†] : 1% [63] [†] : HZ: 0.025% | – HZ: 0.001%* | – – | – | – | – | – |
| Influenza virus (A and B) | Flu | [3] [†] : 2% [67] [†] : 3% | – – | – – | Influenza A +: [79] [†] :anti-GD1b ¹ [80] [†] :anti-GD1b, -GM1, and -GD1a ¹ | – | – | No structural homologies with known gangliosides[68] |

(continued)

Table 22.1 (continued)

| Pathogen | Main disease spectrum (apart from GBS) | Detection rate (%) | | | Anti-glycolipid antibodies | | | Microbial mimic structure |
|------------------------------------|---|---|--------------|----------|---|----------|---|---------------------------|
| | | GBS | HC | NC | GBS | Controls | | |
| Human immunodeficiency virus (HIV) | Three stages: acute infection, clinical latency, AIDS | [75] [†] : 0% (UK) [74] [†] : 55% (Africa) | – 4%* | – – | – | – | – | – |
| Hepatitis E virus (HEV) | Acute hepatitis | [77] [†] : 5% [78] [†] : 11% | 0.5%* 0%* | – 2%* | No antibodies against known glycolipids detected [77] | – | – | – |

Abbreviations: AIDS = acquired immunodeficiency syndrome; bp = base pairs; CMV = cytomegalovirus; FS: Fisher syndrome; GalC, galactocerebroside; GBS = Guillain-Barré syndrome; HC = healthy controls; HHV = human herpes virus; HZ = herpes zoster; Ig = immunoglobulin; NC = controls with other neurological diseases.

*P ≤ 0.05; †Data from a single case report; –: no data available.

Evidence for infection: To compare an acute serological response against a pathogen between different studies we collected data of positive specific IgM results (if available).

[†]Enzyme-linked immunosorbent assay (ELISA); IgM; [‡]Complement fixation test (CFT); Igs (no discrimination between isotypes); [§]Isolation of *Salmonella Typhi* in blood, stool, and/or urine; [¶]ELISA: IgG avidity;

¹Plasma CMV DNA was positive in 62% of cases with CMV infection[¶]; ⁵Anti-GM2 IgM; ¹¹Clinically defined herpes zoster (HZ); ^{*}Polymerase chain reaction (PCR)

Mycoplasma Pneumoniae

Mycoplasmas were first described as infectious agents in humans in the 1940s [17]. They are the smallest self-replicating life forms. Today, we know that *M. pneumoniae* is a frequent cause of respiratory tract infections (e.g. ‘walking pneumonia’) and colonizes the upper respiratory tract of healthy children [6,18]. The incidence of *M. pneumoniae* infections is higher in children than in adults [19]. Interestingly, a wide spectrum of extrapulmonary complications has been described in association with *M. pneumoniae* infection, including encephalitis and GBS [18]. Preceding *M. pneumoniae* infection has been reported in 3–21% of GBS in adults [3,20–22], and in GBS variants [23–25] and related neuropathies [26]. These patients are younger and report respiratory prodromes more often than *M. pneumoniae* seronegative GBS patients. There is no distinct clinical picture and the outcome is generally good, although we recently presented a case series of children who developed severe and complicated disorders within the GBS spectrum after infection with *M. pneumoniae* [10].

In patients with GBS, serum antibodies to *M. pneumoniae* have been found to cross-react with the myelin glycolipid galactocerebroside (GalC) [27,28]. Interestingly, we recently detected anti-GalC antibodies in the CSF of a patient diagnosed as Bickerstaff brainstem encephalitis (BBE) following *M. pneumoniae* infection [29]. Anti-GalC antibodies have been shown to cause demyelinating neuropathy in rabbits that were immunized with GalC [30], and have been associated with demyelination in GBS [28,31], encephalitis [32] and acute disseminated *encephalomyelitis* [33]. However, anti-GalC antibodies could also be detected in patients with *M. pneumoniae* infections but without neurologic symptoms [28,34]. The observation that the isotype IgG of anti-GalC antibodies was more frequent in GBS patients than in patients without neurologic symptoms [28] suggests that a subclass switching of anti-GalC antibodies may pose a risk for GBS after *M. pneumoniae* infection.

Haemophilus Influenzae

H. influenzae is also frequently carried in the upper respiratory tract of humans. The bacteria was first isolated by Pfeiffer in 1893 during an influenza outbreak (‘influenza bacillus’) [35]. *H. influenzae* has encapsulated (type a–f) and unencapsulated (nontypeable) strains, and can cause respiratory infections and severe invasive diseases, particularly in children. In the pre-vaccine era, *H. influenzae* type b (Hib) accounted for 95% of all strains that caused invasive disease. It is this strain that has been reported in association with GBS. The rates of preceding *H. influenzae* infection in GBS vary between 1% and 9% [3,36,37], without significant differences to control subjects. Patients with GBS associated with *H. influenzae* presented with respiratory prodromes, less frequent cranial and sensory nerve involvement, pure motor axonal dysfunction and positivity for anti-GM1 IgG antibodies [36]. A GM1-like structure could be identified in an *H. influenzae* isolate of a patient with axonal GBS [38].

In fact, the rate of positive IgM antibodies against *H. influenzae* was more frequent in Fisher syndrome (FS) patients (11%) and differed significantly compared with controls [37]. Interestingly, in his original description Charles Miller Fisher reported the isolation of *H. influenzae* in the sputum of a 63-year-old man with pneumonia who developed FS [39]. Patients with both FS or GBS who had serologic evidence of a recent *H. influenzae* infection were positive for serum anti-GQ1b and anti-GT1a IgG antibodies [37]. It could be demonstrated that *H. influenzae* isolates from FS patients bear an anti-GT1a/GQ1b epitope [37,40]. Serological evidence for a recent *H. influenzae* infection was also found in 6% of BBE patients [41]. Thus, *H. influenzae* may be involved specifically in the development of the anti-GQ1b antibody syndrome (FS and BBE) [42] because of its suggested molecular mimicry with GQ1b.

Salmonella Enterica Species

In contrast to the bacterial and viral pathogens detected in the respiratory tract, the diagnosis of enteric fever caused by *Salmonella typhi* (typhoid fever) or *Salmonella paratyphi* (paratyphoid fever) is clear-cut because of the detection of the bacteria in the blood stream. Enteric fever can cause a wide range of neurologic symptoms, with the most common being a toxic confusional state or delirium ('nervous fever') [43]. GBS has rarely been described in association with *Salmonella* [43,44]. Single case reports exist on different GBS variants [45–48]. The only analysis of anti-glycolipid antibodies in a BBE patient with detection of *S. paratyphi A* in blood cultures revealed anti-GQ1b IgM and IgG antibodies [48]. However, a GQ1b epitope was not tested in this specific case.

Cytomegalovirus

The most frequent virus associated with GBS is cytomegalovirus (CMV), which emphasizes that molecular mimicry may be not restricted to bacterial infections. CMV is the largest known human herpes virus and can be transmitted via saliva, sexual contact, placental transfer, breastfeeding, blood transfusion, and solid-organ or haematopoietic stem-cell transplantation. The most important clinical manifestations of CMV are congenital and neonatal infections and a mononucleosis-like syndrome [49]. CMV establishes lifelong latency after primary infection in cells of the myeloid lineage. The overall seroprevalence for CMV is 30–70% [50]. Long-term immunosuppression can lead to uncontrolled replication and serious disease [49]. GBS was first associated with CMV in 1967 [51]. Since then rates of preceding CMV infections are reported in 6–15% of GBS cases [3,20,21,52–55]. GBS patients associated with CMV showed distinct clinical features: younger age, more severe disease course indicated by a high frequency of respiratory insufficiency and delayed recovery, cranial nerve involvement (facial palsy) and severe sensory loss [53]. A positive IgM serology against CMV was also found in 6% of FS patients [52]. The electrophysiological subtype is acute inflammatory demyelinating polyneuropathy (AIDP) [21].

CMV has been associated with the occurrence of anti-GM2 antibodies in GBS patients, particularly of the isotype IgM [54,56]. It could be demonstrated that CMV-infected fibroblasts express a GM2-like epitope that was specifically recognized by anti-GM2 IgM antibodies [57]. However, anti-GM2 IgM antibodies could also be found in patients with positive CMV serology but without neurologic diseases [21,52]. Interestingly, CMV DNA was detected in the blood of 62% GBS patients with a positive serology [54]. Although the presence of CMV DNA in blood did not appear to be a significant predictive factor for the outcome, these findings suggested a role of viral replication in the development of GBS. In fact, CMV DNA could also be detected in the CSF in 31% (13 out of 42) of GBS patients [58]. This observation, however, could not be reproduced in another large GBS cohort ($n = 170$), where CMV DNA was detected in only one single case (0.6%) [59]. While there may be a role for anti-GM2 IgM antibodies in the development of GBS after CMV infection, the involvement of viral replication has yet to be established.

Epstein-Barr Virus

Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis ('kissing disease'). The rate of positive IgM against EBV viral capsid antigen in GBS patients has been reported with 1–10% [3,20,21,55]. GBS has also been described after EBV reactivation in the immunocompromised host [60]. However, since over 90% of adults are infected with EBV [61] and because no known anti-glycolipid

antibodies could be detected in EBV-positive GBS cases [3,21] the relation of EBV with GBS is controversial.

Herpes Simplex Virus and Varicella-Zoster Virus

In contrast to CMV and EBV that establish latency in myeloid and B cells, respectively, the human herpes simplex virus (HSV) and the varicella-zoster virus (VZV) persist in the ganglia of cranial nerves and dorsal roots, and are present in virtually every elderly adult [62]. There are case reports that suggest these viruses as triggers of GBS. However, the association is weak [3].

The infection with VZV usually occurs during childhood and may manifest as chickenpox. A spontaneous reactivation of VZV can lead to herpes zoster. The pathomechanisms leading to reactivation of the virus may be associated with a derangement of the immunological status of the host. Interestingly, a recent study found an 18-times increased risk for the development of GBS in Taiwanese patients with herpes zoster during a 2-month follow-up, compared to a matched control population [63]. Thus, these findings first suggest that factors that are important for the reactivation of VZV may be also involved in the development in GBS. Anti-glycolipid antibodies have not been described in these cases.

Influenza Virus and the Flu Vaccine

In 1967, the U.S. national influenza immunization campaign against influenza A (H1N1) estimated an attributable risk of vaccine-related GBS of about one in 100,000 [64]. These events caused considerable public concern. A similar association was suggested for the immunization campaign against influenza A (H1N1) in 2009, but extensive national and international studies found that vaccination was associated with only a very small attributable risk of GBS (1.6 excess cases of GBS per 1,000,000 vaccine recipients) [65]. In fact, vaccination might even reduce the risk of acquiring GBS, as this condition may be caused by influenza [66]. Serologic evidence for an influenza infection has been reported in 2–3% of GBS cases [3,67], but the influenza virus does not share structural homologies with known gangliosides [68]. However, the risk of developing GBS after influenza infection is estimated to be 4 to 7 times higher than after influenza vaccination [69]. No relapses of GBS in patients with a history of this disease have been observed after influenza vaccination [69].

Human Immunodeficiency Virus

The association of GBS with human immunodeficiency virus (HIV) was reported soon after the beginning of the HIV infection epidemic in the 1980s. Peripheral neuropathies in general are common and occur in all stages of HIV infection [70]. The neuropathic complications in HIV infection may result from a variety of pathologic processes, such as immune dysregulation during early stages, opportunistic infections (CMV, VZV, etc.) in late stages, and treatment-related effects by antiretroviral therapy (ART) (toxicity or aberrant manifestation of immune reconstitution after initiation, re-initiation, or change of ART) [71,72]. GBS in association with HIV may occur in the early stage of infection or at seroconversion and may present with more frequent recurrent episodes or the development of chronic inflammatory demyelinating polyneuropathy (CIDP) [73]. Although HIV infection was found in 55% of 32 consecutive GBS cases in Zimbabwe [74], GBS has not been observed in a prospective study of 1,500 HIV-infected patients in Britain [75].

Hepatitis E Virus

The latest discovery of a preceding infection in GBS is hepatitis E virus (HEV). HEV infection is the most common cause of acute hepatitis worldwide [76]. In developed countries, HEV infection usually presents as a self-limiting disease caused by genotype 3. Notably, a Dutch case-control study showed that 5% of GBS patients had serologic evidence for HEV infection, compared with 0.5% of matched healthy controls [77]. HEV RNA was found in the serum of one-third of these HEV seropositive GBS cases and was also found in faeces in one case (all genotype 3). Antibodies against known glycolipids were not detected. Similarly, 10% of patients with GBS from Bangladesh had an antecedent HEV infection [78], indicating that HEV may be a worldwide trigger of GBS. Of note, hepatitis A and B virus infections were not found in GBS patients [3].

Conclusion

GBS is a very rare complication of common infections. The demonstration of a causative pathogen in GBS is rather complex. The presence of a pathogen in a GBS patient does not necessarily implicate that there is an infection that triggered an immune response to the nerves. Almost any pathogen at least once has been reported in a patient with GBS. This questions whether there is a causal relationship between the infectious agent and GBS. In fact, larger, prospective, standardized and controlled studies are lacking for most specific types of infection associated with GBS. More stringent criteria are therefore required to establish the involvement of a pathogen in GBS and to direct the search for the target antigen in the triggering infectious agent.

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23

The Campylobacter Story: A Bumpy Road of 150 Years

Hubert P. Endtz

Landry JBO. Note sur la paralysie ascendante aiguë. *Gazette Hebdomadaire de Médecine et de Chirurgie*, 1859

One of the first descriptions of the Guillain-Barré Syndrome (GBS) appears in the *Gazette Hebdomadaire de Médecine et de Chirurgie*, where Octave Landry describes a study of 5 patients with an acute ascending paralysis.

« La sensibilité et la motilité peuvent être également compromises. Cependant, les troubles fonctionnels portent surtout sur le mouvement et sont alors caractérisés par la diminution graduelle de la force musculaire, avec flaccidité des membres sans tremblement, sans contracture, sans convulsion. Dans la presque totalité des cas la défécation et la miction restent normales. On n'observe aucun symptôme immédiat du côté des centres nerveux, pas de céphalalgie ni de délire. Jusqu' à la fin, les facultés intellectuelles sont complètement conservées. Le début des accidents paralytiques peut être précédé d'un sentiment de faiblesse et de crampes abdominales passagères ».

To the best of our knowledge, this is the first description of preceding gastrointestinal complaints in GBS patients [1].

Escherich T. Beitrage zur Kenntniss der Darmbakterien. Ueber das Vorkommen von Vibrionen im Darmcanal und den Stuhlgangen ser Sauglinge. *Münchener Medizinische Wochenschrift*, 1886

Although *Campylobacters* were identified as human pathogens only in the 1970s, they have probably caused illness in man for centuries. In 1886, Escherich published a series of articles in the *Münchener Medizinische Wochenschrift* in which he reports on spiral bacteria in the colon of children who had died of an intestinal disease that he describes as 'cholera infantum'. Attempts to culture the bacteria on solid medium were not successful. He also saw spiral organisms through a microscope in the stools of 35 out of 72 infants suffering from other enteric diseases. Unfortunately, his report remained unreferenced in the English literature as it was published in German [2].

McFadyean J, Stockman S. *Report of the Departmental Committee*

appointed by the Board of Agriculture and Fisheries to Inquire into Epizootic Abortion. London: His Majesty's Stationery Office, 1913

Campylobacters were described for the first time in association with veterinary disease before they were identified as an important pathogen in human medicine. In 1909, 2 veterinarians reported the isolation of vibrio-like bacteria from aborted fetuses in ewes. Similar bacteria were later also isolated in infectious abortions of bovines and dysentery in calves and swine. *Vibrio fetus* and *Vibrio jejuni* were the names proposed to these newly identified veterinary pathogens [3].

Guillain G, et al. Sur un syndrome de radiculonévrite avec hyperalbuminose du liquide céphalo-rachidien sans réaction cellulaire. Remarques sur les caractères cliniques et graphiques des réflexes tendineux. *Bulletins et mémoires de la Société médicale des hôpitaux de Paris*, 1916

During the First World War, 2 French military doctors, Georges Guillain and Jean-Alexandre Barré, together with André Strohl, all enlisted in the 6th French Army, described the case history of 2 paralysed soldiers with areflexia associated with raised protein values but with normal cell counts in the cerebrospinal fluid. The three published their findings in *Bulletins et mémoires de la Société médicale des hôpitaux de Paris* in 1916. The name of Strohl was dropped for unknown reasons and the disease became known as Guillain-Barré syndrome. André Strohl became a medical physiologist and professor in physiological medicine in Algiers and Paris. Georges Guillain became professor and chair of the department of neurology at the famous Salpêtrière Hospital in Paris. Jean-Alexandre Barré was appointed as professor of neurology in Strasbourg [4].

Levy AJ. A gastro-enteritis outbreak probably due to a bovine strain of vibrio. *Yale Journal of Biology and Medicine* 1946

The first well-documented description of a *Campylobacter* outbreak in humans was published in 1946 and describes a milk-born outbreak of diarrhoea in 2 state institutions involving 355 patients. Organisms resembling 'V. jejuni' were grown in broth cultures of the blood of 13 victims [5].

Vinzent R, et al. Septicémie grave au cours de la grossesse due à un vibriion. Avortement consécutif. *Bulletin de Academie Nationale de Medecine*, 1947

Vinzent and colleagues reported on the case histories of 3 pregnant women with fever of unknown origin. *Vibrio fetus* was isolated from the blood specimens taken from the 3 women. Two out of the 3 aborted within 4 weeks and a necrotizing inflammation was observed on the placenta. In 1957, King described 11 additional bloodstream infections with *Vibrio fetus* and related species, and a full bacteriological, biochemical and serological description of the strains [6,7].

Cambell AMG. The etiology of polyneuritis. *Proceedings of the Royal*

Society of Medicine, 1958

Campbell published the case histories of his own GBS patients and patients from the literature. He found that in about 60% of cases there is a respiratory infection with cough, sore throat and pyrexia before the onset of the polyneuritis. In another 10–20% of the cases the onset is preceded by an episode of diarrhoea. He reports that “recent work has tended to emphasize that the Landry-Guillain-Barré syndrome is a non-specific reaction to several infective agents and is possibly due to an abnormal antigen-antibody response”. In addition, he also reports that the polyneuritis is preceded by diarrhoea by 3–10 days. However, we have to wait until the late 1970s and the isolation of *Campylobacter* to truly understand the far-reaching implications of his observations [8].

Dekeyser P, et al. Acute enteritis due to a related vibrio: first positive stool cultures. *Journal of Infectious Diseases, 1972*

Until 1968, the isolation of *Campylobacter* from human stools was difficult. A breakthrough discovery was made by Dekeyser and Butzler and their team in Brussels, Belgium. They isolated a ‘related vibrio’ from blood samples of a 20-year-old female who was admitted to the hospital with severe diarrhoea and fever. Using a newly developed mechanical filtration technique, they also isolated the ‘related vibrio’ from the stools of diarrhoeal patients. This first faecal isolation of ‘related vibrio’ from human stools was followed by the observation that *C. jejuni*/*C. coli* were isolated from 5.3% of 3,800 stools from children. By the mid-1980s, *Campylobacter* species were recognized as the most common cause of bacterial enterocolitis [9].

Skirrow MB. *Campylobacter* enteritis: a ‘new’ disease. *BMJ, 1977*

The filtration technique was, however, very cumbersome, time-consuming and less adapted to high-throughput diagnostic microbiology laboratories. The development of selective media containing antibiotics to inhibit the residential enteric flora proved to be a real breakthrough. In 1977, Martin Skirrow published a selective media containing vancomycin, polymyxin and trimethoprim and isolated *Campylobacter* spp. in 57 stool samples out of 803 (7.1%) patients with diarrhoea. New isolation protocols and selective media using various combinations of selective antibiotics were rapidly introduced in diagnostic laboratories and *Campylobacter* diarrhoea emerged as a frequent cause of bacterial diarrhoea [10].

Rhodes KM and Tattersfield AE. Guillain-Barré syndrome associated with *Campylobacter* infections. *BMJ, 1982*

These newly developed methods drew the attention of scientists trying to decipher the aetiology of preceding infections in patients with GBS. However, no *Campylobacter* spp. could be cultured from the stools collected from these patients at the onset of the muscular weakness. The first report of a possible link between *Campylobacter* infections and GBS was published in 1982. Rhodes and Tattersfield described the case history of a 45-year-old man admitted to the hospital with severe diarrhoea, who, on the 10th day of hospitalisation, developed a rapidly progressive paresis with areflexia and was diagnosed with the Guillain-Barré syndrome. *Campylobacter* was isolated from his stools on the 7th day of

admission.

Two years later Kaldor and Speed published a serological study of the association of *Campylobacter* infections and GBS. In a retrospective cohort of 56 GBS patients, 21 (38%) had serological evidence of a recent *Campylobacter* infection. The *Campylobacter*-associated GBS cases manifested a significantly more severe form of the disease. They also stated that “antigenic similarities between neural glycopeptides and bacterial capsules may be the possible immunological link in the case of *Campylobacter*”. These observations were followed by to a tsunami of scientific communications and evidence of the role of *Campylobacter* infections preceding the development of GBS [11,12].

Yuki N et al. A bacterium lipopolysaccharide that elicits Guillain-Barré syndrome has a GM1 ganglioside-like structure. *Journal of Experimental Medicine*, 1993

The next flagship discovery came from Aspinall and Yuki for what would become an excellent paradigm and attractive model for the elucidation of host and microbial determinants in GBS: the molecular mimicry hypothesis. The groundwork was led by Aspinall, who conducted extensive structural studies of lipooligosaccharide (LOS) of various *Campylobacter* serotypes and found that the type strain of HS:19 serotype showed structural similarity with both GM1 and GD1a gangliosides. In addition, the structure of 2 HS:19 *Campylobacter* strains isolated from GBS patients showed structural similarity with GM1, GT1a and GD3.

In 1993, Yuki and colleagues published data showing striking homology between the core oligosaccharides of *Campylobacter* LOS and a number of different glycosphingolipids of the ganglioside groups present on neural membranes. These were the first reports of molecular mimicry between human nerve tissue and *Campylobacter* strains isolated from patients with GBS [13,14].

Rees JH, et al. *Campylobacter jejuni* infections and the Guillain-Barré syndrome. *New England Journal of Medicine*, 1995

To confirm the association of *Campylobacter* as an important preceding infection among GBS patients, Rees and colleagues conducted a case-control study involving 96 GBS patients. They found evidence of a recent *Campylobacter* infection in 26% of them as opposed to 1–2% in 2 different control groups. The authors confirmed that the *Campylobacter*-associated cases were more likely to present with severe axonal forms of GBS [15].

Jacobs BC, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology*, 1998

A few years later, a second state-of-the-art case-control study was published by Jacobs and colleagues. They conducted a serological study among 154 GBS patients and 154 sex-matched controls with other neurological diseases. Serological evidence of a recent *Campylobacter* infection was observed in 32% of the GBS patients. They also suggested that the clinical heterogeneity of the syndrome might be a reflection of the great variety of preceding infectious aetiologies.

A very similar study design was used 10 years later in Bangladesh. In a case-control study involving 97 GBS patients and 194 controls, Islam and colleagues found serological and microbiological evidence

of a recent *Campylobacter* infection in 57% of the GBS cases and 3% of the controls with other neurological diseases. The mean age was 21 years and the majority of the cases (67%) were severe and axonal forms of GBS and associated with a high case fatality (14%) and severe disability at 6 months of 29% [16,17].

van Belkum A, et al. A *Campylobacter* gene associated with immune-mediated neuropathy. *Nature Medicine*, 2001

As the molecular mimicry hypothesis was considered an excellent paradigm, the elucidation of the biosynthesis pathways of bacterial ganglioside-like structures became an area of great interest. Godschalk and van Belkum demonstrated for the first time that specific bacterial genes involved in the biosynthesis and transfer of sialic acid were crucial in determining specific LOS loci that subsequently induce anti-ganglioside antibodies and trigger the onset of GBS. Knockout mutants of *C. jejuni* that lacked these genes, unlike the wild types, showed reduced or no reactivity with sera from GBS patients. Thus, a new and putative GBS marker gene was identified in *C. jejuni* [18,19].

Yuki N, et al. Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, 2004

For a long time, the reproduction of the disease in an animal model remained the ‘missing link’ that would help fulfil Koch’s and Witebsky’s postulates. Prior animal studies had already shown that immunization and infection with *C. jejuni* or purified LOS results in a cross-reactive anti-ganglioside immune response. In 2004, Yuki and colleagues demonstrated that the sensitization of rabbits with a brain ganglioside mixture containing GM1, GD1a, GD1b and GT1b leads to high anti-GM1 antibody titres and an acute flaccid paralysis with clinical, electrophysiological and histopathological features resembling GBS. Experiments with purified *Campylobacter* LOS led to similar results. *Campylobacter*-induced GBS is currently the best-studied autoimmune disease implicating molecular mimicry as mechanisms of disease. GBS is therefore a perfect model to study the intriguing aspects of post-infectious immune-mediated diseases [20,21].

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24

The Genetic Basis of Guillain-Barré Syndrome: Susceptibility and Outcome

Karin Geleijns

Introduction

In the last 100 years many great scientific papers have been written about the progress made in understanding the pathogenesis of Guillain-Barré syndrome. The roles of antecedent infection, molecular mimicry and anti-ganglioside antibodies have been extensively described elsewhere in this monograph. In this chapter I focus on the contribution of genetic host factors in the pathogenesis of GBS. Why do we think that host factors are involved? What are ways to assess their contribution to disease susceptibility or severity? What are the results of these studies? What would be a direction for further studies?

Why Do We Think That Genetic Host Factors Are Involved?

In GBS the variety in preceding infections, clinical signs and symptoms, and severity of the disease is large. Even in subgroups of patients who had the same antecedent infection the clinical signs and symptoms are heterogeneous, suggesting a contribution of host factors. Other reasons to think that host factors are involved are reports of GBS within families, the increased recurrence risk of GBS or the low incidence of GBS after a common infection, which I discuss in the next part of this chapter.

Familial Guillain-Barré Syndrome

Saunders M, Rake M. Familial Guillain-Barré syndrome. The Lancet, 1965; Geleijns K, et al. The occurrence of Guillain-Barré syndrome within families. Neurology, 2004

In 1965 M. Saunders and M. Rake reported a brother and sister who had both developed GBS with a time interval of 4 years [1]. They both were 70+ years old and had the same preceding symptoms. Since then a few similar family reports have been published. In the largest report, published in 2004, 20 families in the Netherlands, each with at least 2 members who had GBS, were contacted. As some refused to participate or had uncertain diagnoses, 12 of those families were included in the study [2]. The patients were first-, second- or third-degree relatives. The prodromal illness, clinical features and severity of the disease varied between the affected members within the families. Although this is the largest report of families with GBS, it is not sufficient to prove the occurrence of GBS within families. Factors that may suggest a genetic susceptibility were the slightly more frequent occurrence of GBS within siblings (2.6 fold

increase compared to the expected incidence), the observed earlier onset of GBS in successive generations and patients who had 4 episodes of GBS.

Recurrent Guillain-Barré Syndrome

Kuitwaard et al. Recurrent Guillain-Barré syndrome. Journal of Neurology, Neurosurgery & Psychiatry, 2009

Although GBS is a monophasic disease, some patients, as mentioned above, have more than one episode of Guillain-Barré syndrome. Previous studies estimated a recurrence rate of 2–5% [3,4]. Kuitwaard and colleagues reported the largest cohort of patients with recurrent GBS [5]. They investigated whether the neurological symptoms or antecedent infections were similar in each patient episode and whether the patients with subsequent episodes could be distinguished from those with nonrecurrent GBS. They identified 32 patients who had, in total, 81 episodes of GBS: 7 patients had 3 episodes, 2 had 4 episodes, 2 had 5 episodes, and the other 21 had 2 episodes each. Although the patients with recurrent GBS had different types of antecedent infections, the signs and symptoms during every episode were similar. Other observations were (A) a trend towards shorter intervals between subsequent recurrences, (B) a more severe deficit with each recurrence, (C) all patients with 3 or more episodes were female, and (D) 3 patients with GBS had an autoimmune disease, further indicating that immunological and genetic host factors are involved in the pathogenesis. In comparison with the patients who had nonrecurrent GBS, the patients who had one of more recurrences were younger at first episode, had milder symptoms, and more often had the GBS variant, Miller Fisher syndrome.

Guillain-Barré Syndrome after Outbreaks Part I

Sliman NA. Outbreak of Guillain-Barre syndrome associated with water pollution. British Medical Journal, 1978

The first article that was available for me in English about the incidence of Guillain-Barré syndrome after an epidemic outbreak was the paper by N.A. Sliman. In 1976 in a rural town in Jordan with ~30,000 inhabitants, drinking water seemed to be polluted with *Escherichia coli* during a regular check. At least 5,000 inhabitants developed acute diarrhoea. Only a few stool cultures were positive for shigella and a few blood samples were positive for *Salmonella typhi*. During the third week, patients with peripheral neuropathy were presented at the El-Sult Hospital. Sixteen patients were identified with GBS, of whom 13 had mild symptoms of diarrhoea as a preceding symptom [6]. The outbreak of acute diarrhoea didn't result in a GBS epidemic, suggesting that host factors were involved.

Guillain-Barré Syndrome after Outbreaks Part II

Ang CW, et al. A case of Guillain-Barré syndrome following a family outbreak of Campylobacter jejuni enteritis. The Journal of Neuroimmunology, 2000

Since *Campylobacter jejuni* is the most common antecedent infection in GBS patients it is interesting to look in more detail about the incidence of GBS after an outbreak with this bacterium. Although there have been reports of epidemics of *Campylobacter* that did not result in an outbreak of Guillain-Barré syndrome [7], the estimation is that 1 out of 1,000 will develop GBS after such a *Campylobacter jejuni*

infection [8]. Ang and colleagues reported an outbreak of *Campylobacter jejuni* within a family consisting of parents and 2 sons [9]. The father and his sons had diarrhoea and the serology of these 3 family members indicated a recent infection with *C. jejuni*. Only one son developed GBS. The stool cultures of father and the son without GBS were positive for *C. jejuni*. Only the serum of the GBS patient strongly reacted with the LPS fractions of the cultured *C. jejuni* from his father and brother. Furthermore, only the GBS patient had high titres of IgM and IgG anti-glycolipid antibodies, compared with his father and brother, who had low titres of IgM anti-glycolipid antibodies. HLA-genotyping revealed that the father and unaffected son had HLA-A2/DR4/DQ8 haplotype, which the patient did not have, which suggests that this haplotype might be protective for neurological sequelae.

Genetic Susceptibility Factors

The paper of Ang and colleagues suggests that a certain HLA haplotype might be protective for neurological sequelae after an infection with agents associated with GBS. The highly polymorphic human leucocyte antigen (HLA) system plays a central role in the immune responses by presenting antigens to the immune system. In infectious or autoimmune disorders, as well as in GBS, many studies have been performed to assess whether a certain HLA haplotype is associated with disease susceptibility.

Human-Leucocyte Antigen Class II in GBS

Geleijns K, et al. HLA class II alleles are not a general susceptibility factor in Guillain-Barré syndrome. Neurology, 2005

Since disease susceptibility of autoimmune disease is most closely linked to HLA-DRB1 and HLA-DQB1 alleles, we investigated whether these alleles confer susceptibility to GBS or are related to specific clinical and serological subgroups [10]. One hundred and sixty-four patients clinically and serologically well characterized were genotyped. We found no association between HLA-DRB1 and HLA-DQB1 alleles and GBS. We also reviewed 17 case-control studies which had previously investigated an association between HLA-class I or II antigens and GBS susceptibility and subgroups [11–27]. (See [Table 24.1](#)). With regard to disease susceptibility, most of the studies did not find any association or at most only a weak association that could not be confirmed by other studies. The same held true for subgroup analyses. We concluded that the HLA-system probably does not play a general role in susceptibility to GBS. Maybe this is not so remarkable in that GBS seems not to be a classical autoimmune disorder given that women are not more frequently affected, incidence is not highest during the fertile period and GBS is more often a post-infectious disorder with, most of the time, a monophasic course of disease.

Table 24.1 Overview of case-control studies of HLA-distribution in Guillain-Barré syndrome

| Study group | HLA-class | Cases vs controls | subgroups of patients and its HLA associations |
|---------------------------|--|--|--|
| Geleijns K, et al., 2004b | DRB1 DQB1 | 164 Caucasian GBS patients 207 Caucasian controls | <i>antecedent infection, anti-ganglioside antibodies, disease severity, clinical characteristics, electrophysiological criteria</i> HLA-DRB1*01 is associated with mechanical ventilation |
| Magira EE, et al. | DRB1 DQB1 DQA1 | 47 Chinese AMAN patients 25 Chinese AIDP patients 97 Chinese controls | <i>C. jejuni serology</i> DQ-beta RLD ⁵⁵⁻⁵⁷ /ED ⁷⁰⁻⁷¹ and DR-beta E ^{9V11} H ¹³ associated with susceptibility to AIDP |
| Li H, et al. | DRB1 DQB1 DQA1 | 47 Chinese GBS patients 50 Chinese controls 7 Chinese <i>C. jejuni</i> enteritis | <i>C. jejuni serology, anti-GM1 antibodies</i> no significant differences |
| Ma JJ, et al. | A, B, C DRB1, DQB1 | 81 Japanese GBS patients 87 Japanese controls | <i>C. jejuni serology, anti-GM1 antibodies</i> no significant differences |
| Koga M, et al. | A, B, C DRB1, DQB1 | 35 Japanese GBS patients 58 Japanese MFS patients 112 Japanese controls | <i>C. jejuni isolation, anti-ganglioside antibodies</i> significant association with HLA-B54 and Cw1 and <i>C. jejuni</i> + GBS |
| Monos DS, et al. | DRB1, DRB3 DRB4, DRB5 DQB1, DPB1 | 12 Chinese AIDP patients 31 Chinese AMAN patients 34 Chinese controls | no significant differences between AIDP or AMAN vs controls |
| Rees JH, et al. | DRB1, DQB1 | 97 Caucasian GBS patients 7 Caucasian MFS patients 100 Caucasian controls | <i>C. jejuni serology</i> DQB1*03 associated with <i>C. jejuni</i> + GBS group |
| Chiba A, et al. | A, B, C DR, DQ | 32 Japanese MFS/GBS patients with IgG anti-GQ1b antibodies 51 Japanese controls | <i>no subgroups tested</i> no significant associations |
| Piradov MA, et al. | A, B DR | 30 Slavic GBS patients 150 Slavic controls | <i>no subgroups tested</i> no significant association |
| Yuki N, et al. | A, B, C | 5 Japanese <i>C. jejuni</i> + GBS patients 3090 Japanese controls | <i>no subgroups tested</i> Association between HLA-B35 and <i>C. jejuni</i> and anti-GM1 positive patients with an acute axonal polyneuropathy |
| Hillert J, et al. | DR, DQ, DP | 49 Swedish GBS patients 100 Swedish controls | <i>severity of disease, response to plasmapheresis</i> no significant associations |
| Winer JB, et al. | A, B, C DR | 89 Caucasian GBS patients 210 Caucasian controls | <i>severity of disease, prognosis, antecedent infection</i> no associations |
| Hafez M, et al. | A,B | 32 Egyptian GBS patients 234 Egyptian controls | <i>no subgroups tested</i> A3-B8 significantly increased in GBS |
| Kaslow RA, et al. | A, B, C | 92 USA GBS patients 100 USA controls | A/NJ/76 influenza vaccination no significant association |
| Gorodezky C, et al. | DR | 38 Mexican GBS patients 100 Mexican controls | <i>no subgroups tested</i> HLA-DR3 significantly increased in GBS |
| Latovitzki N, et al. | A, B D | 18 European GBS patients 103 European controls | <i>no subgroups tested</i> no significant associations |
| Stewart GJ, et al. | A, B D | 22 Australian GBS patients 322 Australian controls | <i>no subgroups tested</i> no significant association |
| Adams D, et al. | A, B | 59 Caucasian GBS patients 561 Caucasian controls | <i>no subgroups tested</i> no significant association |

In this table only the significant HLA-associations are represented. The subgroups tested represented are shown in italics. GBS: Guillain-Barré syndrome; AMAN: acute motor axonal neuropathy; AIDP: acute inflammatory demyelinating neuropathy; *C. jejuni*: *Campylobacter jejuni*; MFS: Miller Fisher syndrome

Single Nucleotide Polymorphisms

Another way to assess a genetic contribution to the pathogenesis of GBS is to study single nucleotide polymorphisms (SNPs) in relation to disease susceptibility or severity. These SNPs are widely

distributed throughout the genome and are by definition present in at least 1% of the general population. Within a protein-coding gene these SNPs can be located in (A) the promoter region, which is involved in the transcriptional regulation of the gene expression, (B) the coding region, which is translated to a protein, (C) the intron, which is not translated to a protein but is involved in splicing, and (D) the untranslated region (UTR), which affects the stability of RNA. In this manner an SNP can account for differences in protein levels, altered function of a protein or the absence of a protein.

Immunogenetic Polymorphisms in Guillain-Barré Syndrome

Blum S, McCombe PA. Genetics of Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP): current knowledge and future directions. Journal of the Peripheral Nervous System, 2014

Since 1985 several papers have been published that assessed whether immunogenetic polymorphisms are a susceptibility factor for GBS or are associated with disease severity or other clinical or serological characteristics of GBS. In 2014 Blum and McCombe published a beautiful overview paper in which they listed all performed studies [28]. (See [Table 24.2](#)). The single nucleotide polymorphisms studied were located in genes encoding for cytokines, pattern recognition receptors, proteins involved in complement system, enzymes involved in breakdown of blood-brain barrier, etc. The studies were most of the time single studies performed in small numbers and if studies were repeated the results were different. No clear conclusions can be drawn. In the next part I highlight 2 of these performed studies.

Geleijns K, et al. Mannose-Binding Lectin Contributes to the Severity of Guillain-Barré Syndrome. The Journal of Immunology, 2006

The main question with all the associations between SNPs and disease susceptibility or severity is whether it leads to a functional difference in vivo. In this study we assessed the association between polymorphisms in the gene encoding for mannose-binding lectin (MBL) and disease susceptibility or severity [29]. MBL is a protein involved in the activation of complement systems via the lectin pathway. An association was found between the haplotypes associated with high-MBL activity and GBS susceptibility, and particularly with disease severity. There was also a correlation between high serum levels of MBL and MBL activity in patients with severe weakness, supporting a functional effect of the genetic association in the pathogenesis of GBS.

Table 24.2 List of genetic association studies in Guillain-Barré syndrome other than HLA

| | Genes studied | Link to GBS susceptibility | Authors |
|--------------------------------------|---|---|--|
| CD1 molecules | CD1 a | yes no no | Caporale CM, et al. 2006 Kuijf ML et al. 2008 Wu LY et al 2012 |
| | CD1D CD1E | no yes no no | Caporale CM et al. 2006 Caporale CM et al. 2006 Kuijf ML et al. 2008 Wu LY et al 2012 |
| Pattern recognition receptors | CD14 TLR4 | no no yes | Geleijns K et al. 2004 Geleijns K et al. 2004 Nyati KK et al. 2010 |
| Fcγ receptors | FCGR2A FCGR3A FCGR3B FCRL3 NRC31 | yes no yes no link to severity no link to severity link to severity yes link to disease course | Sinha S et al. 2010 Vedeler CA et al. 2000 Sinha S et al. 2010 Vedeler CA et al. 2000 van Sorge NM et al. 2005 Sinha S et al. 2010 Vedeler CA et al. 2000 van Sorge NM et al. 2005 Sang D et al. 2012 Dekker MJ et al. 2009 |
| Killer immunoglobulin-like receptors | KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1 | no | Blum S et al. 2013 |
| GM markers | IGHG1 | yes no | Feeney DJ et al. 1989 Pandey JP et al. 2005 |

(continued)

Table 24.2 (continued)

| | Genes studied | Link to GBS susceptibility | Authors |
|--------------------------|---------------|-----------------------------|-------------------------|
| KM markers | IGHG2 | yes | Feeney DJ et al. 1989 |
| | | no | Pandey JP et al. 2005 |
| | IGHG3 | yes | Feeney DJ et al. 1989 |
| | | no | Pandey JP et al. 2005 |
| | IGKC | link to antibody production | Pandey JP et al. 2005 |
| Complement system | | | |
| | MBL2 | link to severity | Geleijns K et al. 2006 |
| Cytokines | | | |
| | TNFA | yes | Ma JJ et al. 1998 |
| | | yes | Wu LY et al. 2012 |
| | | link to severity | Geleijns K et al. 2007 |
| | | link to AMAN | Zhang J et al. 2007 |
| | | link to AMAN | Prasad KN et al. 2010 |
| | | link to AMAN | Jiao H et al. 2012 |
| | IL10 | yes | Myrh KM et al. 2003 |
| Apoptosis | | | |
| | FAS | link to antibody production | Geleijns K et al. 2005 |
| Matrix metalloproteinase | | | |
| | MMP9 | link to severity | Geleijns K et al. 2007 |
| Alpha 1-antitrypsin | | | |
| | SERPINA1 | yes | McCombe PA et al 1985 |
| Apolipoproteine E | | | |
| | APOE | no | Pritchard J et al. 2003 |

Adapted from [28].

Wu et al. The effect of TNF-alpha, FcγR and CD1 polymorphisms on Guillain-Barre syndrome risk: evidences from a meta-analysis. *The Journal of Immunology*, 2012

Since the small sample size, heterogeneity of GBS and the expected small effect of individual SNPs, a meta-analysis can improve the power of genetic association studies. In this meta-analysis Wu and his colleagues included genetic association studies if a polymorphism was assessed in more than 2 case-control studies [30]. The largest sample size was for TNF-alpha 308A/G polymorphism: 713 cases and 729 controls, consisting of 4 Asian cohorts and 1 Caucasian. This polymorphism was significantly associated with disease susceptibility in this analysis. In a previously performed study by Prasad and colleagues, the A-allele of this polymorphism was associated with higher circulating TNF-alpha levels in blood in GBS patients with the AMAN variant, suggesting a possible contributing factor to the pathogenesis of GBS [31]. In their meta-analysis Wu and colleagues did not find an association between CD1 genes or FcγR polymorphisms and a risk of developing GBS.

Future Directions

At the start of my PhD project there were no high-throughput techniques to detect SNPs. In the last decade genome-wide assays studies (GWAS) have been performed in infectious and autoimmune diseases, and nowadays whole-exome sequencing is used to discover genetic causes of diseases. Because of the complex genetic traits of GBS and the expected small effects of individual genes, genetic association studies will require large sample sizes of clearly defined subgroups of GBS patients. Another way to search for the genes or gene networks involved is to perform functional studies like that done by Chang and colleagues [32].

Identification of Gene Networks and Pathways in Guillain-Barré Syndrome

Chang KH, et al. Identification of gene networks and pathways associated with Guillain-Barré syndrome. *PLoS ONE*, 2012

To identify gene networks and pathways in Guillain-Barré patients, Chang and colleagues drew blood from Taiwanese GBS patients within 1–2 weeks after disease onset and from 7 healthy volunteers [32]. They performed a genome-wide mRNA expression data of peripheral blood leukocytes by Affymetrix Human Genome U133 plus 2.0 Array. Two hundred and fifty-six genes reached the minimum fold change (> 2), of which 246 genes were upregulated and 10 downregulated. These 256 genes were subjected to a network analysis clustered in the networks by (A) disease and disorder, (B) molecular and cellular functions or (C) physiological system development and function. In these networks they found several interesting genes, a few of which I highlight with potential therapeutic options. Matrix metalloproteinase-9, detected in damaged nerves and associated with disease severity and electrophysiological changes in GBS patients, could be a potential target molecule for therapy since in the animal model experimental autoimmune neuritis (EAN) administration of a MMP-9 inhibitor decreased the disease severity [33–36]. The expression of a member of the cyclooxygenase family encoded by PTGS2 was also upregulated. Since administration of COX inhibitor decreased disease parameters at several levels in EAN this could also be an interesting therapeutic target [37–39]. With regard to canonical pathways, I highlight the ERK/MAPK pathway that is involved in production of proinflammatory cytokines and demyelination [40]. Blocking this pathway may be also a therapeutic target. In my opinion this study shows another possibility to further dissect the genetic contribution in the pathogenesis of GBS.

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CLINICAL INVESTIGATION

25

Guillain-Barré Syndrome—Pathology

Claudia Sommer and Klaus Toyka

Introduction

What is the essence of GBS, and can any part of it be understood by looking at pathology? Which parts of the nervous system have been studied, and are these the ones that give us insight into the formal pathogenesis and pathophysiology of GBS?

The early reports starting some 60 years ago include mostly postmortem evaluations with very limited clinical and electrophysiological data. With the advent of artificial ventilation and other advances in intensive care medicine, fortunately, fewer patients run a lethal course nowadays in optimal clinical settings. The remaining lethal cases are autopsied very rarely. This causes a fundamental bias when analyzing older studies. Over the last 20 years, patients fulfilling contemporary diagnostic criteria do not have sural biopsies done except with a clear research protocol. Hence few studies have reported on the sural nerve and only occasionally on motor nerves. Technically, electron microscopy (EM) and teased fibre preparations dominated the field later, followed by light microscopic immunopathology. Most recently, skin biopsies entered the field, and findings were correlated to sural nerve pathology. We here describe the pivotal reports delineating the inflammatory nature of GBS published between 1948 and 1996 and discuss some of the later studies elucidating disease mechanisms in GBS, in comparison to its animal model experimental autoimmune ('allergic') neuritis (EAN), where applicable.

Sural nerve biopsy limits information to a very short moment in time, to a rather small area of nerve and to a less affected part of the peripheral nervous system (PNS). The very few reports on motor nerves underscore this dilemma. Despite these limits, our current hypotheses on the immunopathology have become more precise based on data gained from (A) numerous diligent EAN studies, (B) data from ex-vivo studies on the functional role of autoantibodies and proinflammatory factors (cytokines, chemokines, complement) and (C) data on other biomarkers. Now we may be able to place these findings from human GBS neuropathology collectively into a telling context.

Early Autopsy Studies Find a Polyradiculoneuropathy

Haymaker WE and Kernohan JW. The Landry Guillain-Barré syndrome; a clinicopathologic study of 50 fatal cases. *Transactions of the American Neurological Association*, 1948;
Haymaker WE and Kernohan JW. The Landry-Guillain-Barré syndrome; a clinicopathologic report of 50 fatal cases and a critique of the literature. *Medicine*, 1949

Haymaker and Kernohan's 1948 report was the first on a large and quite heterogeneous group of 50

patients with an acute and ultimately lethal PNS disease [1]. More than a quarter of the group had findings that would not be compatible with later definitions of GBS. These included CSF cell counts of up to 110 cells per microlitre and perivascular CNS infiltrates; some had meningeal lymphocytic infiltrates. Haymaker and Kernohan in their 50 autopsy cases, most of whom had died of respiratory failure, described perivascular lymphocytes in the white matter in 25% of their cases [1,2]. They found mild cellular infiltration in the meninges in some cases. Anterior horn cells were affected by mild chromatolysis in about the same percentage. In the PNS spinal root, infiltration was predominant, which they considered a secondary feature and which led them to the assumption that the patients did not have an inflammatory type of neuropathy.

Since their patients had died between 2 and 46 days after the onset of disease, the authors had the chance to look at early and late pathology and to speculate on the sequence of events in the peripheral nervous system. They describe nerve oedema during the first 3 to 4 days, focal swelling of myelin sheaths and irregularity of axon cylinders on day 5, lymphocyte infiltration on day 9 and the presence of macrophages on day 11. Schwann cell proliferation was observed on day 13. In their patients with a pure motor phenotype, only the anterior roots were affected; in those with sensorimotor deficits, they found the same pathology in anterior and posterior roots. Interestingly, the authors interpreted the late and rather scarce presence of inflammatory cells in peripheral and cranial nerves as related to regeneration, not inflammation, and named the disorder a polyradiculoneuropathy—not a neuritis.

What then had these patients suffered from in terms of our contemporary nomenclature? Obviously, a selection of cases by time of death imposes a massive bias and conclusions need to be drawn with care. This was recognized early and led to the landmark experiments on actively immunized rabbit EAN by Waksman and Adams in the early 1950s, which produced enormous momentum to the field [3].

GBS: A Polyneuritis After All?

Krücke W. Die primär-entzündliche Polyneuritis unbekannter Ursache. In Lubarsch O, Henke F, Rössle G, Hrsg. *Handbuch der speziellen pathologischen Anatomie und Histologie; Erkrankungen des peripheren Nervensystems*. Berlin: Springer, 1955; Asbury AK, et al. The inflammatory lesion in idiopathic polyneuritis. Its role in pathogenesis. *Medicine*, 1969

The first comprehensive review of the histopathology GBS and other types of acute inflammatory polyneuropathies was published by Wilhelm Krücke in 1955 [4], with the inclusion of several personal cases. The author discusses in detail the paper by Haymaker and Kernohan but also elaborates on the extensive pathology literature from France, Germany, England and the United States on reported cases back to 1898. In his treatise he favours an ‘allergic’ hypothesis because of the type of lesions, but points out that only experimental work on animals models would tell. He also makes the point that acute inflammatory polyneuritis and parainfectious and postinfectious polyneuritis have the same pathological features and may therefore be the same disease. Of note, the landmark study by Waksman and Adams had not yet appeared at the time of Krücke’s writing. In later years he extensively studied the neuropathology of EAN with J.M. Schröder in comparison to ‘allergic’ polyneuritis in humans.

From their 19 autopsy cases in Boston, Asbury, Arnason and Adams first make the point that GBS is a ‘neuritis’ after all [5]. The authors find that both the temporal course and the basic pathology are well modelled by EAN as described in the same institution [3]. They show massive lymphocyte infiltration in the radial and femoral nerve of 1 patient, lymphocytic and polymorphonuclear infiltrates in the anterior

roots of another, and lymphocytic and polymorphonuclear infiltrates in the cranial and peripheral nerves of others. Even in muscle sections, they found inflammatory infiltrates around the terminal motor nerve branches. Myelin breakdown was observed in motor and sensory nerves. Retraction of myelin at the nodes of Ranvier led to nodal gaps, indicating focal demyelination. In a patient with severe root inflammation, anterior and posterior horn cells were pathologic, and there was astroglial proliferation in the spinal cord. Inflammatory infiltrates had a perivascular preference in several cases. Of interest, several patients survived the GBS and died of other causes later. Denervation atrophy of muscles was seen in those that survived the longest. One lady had recovered from GBS and was able to walk with crutches. She had lived 5 more years before dying from a stroke. Her autopsy showed segmental demyelination with insufficient remyelination, and there were still focal inflammatory infiltrates surrounding endoneurial vessels. These consisted of lymphocytes and occasional plasma cells. A similar pathological picture was observed in a patient surviving for 6 years. The authors conclude from these observations that low-grade inflammatory activity may persist, and that a hypothetical flare-up of this process might underlie recurrent polyneuritis. The authors also conclude that GBS, like EAN, is a “cell-mediated immunologic disorder, in which peripheral myelin is attacked by specifically-sensitized lymphocytes”. Of note, macrophages were not specifically addressed in these light-microscopic analyses nor were they the focus in early EAN pathology.

Are Macrophages the Culprit?

Wiśniewski H, et al. Landry-Guillain-Barré syndrome. A primary demyelinating disease. *Archives of Neurology*, 1969; Prineas JW. Acute idiopathic polyneuritis. An electron microscope study. *Laboratory Investigation*, 1972; Matsuyama H and Haymaker W. Distribution of lesions in the Landry-Guillain-Barré syndrome, with emphasis on involvement of the sympathetic system. *Acta Neuropathologica*, 1967

Wiśniewski and colleagues reported on a young female patient who had progressive tetraparesis and eventually respiratory failure [6]. She died from acute cardiac arrest and had a postmortem examination only 4 hours after her death. No pathology was seen in the CNS (*vide supra*, report by Haymaker & Kernohan [1]). In the PNS intermodal demyelination was found, with macrophages entering the myelin sheath, phagocytosing myelin debris, and presumably activated lymphocytes were identified near the lesions. In addition, net-like and vesicular myelin changes were noted. These authors cite the earlier observations by Krücke [4]. Shortly thereafter, the sural biopsy from a Japanese boy with GBS was reported by Miyakawa [7], including EM presenting all the features described by Wiśniewski’s group.

In the single-author study by Professor John Prineas of Sydney, sural nerves obtained by biopsy were studied, allowing better tissue preservation and excluding terminal disease pathology at autopsy [8]. Of note, 6 out of 10 patients had received corticosteroids before the biopsy, which may have downscaled any inflammatory signs. Perivascular lymphocytic infiltrates were seen but were considered minor. Two types of myelin degeneration could be observed: one resembling Wallerian degeneration, the other one showing myelin debris as small and aligned along the nerve fibre, with myelin phagocytosed by an invading mononuclear cell leaving the axon intact. Prineas named this ‘active primary demyelination’. On EM the pivotal findings were macrophages penetrating the myelin sheaths through gaps in the basement membrane and leading to lysis of major dense lines while stripping away the outermost laminae of the myelin sheaths. The same cells then phagocytosed this myelin, such that debris could be seen in their cytoplasm. Macrophage processes were found particularly to be burrowing their way along minor dense lines

(intraparallel lines). The less commonly observed vesicular dissolution of myelin appeared to be mediated by macrophages in the presence of lymphocytes. Demyelinated axons were then surrounded by polymorphic mononuclear cells. Prineas concludes that amongst the possible pathomechanisms, an antibody-mediated process and a direct cytotoxic attack by lymphocytes were both unlikely, while the ‘chief effector agents’ were supposed to be macrophages ‘with a specific affinity for myelin’. Prineas also showed the preferential location of macrophage attack at internodes near the paranode (Figure 25.1).

In a second large series from Bordeaux, France [9], the main findings of Prineas were confirmed on 65 patients while in some patients mononuclear cells had invaded the fibre between the myelin and axon and occasionally inside the axon. This was later shown to be a typical feature for the Chinese GBS-like syndrome named acute motor axonal neuropathy (AMAN) [10]. A variant of an axonal type of GBS was first described by Feasby and colleagues in 5 Canadian patients with clinically quadriplegic acute GBS and completely inexcitable peripheral nerves on electrophysiological examination [11]. Nerve biopsies showed predominant axonal damage. One patient died and on autopsy was found to have massive axonal degeneration without inflammation or demyelination [11].

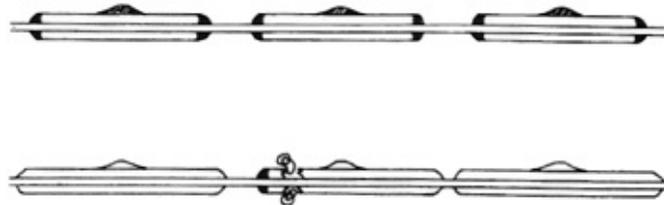


Figure 25.1 Simple cartoon proposing how in GBS a focal attack of mononuclear cells at the internode may widen the node of Ranvier and amputate part of the Schwann cell (bottom) as compared to a primary Schwann cell disease (top). Modified with permission from [8].

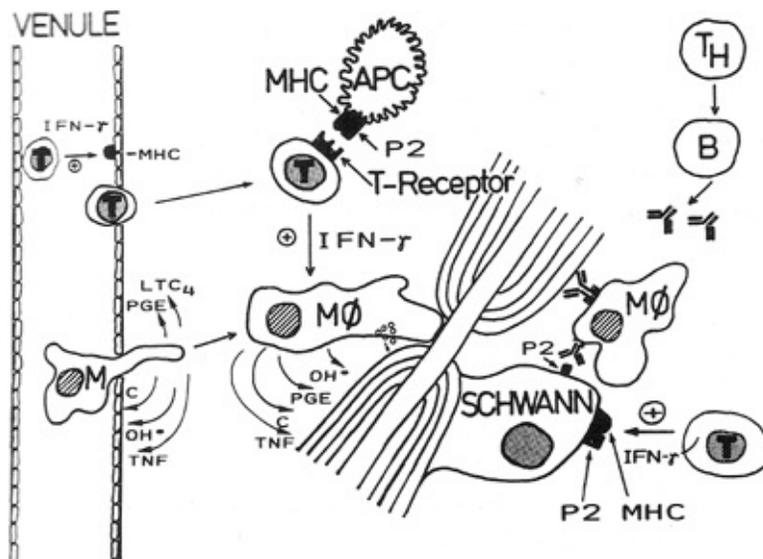


Figure 25.2 Simplified scheme showing some major putative mechanisms in immune-inflammatory polyneuropathies. APC, = antigen presenting cell; TH = T-helper lymphocyte; B = B-lymphocyte coated with antibodies and once transformed to plasma cells secrete antibodies directed at myelin antigens (AG), e.g. protein P2. Macrophages may directly attack nerve fibres by producing proinflammatory factors: OH·= hydroxyl radicals; PGE = prostaglandin E and other eicosanoids; c = complement; TNF = tumor necrosis factor family; IFN γ = Interferon gamma; LTC $_4$ leucotriene C $_4$. Macrophages may also act by binding antibodies (arming) through their Fc receptor; granules secreted from the macrophage cytoplasm indicate potentially myelinotoxic lysosomal enzymes; Schwann cells could also be attacked by cytotoxic T cells since they express major histocompatibility complex II (MHC) and myelin antigens. Note that rare mast cells are not depicted. Modified with permission from [27].

The question as to whether the autonomic nervous system is also part of GBS was addressed by several authors. In the study by Asbury, Adams and Arnason (*vide supra*) and in a report by Matsuyama and Haymaker, inflammatory cell infiltrates were described in sympathetic ganglia [12].

Much later, pathological studies on GBS sural nerves and numerous rat EAN experiments led to several new observations which allowed putative immune mechanisms to be proposed including activation of macrophages armed by myelin-specific antibodies (Figure 25.2). More recently, using modern MRI technology and specific labelling techniques, Stoll and colleagues followed hematogenous macrophages dynamically while they were infiltrating their nerve target ('caught in the act')[13].

Evidence from Sural Nerve Biopsies: Inflammatory Infiltrates

Schmidt B, et al. Inflammatory infiltrates in sural nerve biopsies in Guillain-Barré syndrome and chronic inflammatory demyelinating neuropathy. *Muscle Nerve*, 1996

With the advent of immunohistochemistry, inflammatory infiltrates in nerve biopsies could be more precisely differentiated. In a 1996 study in Würzburg, Germany, Schmidt and colleagues took sural nerve biopsies from 22 GBS patients with moderate to very severe disability. All but 2 had a full course of IVIg or plasmapheresis treatment before the biopsy was taken and 5 had no overt sensory deficit. Schmidt and colleagues identified and quantified endo- and epineurial perivascular T-lymphocytes in most specimens (Figure 25.3)[14]. Most of these were of the CD4 type. Macrophages (CD 68) were abundant and often appeared in large clusters, mostly associated with endoneurial blood vessels. Other macrophages were diffusely scattered throughout the endoneurium. The number of T cells was correlated with clinical data. Patients with clinical sensory involvement or with a later time point of biopsy had higher lymphocyte numbers in the sural nerve than their counterparts. Also, macrophage numbers were higher in biopsies taken later in the disease. Surprisingly, the numbers of macrophages were not higher in patients with hyperacute courses. On the contrary, those cases had fewer macrophages than patients with acute to subacute GBS. Thus, the old idea of inflammatory cells being involved first in degeneration and subsequently in regeneration [1,2] might be supported by these findings. One permanently disabled patient slowly deteriorated after 10 years at which time the other sural nerve was biopsied. Numbers and patterns of inflammatory cells were in about the same order as during the acute stage.

Infiltrating macrophage clusters as observed here were also seen in sural nerves from the 13 patients with CIDP [14], which was later confirmed by a larger study, and this finding turned out to become a useful diagnostic marker [15].

Are Motor Nerves More Informative in GBS?

Hall et al. Motor nerve biopsy in severe Guillain-Barré syndrome. *Annals of Neurology*, 1992

Given that GBS is clinically more a motor than a sensory neuropathy, a motor nerve might be more informative as to the pathophysiology than a sensory nerve. Along these lines, Hall and colleagues describe the biopsy findings of a terminal branch of the musculocutaneous nerve in a 55-year-old GBS patient with hyperacute GBS after a respiratory infection, needing artificial ventilation arising already on day 2 [16]. The patient had a very severe course and responded poorly to therapeutic plasmapheresis. He even further deteriorated, ultimately showing near quadriplegia, external ophthalmoplegia and facial and

bulbar palsy. In order not to miss a diagnosis, a musculocutaneous nerve biopsy was performed on day 16. Demyelination was patchy, with one fascicle much more affected than the second one. Some lymphocytes could be detected, but the majority of inflammatory cells in the endoneurium and in the epineurium were macrophages. These were laden with myelin debris. Direct myelin stripping by macrophages could be detected, but was rare. In the demyelinated axons, the density of neurofilaments was increased. In spite of the massive demyelination, there was no indication of axonal degeneration. The authors concluded that some of their findings might be due to the late time point, and because the patient had already been treated. This might explain the low number of lymphocytes and the few myelin stripping macrophages.

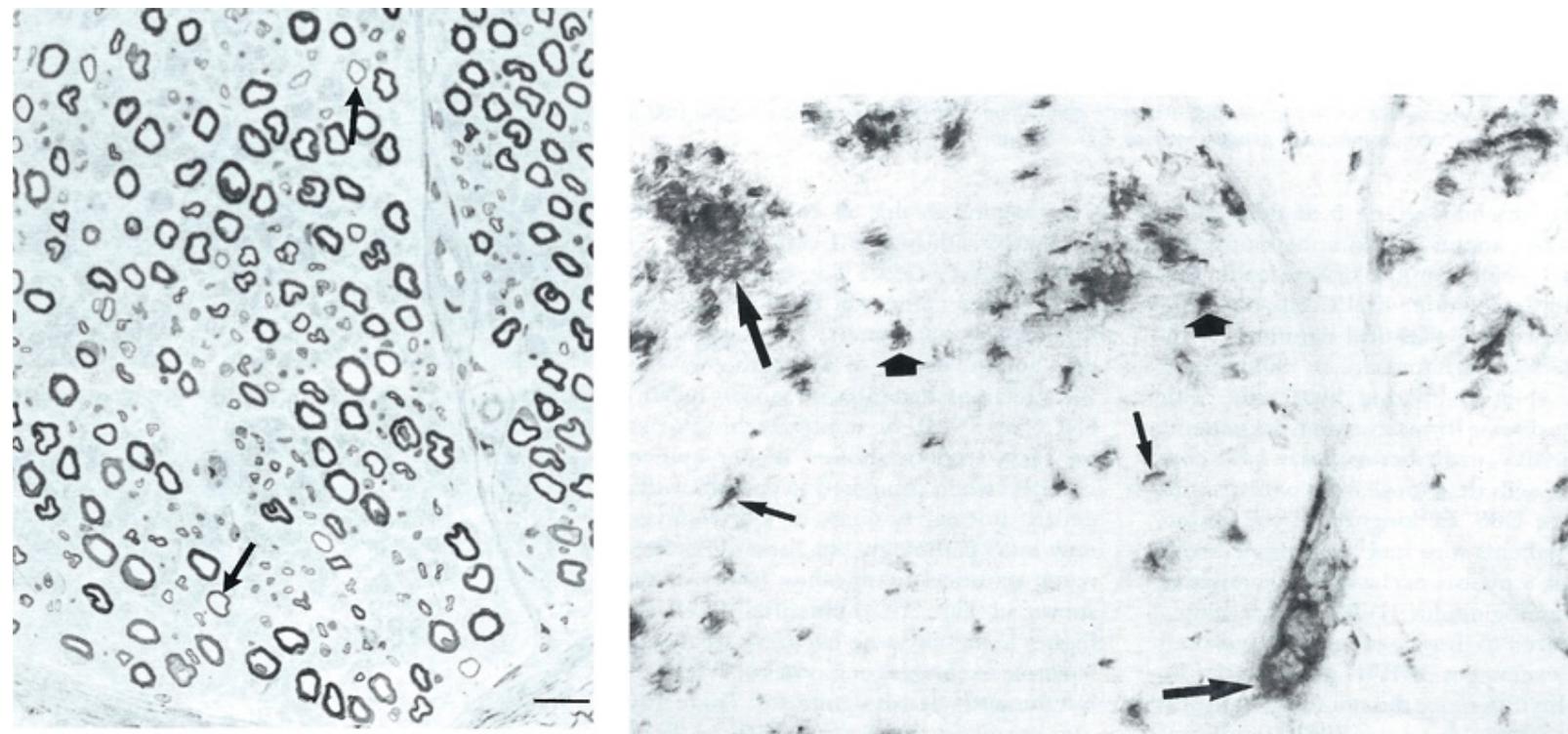


Figure 25.3 Sural nerve from a markedly affected GBS patient. Left: Spur medium embedded nerve stained with toluidine blue showing mild demyelination and mild endoneurial oedema. Right: CD 68+ macrophages were scattered (arrowheads) or in large clusters. Many slim macrophage processes are seen (small arrow) which were not quantified avoiding overestimating macrophage numbers. Bar = 20 μ m. Modified with permission from [14].

Can We Understand the Immunopathogenesis from Sural Nerve Biopsies?

Hafer-Macko CE, et al. Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Annals of Neurology*, 1996; Kiefer R, et al. Enhanced B7 costimulatory molecule expression in inflammatory human sural nerve biopsies. *Journal of Neurology, Neurosurgery and Psychiatry*, 2000

In the series by Schmidt and colleagues ([14], *vide supra*) immunohistochemistry techniques were first included in a large series of biopsies. This was within a period when researchers searched for relevant cofactors in the immunopathogenesis of GBS.

The direct demonstration of the role of circulating ganglioside antibodies and of complement is covered in the chapters by Plomp and Willison, by Yuki and by Uncini and Kuwabara. This field started

with 2 independent observations in the Miller Fisher variant in 1995 [17,18] later followed by similar experiments in GBS (AIDP). Here, active complement had a neurotoxic effect on top of and separate from the immunopharmacological blockade by ganglioside antibodies alone.

Activated complement C3a and C5a had first been shown in the cerebrospinal fluid of GBS patients by Hartung and colleagues [19], which prompted a formal study in EAN by Stoll and colleagues into its role as an important pathogenic cofactor [20]. Stimulated by these investigations, Hafer-Macko and colleagues investigated the presence of complement in GBS by light and electron microscopy [21]. Three subjects with GBS (AIDP) and early death (3 to 6 days after onset) were autopsied and showed a rim of the complement activation marker C3d and the terminal complement complex neoantigen C5b-9 along the outer surface of the Schwann cells. On EM of the positive fibres showed mild vesicular changes of the outermost myelin lamellae. Of note, in the hyperacute patient this was seen in some fibres with no macrophages as yet. Vesicular degeneration was seen before the invasion of macrophages. The authors proposed complement activation as a definite mechanism in GBS.

Kieseier and colleagues examined 5 sural nerves from the Würzburg sural nerve collection for the presence of novel proinflammatory mediators, namely metalloproteinases MMP 7 and MMP 9 that could be produced by macrophages and endothelial cells candidates for causing breakdown of the blood-nerve barrier [22]. The authors showed ring-shaped immunoreactivity around epineurial blood vessels ([Figure 25.4](#)). Similar findings were obtained in the sciatic nerve of adoptive transfer EAN rats. Moreover, the expression of MMP-9 and of its proteinase activity was highest at the peak of the model disease ([Figure 25.5](#)).

There were also attempts to further elucidate the role of macrophages as antigen presenting cells in nerve tissue in GBS and other inflammatory neuropathies. By PCR and immunocytochemistry upregulation and increased expression of costimulatory molecules, B7-1 and B-2 appeared in GBS biopsies but not in control biopsies from hereditary neuropathies, indicating that TH-1-lymphocytes may have been activated in situ by this mechanism [23].

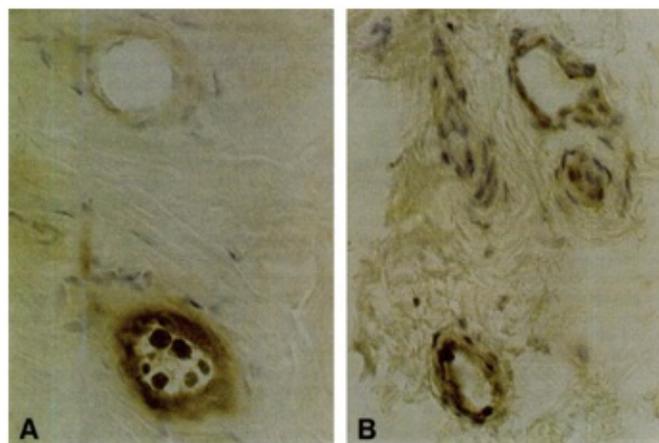


Figure 25.4 in 4A MMP-9 and in 4B MMP-7 show epineurial perivascular immunoreactivity.

Reproduced with permission from [22].

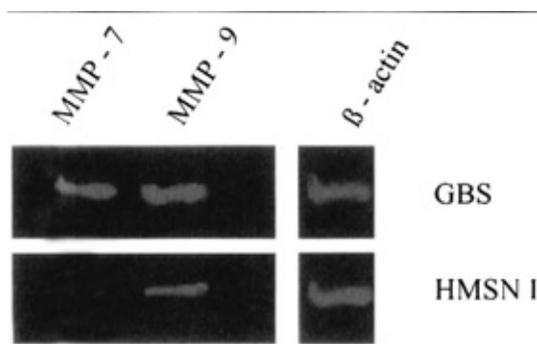


Figure 25.5 Qualitative PCR blots are shown in a sural nerve extract from a patient with GBS and a control with a hereditary neuropathy.

Reproduced with permission from [22].

GBS Pathology: Skin Innervation

Ruts L, et al. Unmyelinated and myelinated skin nerve damage in Guillain-Barré syndrome: correlation with pain and recovery. *Pain*, 2012

Given that GBS is a primary demyelinating disease, involvement of the unmyelinated axons in the skin would only be expected in cases with secondary axonal damage, or in cases that are clinically diagnosed as GBS but that may in fact belong to the AMAN spectrum. However, as always, one does not know for sure until the question is properly examined. Ruts and colleagues investigated skin biopsies from 32 GBS patients [24]. Biopsies were taken from the distal leg and from the lumbar region at 2 time points, the first in the acute phase of the disease and the second at a 6-month visit. Patients in the acute phase were biopsied between week 1 and week 3 after disease onset. Twenty-four patients were available for follow-up. Assessing the density of intraepidermal nerve fibres, that are generally considered to be C-fibres, the authors found a reduction of intraepidermal nerve fibre density (IENFD) in 41% of their patients in the distal leg, and even in the lumbar region, the average IENFD was reduced compared to normal controls. IENFD was lower in those patients who were biopsied in the third week compared to those biopsied in the first week, indicating a decline over time. Interestingly, even 3 patients with ‘pure motor’ GBS (who, however, had a complaint of pain) had reduced IENFD values. Overall, pain intensity was higher in patients with lower IENFD.

At follow-up, patients still had a lower median IENFD than in controls, both at the distal and at the lumbar biopsy site. In some patients, IENFD even had decreased further at 6 months. Only 4 patients recovered to normal values at the distal leg. In those patients who had normal IENFD in the acute phase, values mostly remained normal at follow-up. Looking at the clinical data, low IENFD in the acute phase correlated with a poorer GBS disability score at 6 months and with overt dysautonomia.

Since the dermis, particularly from proximal regions, contains myelinated nerve fibres, the authors were also able to assess myelination and the morphology of Ranvier nodes. As expected in a demyelinating disease, the stain for myelin basic protein was weaker than in control nerves. Intriguingly, T lymphocytes and macrophages were seen surrounding dermal nerve bundles with degenerated myelin sheets, and there seemed to be tight contact between some of these inflammatory cells and the nerve fibres. Thus, the minimally invasive skin biopsy may be another method to catch macrophages ‘in the act’ in GBS and related diseases.

Does Every Acute Auto-Immune Polyradiculoneuritis Qualify as GBS?

Doppler K, et al. Destruction of paranodal architecture in inflammatory neuropathy with anti-contactin-1 autoantibodies. *Journal of Neurology, Neurosurgery and Psychiatry*, 2015

Some patients experience an acute polyradiculoneuropathy fulfilling all features of GBS; they even improve upon the standard treatments, but they later have relapses or a chronic progressive neuropathy. This course of events has been named 'CIDP with acute onset', or, formerly, 'chronic GBS'. Recently, different types of autoantibodies have been found to be associated with acute onset CIDP (aCIDP), among them antibodies to paranodal proteins (for a review of this, see [25]).

Doppler and colleagues studied 4 patients with a GBS-like onset of a disease that later appeared like CIDP, in whom they found high-titre antibodies to the paranodal protein contactin-1 [26]. These patients responded to IVIg as primary treatment for their assumed GBS, but then relapsed, and later had good responses mainly to plasmapheresis and Rituximab, that is, treatments which reduce the autoantibody load from the system. Skin biopsies were available from 2 and sural nerve biopsies from 3 of the patients. The sural nerve biopsies showed signs of axonal degeneration and numerous endoneurial macrophages, but not the typical signs of demyelination like thinly myelinated nerve fibres or onion bulbs, although the patients had clear demyelinating features in nerve conduction studies. In contrast, skin biopsies revealed elongated Ranvier nodes as a sign of primary demyelination, whereas 21 concomitantly examined patients with GBS did not show this finding. In 1 patient, a follow-up skin biopsy available 6 years later showed complete depletion of myelinated fibres. Furthermore, in the patients with contactin-1 antibodies, the nodal and paranodal architecture was profoundly altered. These patients had loss or destruction of the immunoreactivity for caspr and neurofascin, which was found neither in the 21 patients with GBS nor in 49 patients with CIDP. Sodium channels were no longer clustered at the node. In summary, in a patient with a GBS-like disease that later turns out to have a CIDP-like course, paranodal antibodies may be involved in the underlying pathology. Sural nerve biopsy may show a misleading 'axonal' pattern.

Summary and Conclusions

There is abundant evidence that GBS is an immune-mediated, acute or subacute PNS disorder. Several immunological markers have been identified, some of which are strong candidates as pathogenic factors.

At various meetings, the indication for nerve biopsy has been discussed between experts in the field. With our present armamentarium of clinical and electrophysiological testing, and new diagnostic procedures such as PNS-magnetic resonance imaging and high-resolution ultrasound, the indication can be questioned. Only in the context of new research hypotheses may nerve biopsies be justified. Unfortunately, postmortem examinations have become so rare that clinical-neuropathological correlations have become an exception.

Acknowledgements

We thank our late friends and colleagues Byron Waksman, John W. Griffin and W. Ian McDonald for very helpful discussions and collaborative work over the past 35 years in elucidating some principal features of GBS and its animal models. We also thank our mentors, partners and former trainees in the research on GBS, including J. Michael Schröder, John Pollard, John Prineas, Hugh Willison, Richard Hughes, RDM Hadden, Guido Stoll, Hans-Peter Hartung, Ralf Gold, Brigitte Buchwald, Graham Harvey, Reinhard Kiefer, Beate Schmidt, Jürgen Zielasek, Juan Archelos and Bernd Kieseier, for their invaluable contributions.

We also thank the international experts who founded the Peripheral Nerve Study Group in the mid-1970s, which became the Peripheral Nerve Society in 1994, for fostering a face-to-face exchange of data and hypotheses that moved the field forward.

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26

The CSF in GBS, from Historical Origins to Future Perspectives

Axel Petzold

Introduction

At the time of writing this centenary chapter, the diagnostic value of cerebrospinal fluid (CSF) testing in Guillain-Barré syndrome (GBS) has, ironically, declined, whilst the diagnostic value of electrophysiological tests have been on the rise. The irony here is that the CSF examination was the test Guillain himself brought to the historical arena whilst electrophysiology was used by the historically neglected Strohl. One hundred years later, data from electrophysiological studies have profoundly shaped our concept of GBS and what little the CSF has contributed to the story my personal 'Top 10' will tell.

Quincke, Germany, 23rd July 1891

The first lumbar punctures (LP) were performed by Heinrich Quincke at the University of Kiel, Germany [1]. He used the technique for treatment of hydrocephalus in children. The idea stood the test of time. To treat hydrocephalus there are at present routine neurosurgical techniques to insert a range of shunts into the ventricles or lumbar canal which enable controlled CSF outflow by means of reservoirs and pressure-sensitive valves. Another observation Quincke made was that of a traumatic tap. His fourth attempt of an LP in a 7-year-old girl on the 23rd of July 1891 resulted in documented blood contamination [1]. Again his observation stood the test of time. Presently, we can distinguish a traumatic tap from any intracranial bleed, and those who believe that a small yellow drop cannot readily be seen in a large red puddle make use of calibrated spectrophotometry to this purpose [2].

Quincke himself gave credit to Essex Wynter for publishing the first description of the technique of lumbar puncture [1]. Heinrich Quincke was present at the ninth Jubilee of the University of Glasgow (1901) as a German delegate and received an honorary degree.

Essex Wynter, United Kingdom, February 1889

The first publication on the LP technique was by Walter Essex Wynter at the now-demolished Middlesex Hospital in London [3]. The first time Essex Wynter tried the technique was in a 3-year-old boy with tubercular meningitis in February 1889. Four drachms of liquor were collected, besides what was lost due to leakage. Following the procedure the boy transiently improved before passing away. Autopsy

showed an intact spinal equina, but no trace of the LP in the theca. Such traces were found in the next 3 cases, none of whom survived due to the primary disease, tuberculosis. Based on the results of the meticulous post-mortem examinations, Essex Wynter summarises “though none of these cases were ultimately successful, no harm in any one resulted from interference.”

Intriguingly, Essex Wynter documents that there was no trace of albumin or sugar in 2 of his 4 cases. Retrospectively, this was most likely related to the analytical sensitivity of the tests used at the time, but his observation is a good reminder that absence of evidence is not evidence for absence.

La VI^e Armée, France 20th September 1916: *la dissociation albumino-cytologique*

On the 20th of August 1916 a 25-year-old soldier presented with weakness of his upper and lower limbs. He came from the famous French Sixth Armée, a corps composed of various disparate French armies. The summer is sorely remembered by the Battles of the Somme with more than one million soldiers killed on both sides between the 1st of July and 18th of September 1916. The Sixth Armée was under the command of General Fayolle, who was joined by the British forces. The walking distance of this soldier was reduced to only 200–300 meters [4]. The LP revealed a clear CSF with an albumin concentration of 2.5 g/L and 2–4 lymphocytes. The LP was repeated on 19th September and an increase of albumin was noted without an increase of the white cell count (WCC). Fifty-nine days after his first symptoms he was able to walk again and was sent home as a convalescent soldier on the 30th of September.

About a week later, 28th August, another soldier felt unusually tired and weak after a 15-km march. He was aged 33 years and seen by the neurologist on 5th September, at which time there was clear weakness of the lower limbs. Again the LP revealed a clear CSF with an albumin concentration of 0.85 g/L and 3–4 lymphocytes. Again a repeat LP on 20th September showed an increase of albumin without a change of the WCC. He started to improve 31 days after his first symptoms and was evacuated behind the battle lines on 1st October.

Surely, whilst the British attacked Flers-Courcelette (15–22 September), whilst the Canadian and New Zealand divisions made their debut, whilst the French attacked Frégicourt and Rancourt, there were 3 French neurologists discussing CSF results from 2 LPs taken within 24 hours with results which had never been observed before. The term ‘*dissociation albumino-cytologique*’ was coined.

Later, Guillain refers to the “hyperalbuminosis of the CSF in the absence of cytologic reaction” [5]. He believes this to be such a consistent feature of the disease that he states, “I refuse to recognize radioculoneuritis with hyperlymphocytosis or hypernucleosis as belonging to the syndrome” [5]. He concludes that “because the virus of polyradiculoneuritis with albuminocytologic dissociation does not destroy nerve paths, progressive improvement and eventual recovery of the patient will be observed” [5].

Guillain also recognised that there was no value in analysing CSF obtained by the more risky suboccipital puncture as opposed to a LP [6].

The perceived relevance of all the ‘Top 10’ described in this monograph will change over time. Some observations will slowly fade into oblivion; others will be rediscovered as being of core relevance. The ‘*dissociation albumino-cytologique*’ deserves to remain in the Top 10 because it was the first reproducible biomarker discovery of its kind, made by 3 neurologists under exceptional circumstances on the 20th of September 1916.

Timeless: Rochester and Rotterdam

Not everyone found the CSF total protein to be elevated consistently. But already the detailed French observations on the first 2 patients permitted to hypothesise that the sensitivity of this test was less in a very early LP compared to a later LP [4], an observation shared by a retrospective study from the Mayo Clinic in Rochester [7]. Extending on these observations, a prospective Dutch trial found an increased CSF total protein only in 50% of patients with GBS if the LP was taken in the first week after symptom onset [8]. The sensitivity increased to 80% if the LP was taken after 2–3 weeks [8].

Some authors suggested a prognostic value of high CSF total protein [9,10] or CSF pleocytosis [11], findings which were not generally confirmed [12–18]. Most authors agree that, taken together, routine CSF findings are of no prognostic value in GBS (reviewed in [19]). Notably all of these studies relied on essentially cross-sectional CSF data and indirect correlative evidence.

One conclusion from these studies is that the main limitations of CSF research remains that serial LPs are neither feasible nor ethical. Therefore, and in contrast to blood sampling and electrophysiological tests, it will remain extremely difficult to obtain valuable longitudinal data.

Another conclusion is that the CSF composition from an early LP will be very different from the CSF composition of a later LP. There might be other advantages and disadvantages to both time points we are not necessarily aware of yet.

Sendai, Japan 2006: CSF Tau Protein

A fascinating observation on the CSF comes from the Tohoku University Graduate School of Medicine in Sendai, Japan [20]. The authors had access to CSF samples taken at an average of about 7 days after onset of GBS with quantitative clinical follow-up data over 6 months. Some did not make as excellent a recovery as Guillain would have insisted to be the case in GBS. These patients were not able to walk without problems and were classified as having a poor outcome. The authors compared the CSF data from those with good outcome ($n = 20$) with those with poor outcome ($n = 6$). The first interesting finding was that the CSF total protein was about half in the latter. The second was that the CSF tau protein concentration was significantly higher in patients with poor outcome (341.7 ng/mL) if compared to those with good outcome (159.6 ng/mL, $p = 0.00026$) [20].

Statistically, ordinal logistic regression analysis showed the CSF tau protein concentration to be the only significant predictor for poor outcome. The other factors included into the statistical model were age at onset, time to nadir, need for ventilatory support, axonal pattern on electrophysiology and timing of LP [20].

The data extends on earlier work on CSF tau protein in GBS by taking a prognostic as opposed to diagnostic perspective [21]. For independent interpretation of these data one needs to remember that tau protein is expressed not only in neurons and their axons, but also in glial cells. Therefore an increase of CSF tau protein can be caused by release from a number of cells. Consequently, an increase of CSF tau concentration will not permit one to be absolutely certain about the cellular origin [22]. It will be interesting to learn if future studies will investigate post-translational modifications and proteolytic breakdown products of this fascinating protein.

London, UK, 20th December 2001: CSF Neurofilament Proteins

Personally, I find it paradoxical to write now about neurofilaments in the Top 10 of GBS because it relates to a moment of complete despair just before Christmas 2001. After 4 years of work with

neurofilament proteins I finally had completed collecting the reference population data for the neurofilament heavy chain (NfH). After analysing the data I was disappointed (Figure 26.1). There was a large scatter of CSF NfH data [23]. The reason for my disappointment was that I had hoped to have chosen an excellent control group, a well-defined demyelinating peripheral nervous disease, to test the hypothesis that CSF NfH levels were a biomarker for neuroaxonal degeneration of the central nervous system. In GBS I had expected normal CSF NfH levels. Did I have to accept the null hypothesis and did this mean that the test I had spent the last 4 years on developing was of little value?

Neurofilament (Nf) proteins are highly specific to the neuroaxonal compartment. Neurons of the central nervous system (CNS) express at least 4 types of Nf proteins, α -internexin, a light chain (NfL), a medium chain (NfM) and a heavy chain (NfH) [24]. In addition, peripherin is expressed in the peripheral nervous system (PNS). Therefore the hypothesis was that an increase of the CSF concentration of either NfL, NfM, NfH or α -internexin would provide indirect evidence for neurodegeneration in the CNS. Likewise, an increase of blood peripherin levels would indicate axonal degeneration affecting the PNS. So the choice of a disease known to represent a demyelinating condition of the PNS seemed ideally suited for testing my hypothesis on CSF NfH.

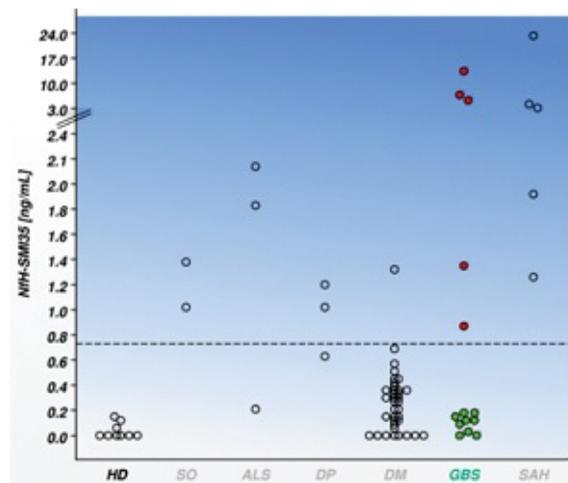


Figure 26.1 Scatterplot of CSF levels in patients with cluster headache (HD), space-occupying lesions (SO), amyotrophic lateral sclerosis (ALS), disc prolapse (DP), demyelinating disease (DM), Guillain-Barré syndrome (GBS) and subarachnoid haemorrhage (SAH). The horizontal reference line (dotted) represents the cut-off (0.73 ng/mL) derived from the reference population; the y-axis is split at 2 ng/mL. Figure reproduced with permission from reference [23].

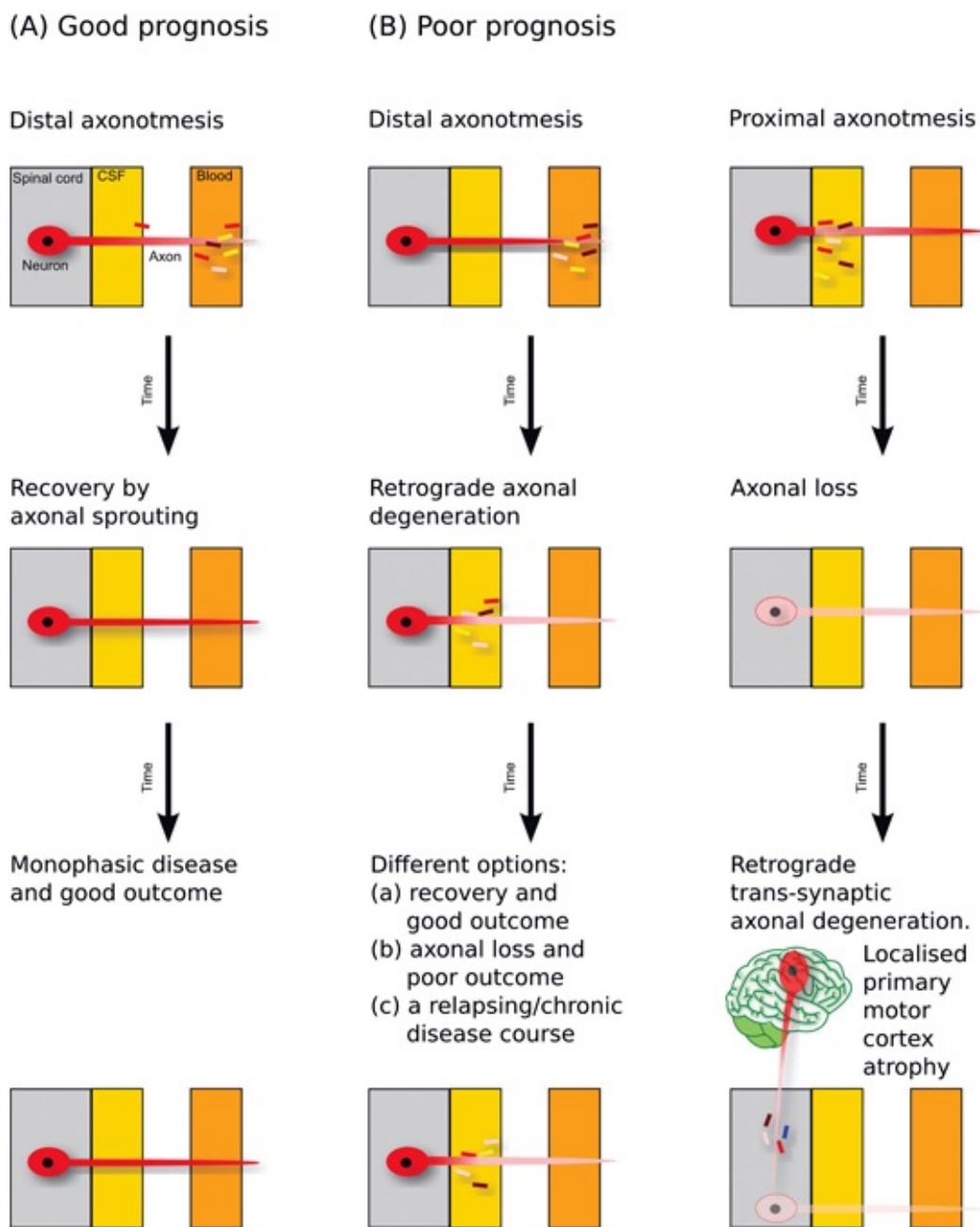


Figure 26.2 Hypothetical relationships of body fluid Nf levels prognosis and disease course in GBS. (A) A good prognosis for a patient with a monophasic disease course who suffers from demyelination alone and/or distal axonotmesis and makes a full recovery. (B) A poor prognosis may follow extensive distal axonotmesis leading to retrograde axonal degeneration and loss or be caused by proximal axonotmesis. In those cases where the motor neuron is lost, trans-synaptic retrograde axonal degeneration might follow and should be demonstrable by longitudinal structural imaging evidence of localised atrophy in the pyramidal tracts and corresponding area of the primary motor cortex.

In retrospect, it appeared that patients with high CSF NfH levels (red dots in Figure 26.1) had a poor outcome [25]. In contrast, patients with normal CSF NfH levels (green dots in Figure 26.1) made a good recovery. But, it took over a decade to better understand the bimodal distribution of the data [22,26–28]. A similar bimodal observation was made for CSF NfL levels [29]. Figure 26.2 summarises my current hypotheses on the relationship of various CSF Nf proteins and GBS.

Japan and Europe (1983–2013): CSF Glial Protein Biomarkers

Glial fibrillary acidic protein (GFAP) and S100B are 2 biomarkers which have been used to provide indirect evidence for glial pathology in GBS [22,28,30]. Both proteins are elevated in the CSF in GBS. It

is likely that this is not a specific phenomenon for GBS, but part of the general glial response to inflammation. As with all measurements, there is a need to be careful that good laboratory standards are followed [31,32]. Potential future studies will need to relate the longitudinal profile of GFAP and S100B to the clinical course. These data will decide if CSF glial protein biomarkers will move from the bottom to the top of the Top 10 list on CSF in GBS.

Never Ending: CSF Anti-myelin Protein Auto-antibodies

One key pathological feature of GBS is damage to the myelin sheath. Conceptually, this has been related to presence of anti-myelin protein antibodies. In fact, over half of the GBS patients from one study did have CSF anti-myelin basic protein (MBP) IgG and IgM auto-antibodies [33]. Findings of auto-antibodies directed against myelin proteins have been regarded as nonspecific, because they are also found in many other diseases. Accepting that some auto-antibodies (anti-MBP) are of little diagnostic value in GBS, there are others which are of high diagnostic value [34]. It will be interesting learning about the longitudinal pattern of such auto-antibodies in, for example, patients who do not follow a monophasic disease course.

Forever: Intrathecal IgG

One pertinent question for CSF research is why there are so few high affinity antibodies detected in the CSF. With the exception of IgG specific to neuroinvasive infections and their complications, such as subacute sclerosing panencephalitis (SSPE), the yield is generally low in autoimmune disease.

It almost seems a paradox that even in a specific CNS disease such as neuromyelitis optica, the use of a highly sensitive cell-based immunoassay for aquaporin 4 auto-antibodies now reaches over 90% diagnostic sensitivity for blood samples yet remains much lower for CSF. Over half a century of research for a diagnostic auto-antibody in multiple sclerosis has not yet brought a result [35]. Why does the lumbar CSF seem to be dominated with intrathecally produced IgG which seems to act more as a smokescreen than to deliver the holy grail? Could it be that high affinity auto-antibodies directed at CNS tissue bind so strongly to the parenchyma that chances are minute for them to be detected with current analytical techniques? This is reminiscent of the absence of albumin and sugar in the CSF of 2 of the patients from Essex Wynter at the end of the 19th century.

Presently, intrathecal IgG trails my table of the CSF Top 10 in GBS.

The Future: A Cocktail from Glasgow

Post-translational modifications govern the interface between protein transcription and translation. For neurofilaments, undoubtedly phosphorylation is of key relevance [24], but I would be hesitant to place this among the Top 10 for GBS. A much more promising mechanism for GBS is glycosylation [36].

Glycosylation mainly targets Asp → Asp-glycan, Ser → Ser-glycan, Thr → Thr-glycan, Hyl → Hyl-glycan, Hyp → Hyp-glycan. Of the 3 types, only N- and O-glycosylation occur in humans. Importantly, protein glycosylation is a hallmark in autoimmune disease. A change of protein glycosylation may trigger an autoimmune attack [37]. Interestingly, glycolysation can differ between tissues with evidence for CNS specific glycosylation patterns. Would it not be wonderful if there were an anatomical map of glycosylation patterns of the PNS and CNS for those protein biomarkers thought to be of relevance in the

disease? Therefore post-translational modifications which only occur in vivo, such as glycosylation, phosphorylation, citrullination, N- and C-terminal modifications will be in my future Top 10.

The interpretation of body fluid Nf levels will need to consider at least 5 proteins (α -internexin (blue), NfL (light red), NfM (bright red), NfH (dark red) and peripherin (yellow) in 2 body fluid compartments (CSF, blood). In the acute phase, proximal axonotmesis within the CSF compartment leads to high CSF NfL, NfM and NfH levels indicate early axonal loss. In cases of where trans-synaptic retrograde axonal degeneration follows, one should also test for CSF α -internexin levels. A rise of CSF α -internexin level should precede visible atrophy on structural imaging modalities. In contrast, high blood peripherin levels in the absence of high CSF NfL, NfM and NfH levels are indicative of distal axonotmesis only. In those cases where axonal sprouting does not occur, distal axonotmesis may continue to develop to retrograde axonal degeneration. This should result in a late increase of CSF levels. Once the motor neuron is lost, trans-synaptic retrograde axonal degeneration may follow. There is also the possibility for a relapsing or chronic disease course to develop, which should be paralleled by a persistent or intermittent rise of blood peripherin levels. One may also expect that the stoichiometry of CSF NfL:NfH levels will change towards NfL, the smaller and therefore less resource-demanding protein to be expressed by the neuron.

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27

The Electrophysiology of Guillain-Barré Syndrome: Our Shared Journeys

Richard A. Lewis, David R. Cornblath and Austin J. Sumner

Introduction

This chapter is reflections on the Guillain-Barré syndrome (GBS) with an emphasis on the electrophysiologic aspects and comes from the exciting time when we were all at the University of Pennsylvania under the chairmanship of Professor Arthur K. Asbury. This was a time and place when investigations of GBS flourished, and electrophysiology was a major component of this exploration. Although we each subsequently moved to different institutions, we continue to share an excitement in investigating and thinking about the physiologic aspects of GBS and other inflammatory neuropathies.

The physiologic hallmark of GBS is conduction block. The rapid onset of symptoms and the frequently observed early recovery of weakness can be attributed to the development and reversal of conduction block. For the 3 of us, understanding the clinical and pathophysiologic nature of conduction block has played a prominent role in our careers.

McDonald WI. The effects of experimental demyelination on conduction in peripheral nerve: a histological and electrophysiological study. II. Electrophysiological observations. *Brain*, 1963.

The McDonald paper was particularly important in the understanding of the electrophysiology of acquired demyelinating polyneuropathies [1]. The extreme dispersion of dorsal root responses illustrated that individual axons conducted at a wide range of velocities indicative of the heterogeneity of demyelination across the population of axons. It also demonstrated that demyelination was distributed multifocally along the length of peripheral nerves, thought to be a consequence of variations in blood-nerve permeability, particularly in nerve roots and nerve terminals. Differential effects on motor versus sensory axons were also recognized. These concepts proved to be particularly applicable to the electrophysiology of acute demyelinating polyradiculoneuropathy. (Please note the reference to the M. Med. Sci. thesis of one A.J. Sumner.)

Asbury AK, et al. The inflammatory lesion in idiopathic polyneuritis: its role in pathogenesis. *Medicine*, 1969.

This careful pathologic investigation of 19 fatal cases of GBS—including both early death and those who initially survived but died later—emphasized the importance of T cells in the development of the disease [2]. The paper was seminal in its impact. It fit nicely with studies of experimental allergic neuritis [3] which also emphasized the role of T cells. Figure 13 from the paper has been shown at many meetings and is a standard at GBS talks worldwide. This paper countered the hypothesis of Haymaker and Kernohan, who considered humoral immunity as an initiating event [4].

Wiederholt WC, et al. The Landry-Guillain-Barré-Strohl syndrome or polyradiculoneuropathy: historical review, report on 97 patients, and present concepts. *Mayo Clinic Proceedings*, 1964.

This early electrodiagnostic paper was one of many which emphasized the notion that the primary lesion in GBS was demyelinating with secondary axonal degeneration [5]. It showed the power of electrodiagnosis in being able to study individuals over time and at many sites in order to best understand the evolution of disease. Wiederholt and colleagues' concepts are still relevant today.

Experimentally-induced conduction block—several publications

In the early 1980s, a series of papers appeared which changed the field [6,7,8,13,14,15]. Using the intraneural injection technique, it was possible to show first that sera from various animal models (EAN, EAE and anti-GalC) and second that sera from GBS patients produced an acute lesion characterized by conduction block associated with minor pathological features, which evolved to complete conduction block and a demyelinating lesion with recovery over time. This experimental approach provided crucial insights into the pathophysiology of GBS. It was a remarkable experience to observe the development of conduction block as the earliest electrophysiologic consequence of the intraneural injection of these antibodies (Figure 27.1) as well as the restoration of conduction which corresponded at around 8 to 14 days (Figure 27.2), to the appearance of 2 to 8 myelin lamellae around each previously demyelinated axon. LaFontaine and co-workers [8], using the elegant biophysical techniques pioneered by Rasminsky, Bostock and Sears demonstrated that conduction block could develop solely from paranodal disruption without (or prior to) segmental demyelination [9,10]. These experiments demonstrated that serum factors from experimental animals or GBS patients could produce demyelination and, along with the concurrent evidence that plasma exchange had a beneficial effect in patients with GBS [11,12], rekindled the concept that humoral immunity was an important component in the pathophysiology of the disease.

As the pathophysiology of GBS was being investigated, clinical electrodiagnosis of GBS was evolving with recognition of the importance of conduction block, that early changes were particularly found in the most distal (low compound motor action potential [CMAP] amplitudes and prolonged distal motor latencies [DML]) and most proximal segments (absent or prolonged F-wave responses).

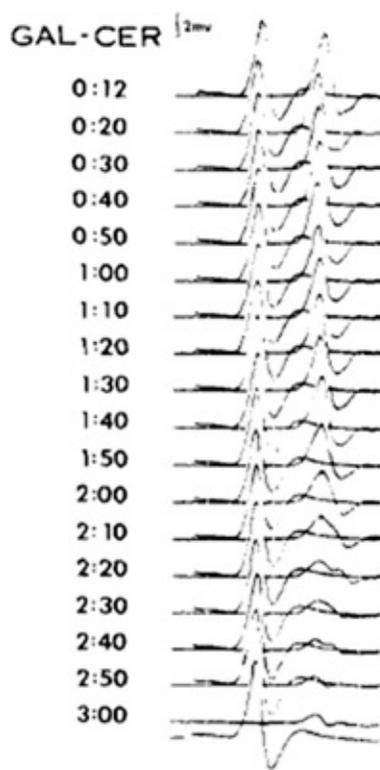


Figure 27.1 Development of acute conduction block induced by intraneural injection of anti-Gal-Cer serum (Modified with permission from Figure 1 of [6]). Serial recordings of rat hind foot with stimulation at the ankle and hip after injection on anti-Gal-Cer in mid-thigh region of sciatic nerve. Note the progressive drop in amplitude from ~1 hour to 3 hours post-injection

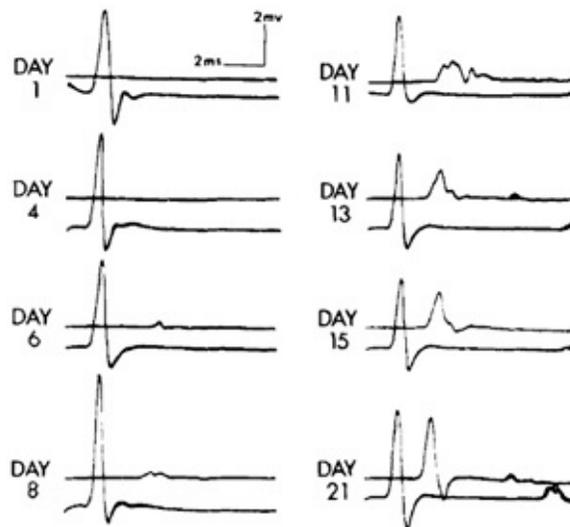


Figure 27.2 Recovery of conduction block after intraneural anti-Gal-Cer serum. (Modified with permission from Figure 6 of [6].) Serial recordings of recovery from complete conduction block induced by anti-Gal-Cer serum. The first evidence of recovery occurred on day 6 with near full recovery by day 21.

Albers JW, Donofrio PD, McGonable TK. Sequential electrodiagnostic abnormalities in acute inflammatory demyelinating polyradiculopathy. *Muscle & Nerve*, 1985.

This series of 180 electrodiagnostic studies of 70 patients with GBS [16] expanded on previous studies by Lambert and Mulder [17] and McLeod [18], and showed that in the first 2 weeks motor amplitude reductions were the most conspicuous early change but by week 5, 87% of patients had conduction slowing suggestive of segmental demyelination. They also recognized the sural sparing phenomenon in

sensory conduction studies. They noted that 3% of patients had only axonal degeneration, and they raised the question of a pure axonal GBS.

Feasby TE, et al. An acute axonal form of Guillain-Barré polyneuropathy. *Brain*, 1986.

Soon after publication by Albers and colleagues, Feasby and co-workers reported 5 patients with axonal GBS [19]. Many in the field were sceptical that this represented a true axonal form of GBS, although both physiologically and pathologically there was no evidence of segmental demyelination or inflammation. Many considered that this may have been the result of severe, aggressive demyelinating GBS and that the findings represented the sequel of this rapidly progressive disease. However, the dramatic findings from northern China made it clear that there was clearly an axonal form of Guillain-Barré syndrome (see below). In support of the axonal form, Hahn and colleagues showed that in experimental animals EAN could be a continuum from pure demyelinating forms with low amounts of antigen to pure axonal forms from high amounts of antigen [20].

Identifying AMAN and AMSAN—several publications

In the 1990s a series of reports [21–25] identified the acute motor axonal neuropathy (AMAN) and the acute motor and sensory axonal neuropathy (AMSAM) forms of GBS, differentiated these from AIDP and identified the nodal changes seen in those disorders. The physiologic consequences of the immune attack on the node of Ranvier has brought to light the importance of conduction block and how nodal pathology and demyelination can have overlapping physiologic findings. This concept has been extended to include more chronic disorders such as multifocal motor neuropathy and forms of chronic inflammatory demyelinating poly neuropathy with antibodies directed against paranodal constituents contactin and neurofascin-155 (see below).

Uncini A, Kuwabara S. Nodopathies of the peripheral nerve: an emerging concept. *Journal of Neurology, Neurosurgery and Psychiatry*, 2015. Querol L, et al. Antibodies to contactin-1 in chronic inflammatory demyelinating polyneuropathy. *Annals of Neurology*, 2013. Querol L, et al. Neurofascin IgG4 antibodies in CIDP associate with disabling tremor and poor response to IVIg. *Neurology*, 2014.

The demonstration of different forms of GBS, from AIDP to AMAN, AMSAN, and Fisher syndrome [26–28] has forced us to reconsider electrodiagnostic criteria for GBS from the days when GBS and AIDP were synonymous [29,30] to those that attempt to differentiate the axonal forms from the demyelinating forms [31,32]. This issue was thoroughly reviewed by Uncini and Kuwabara, with a call for more reliable electrodiagnostic criteria.

Uncini A, Kuwabara S. Electrodiagnostic criteria for Guillain-Barré syndrome: A critical revision and the need for an update. *Clinical Neurophysiology*, 2012.

Criteria have been proposed that attempt to come to grips with the problem of differentiating acute axonal forms of GBS from the AIDP form of GBS in the first week after disease onset [33]. Some propose 2 studies, one within 2 weeks and one after 3 weeks [34]. Others propose that only one study is needed [35]. It is anticipated that the international GBS Outcome Study will provide important information that will lead to an optimal electrodiagnostic approach.

Conclusion

Electrodiagnostic studies remain a crucial investigation of patients with suspected GBS. They have diagnostic and prognostic value. In addition, the neurophysiologic changes in GBS have led to an important understanding of the disease process. As we recognize the complexity of GBS, our approach to electrodiagnostic studies and interpretation is becoming increasingly sophisticated.

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The Emerging Concept of Nodo-Paranodopathies in Guillain-Barré Syndrome and Related Disorders

Antonino Uncini and Satoshi Kuwabara

Introduction

The term ‘nodo-paranodopathy’ was originally proposed to better characterize neuropathies with anti-gangliosides antibodies and overcame some inadequacies of the classical dichotomous classification into demyelinating and axonal [1]. More recently this categorization has been extended to include neuropathies of different aetiology (dysimmune, inflammatory, ischaemic, nutritional and toxic) in which the involvement of the nodal region is central in the pathogenesis [2]. The following studies epitomize the research journey that has led, over the past 20 years, to the conceptualization of dysimmune nodo-paranodopathies. [Figure 28.1](#) illustrates the organization of the nodal region with reference to the key molecular players of this concept.

Hafer-Macko C, et al. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Annals of Neurology*, 1996

By the middle of the 1990s the connections between an acute, motor, primarily axonal subtype of GBS (acute motor axonal neuropathy [AMAN]), preceding *Campylobacter jejuni* infection and anti-ganglioside antibodies were becoming clearer. The Johns Hopkins University group produced detailed pathological studies of fatal GBS cases from the Hebei Province in China providing remarkable insights into the pathogenesis. In 3 seminal papers the authors described extensive Wallerian-like degeneration (with only minimal demyelination and inflammation), almost exclusively of motor fibres, and showed that the earliest and mildest changes consisted of lengthening of the node of Ranvier with, in some instances, involvement of paranodal myelin [3,4]. Most importantly, they demonstrated IgG and complement deposition along the axolemma of motor fibres, particularly at the nodes of Ranvier, prior to the development of Wallerian-like degeneration [5]. It was suggested that “simple binding of antibody, alone or with subsequent activation of complement at the nodes of motor fibres, can be sufficient to impair conduction”. These findings could well explain the puzzling observations of fatal AMAN cases showing only minimal pathologic changes and why some patients rapidly recovered in spite of an initial severe paralysis. The foundation for the concept of nodo-paranodopathy was laid.

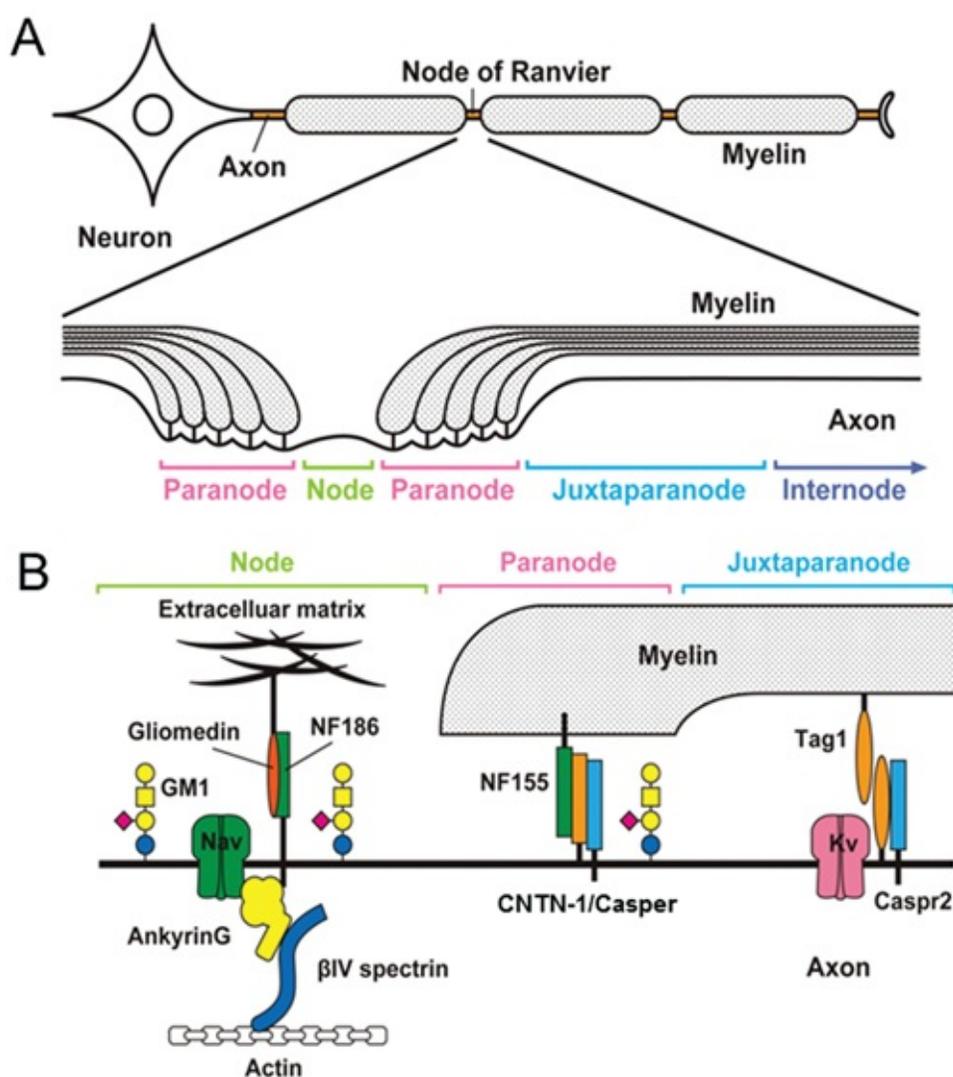


Figure 28.1 (A) Anatomical organisation of myelinated nerve fibre and its subdomains. (B) Simplified molecular organisation at nodes, paranodes, and juxtaparanodes. NF, neurofascin, CNTN-1, contactin-1, Caspr, Contactin-associated protein. TAG-1, transient axonal glycoprotein 1. GM1 ganglioside is enriched at nodal and paranodal axolemma. Modified with permission from Uncini and Kuwabara 2015 [2].

Kuwabara S, et al. Acute motor axonal neuropathy is not only characterized by axonal degeneration. *Annals of Neurology*, 1998

AMAN was initially thought to be characterized pathophysiologically by simple axonal degeneration, and its electrodiagnosis was based on the absence of demyelinating features and reduced compound muscle action potential (CMAP) amplitudes (Figure 28.2A) [6]. In 1998 Kuwabara and colleagues reported AMAN patients with antibodies against ganglioside GM1 who had conduction block (CB) in distal and intermediate nerve segments and conduction slowing which promptly resolved without the development of excessive temporal dispersion (TD) (Figure 28.2C, 28.2D) [7]. This feature, named ‘reversible conduction failure’ (RCF) to distinguish it from demyelinating CB, mimics demyelination on early electrophysiological studies, but sequential recordings did not demonstrate the development of slow components characteristic of demyelination (Figure 28.2E). The authors suggested that conduction failure was caused by “impaired physiological conduction at the node of Ranvier” and that AMAN patients are not only characterized by axonal degeneration but also by RCF [7]. These important electrophysiological observations well correlated with the pathological findings showed by the Johns Hopkins group but remained somewhat disregarded by the neurological community in Western countries.

Capasso M, et al. Acute motor conduction block neuropathy. *Neurology*, 2003

Capasso and colleagues reported 2 patients with antecedent diarrhoea and high titres of IgG antibodies to GM1, GD1a and GD1b who acutely developed symmetric weakness without sensory symptoms. Electrophysiology showed a reduction of distal CMAP amplitudes and early partial motor CB with focal conduction slowing in intermediate nerve segments [8]. In these patients distal CMAPs normalized, and CB and conduction slowly resolved in 2 to 5 weeks in parallel with strength recovery without the development of excessive TD or denervation at electromyography. These patients were initially considered a rare GBS subtype named acute motor conduction block neuropathy (AMCBN). AMAN and AMCBN have in common antecedent *C. jejuni* enteritis and anti-ganglioside IgG antibodies. AMCBN patients show the RCF pattern in most nerves. Therefore, it was hypothesized that AMCBN was an 'arrested AMAN', in which anti-ganglioside antibodies bind to the nodal axolemma and induce RCF not progressing to axonal degeneration [8]. Similar patients were reported under different names (reviewed in Uncini and Kuwabara, 2012) [9]. The description of patients with conduction failure evolving to axonal degeneration (Figure 28.2B) or showing RCF and axonal degeneration co-occurring in the same or different nerves confirms that AMCBN, AMAN with RCF and AMAN with axonal degeneration are a pathophysiological continuum [10–12]. This explains why recovery in AMAN may be either rapid and complete or prolonged with poor outcome in a dichotomous pattern according to the relative amount of axonal degeneration and RCF in each patient [13].

At this point AMAN was electrophysiologically characterized not only by axonal degeneration but also by a reversible failure of conduction, and that both processes could be due to an immune mediated attack to the nodal axolemma became quite certain.

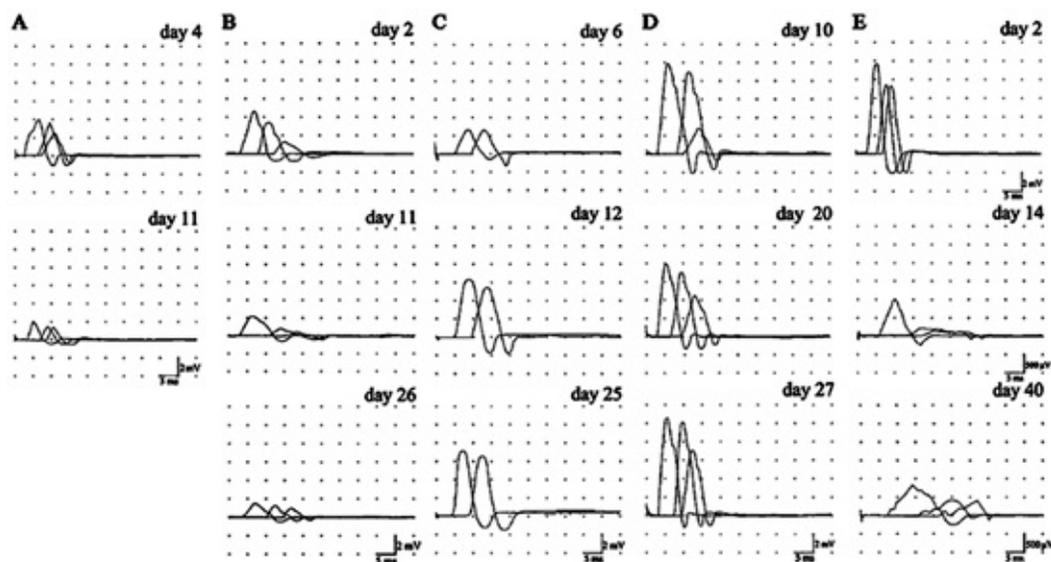


Figure 28.2 Spectrum of motor nerve conduction abnormalities in GBS. Superimposed compound muscle action potentials (CMAPs) recorded from the abductor digiti minimi after ulnar nerve stimulation at wrist, below-elbow and above-elbow, and from the abductor pollicis brevis after median nerve stimulation at wrist and elbow. (A) AMAN with axonal degeneration. Ulnar nerve. Distal CMAP amplitude was already decreased (4 mV) on day 4 and further decreased (2 mV) on day 11. The patient had IgG anti-GM1 and anti-GD1a. (B) AMAN with conduction failure followed by axonal degeneration. Ulnar nerve. Note on day 3 the gradual reduction of CMAP amplitudes from elbow and axilla stimulation followed on day 7 by reduced amplitudes of all CMAPs. The patient had IgG anti-GM1. (C) AMAN with reversible distal conduction failure pattern. Median nerve. On day 6 distal and proximal CMAP amplitudes were reduced (2.6 mV). On day 12 distal CMAP was 142% increased returning within the normal range. There was no excessive temporal dispersion of proximal or distal CMAP in all recordings. The patient had IgG anti-GD1b. (D) AMAN with reversible conduction failure pattern in intermediate nerve segments. Ulnar nerve. On day 10 there was a partial

CB across the elbow which improved on day 20 and resolved at day 27 without the development of excessive temporal dispersion. The patient had IgG anti-GM1, anti-GD1a and anti-GD1b. (E) Acute inflammatory demyelinating polyneuropathy. Ulnar nerve. On day 2 all conduction parameters were normal. On day 14 all CMAPs were dispersed, distal CMAP amplitude was greatly reduced (1 mV), distal motor latency was increased (5.7 ms), the CMAP amplitude ratio between below-elbow and wrist stimulation was 0.2 and conduction velocities were reduced (20 m/s in the below-elbow wrist segment and 26 m/s across the elbow). On day 40 the CMAP amplitude ratio between below-elbow and wrist stimulation was 0.5 but all CMAPs were still reduced in amplitude and dispersed, DML was further increased (7.2 ms) and conduction velocities reduced (19 m/s in the below-elbow wrist segment and 16 m/s across the elbow). The patient did not have anti-ganglioside antibodies. Used with permission from Uncini et al. 2010 [15].

Uncini A, et al. Pitfalls in electrodiagnosis of Guillain-Barré syndrome subtypes. *Journal of Neurology Neurosurgery & Psychiatry*, 2010

The recognition that RCF was a characteristic of AMAN led to a reconsideration of the sensitivity and specificity of the 2 most commonly used criteria sets in the electrodiagnosis of GBS [6,14]. In an Italian population, at first test, the electrodiagnosis was almost identical with both criteria sets: 65–67% of patients were classifiable as acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and 18% as axonal GBS; 14–16% were equivocal [15]. At follow-up, 24% of patients changed classification: AIDP decreased to 58%, axonal GBS increased to 38%, and equivocal patients decreased to 4%. The majority of shifts were from AIDP and equivocal groups to axonal GBS, and the main reason was the recognition by serial recordings of the RCF as expression of axonal pathology. All patients who shifted to the axonal group had antibodies to gangliosides. Similar results were reported in Japanese series [11,16]. These studies demonstrate that in early GBS it may be impossible to distinguish between AIDP and axonal GBS in some patients and indicate that the lack of distinction between demyelinating CB and RCF by serial conduction studies may fallaciously classify patients with axonal GBS as having AIDP. Moreover, these observations explain why, especially in Western countries, antibodies to gangliosides were thought to be associated with AIDP.

Currently employed electrophysiological criteria for diagnosis of GBS subtypes do not require serial studies and do not include RCF as expression of axonal pathology, and therefore are inadequate for the correct diagnosis of GBS subtypes. This highlights the necessity of developing more reliable criteria or a different categorization of axonal GBS with anti-ganglioside antibodies [9].

Susuki K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibres. *The Journal of Neuroscience*, 2007; McGonigal R, et al. Anti-GD1a antibodies activate complement and calpain to injure distal motor nodes of Ranvier in mice. *Brain*, 2010

The most clinically relevant animal model of AMAN was developed by a Japanese group led by Yuki [17]. The sensitization of rabbits with purified GM1 induced flaccid monophasic weakness, high titres of anti-GM1 or anti-GD1b IgG antibodies, IgG deposition on motor axons and Wallerian-like degeneration without prominent demyelination or inflammation. In a further study the same group showed that IgG antibodies bound to GM1, which is strongly expressed at the nodes of Ranvier, and activated complement resulting in the formation of the membrane attack complex at the nodal axolemma and destruction of nodal molecular complexes including Na_v channels [18]. The autoimmune processes caused lengthening of the nodes and paranodal detachment of myelin sheath identical to the early pathology found in AMAN

patients. Anti-GD1a antibodies disrupt the nodes by the same mechanisms. In an ex vivo mouse preparation it was shown that the nodes of Ranvier were targeted by anti-GD1a antibody [19]. Complement deposition was associated with complete loss of nodal protein staining, including voltage-gated sodium channels, and nerve-conduction failure. Both morphological and conduction abnormalities were prevented by eculizumab (a humanised mouse monoclonal antibody which prevents the formation of the terminal complement complex), whereas inhibition of the protease calpain preserved the immunostaining profiles of nodes of Ranvier without protecting nerve conduction.

The above findings indicate that the early immunopathologic stage of AMAN is characterized by an attack to the nodal region and failure of nerve conduction which may still be promptly reversible as in AMAN with RCF or AMCBN. If the immune reaction progresses, calcium entry in the axon triggers protease activation and consequent axonal damage and Wallerian-like degeneration. Macrophages subsequently move from the nodes into the periaxonal space, scavenging the injured axons (Figure 28.3). Experimental models have thus confirmed that in AMAN the major injury site of the antibodies is the nodal region, clarified the immunopathologic cascade and furnished an explanation for the electrophysiologic and clinical correlates.

Susuki, et al. Dysfunction of nodes of Ranvier: a mechanism for anti-ganglioside antibody-mediated neuropathies. *Experimental Neurology*, 2012

The careful reconsideration of different experimental models of neuropathies associated with antibodies to GM1, GD1a and GD1b indicated a common pathophysiologic mechanism characterized by complement mediated dysfunction and disruption of the nodes of Ranvier [20]. In recent years evidence has accumulated that dysfunction/disruption of the nodal region is a pathogenic mechanism not only in AMAN but also in other acute neuropathies with antibodies to gangliosides. RCF has been described in motor and sensory fibres of patients with acute motor and sensory axonal neuropathy (AMSAN) and, similarly to AMAN, abnormally elongated nodes were found in the dorsal roots of patients with AMSAN [21,22]. RCF in motor and sensory fibres has also been reported in patients with the pharyngeal-cervical brachial subtype of GBS [23]. RCF restricted to sensory fibres has been reported in patients with promptly reversible acute sensory ataxic neuropathy and IgG anti-GD1b or -GQ1b antibodies and in the Miller Fisher syndrome which is also known to have a good prognosis [24,25]. Moreover, the sensory nerve biopsy of an anti-GQ1b positive patient with the Miller Fisher syndrome showed lengthening of nodes of Ranvier, myelin splitting and macrophage invasion of the internodal axon without any features of demyelination strikingly similar to the pathological features found in motor fibres of AMAN patients [26].

The experimental and clinical electrophysiological observations indicated that RCF can be found also in sensory fibres and that in different acute anti-ganglioside antibody mediated neuropathies the main site of injury is the same: the excitable axolemma of the nodal region.

Uncini A, et al. Nodo-paranodopathy: beyond the demyelinating and axonal classification in anti-ganglioside antibody-mediated neuropathies. *Clinical Neurophysiology*, 2013

The traditional classification of neuropathies into axonal or demyelinating might generate confusion in diagnosing GBS subtypes. AMAN is classified as an axonal neuropathy because the primary attack is

directed towards the nodal axolemma, leading ultimately to axonal degeneration. However, the term 'axonal' may be misleading as in common neurological knowledge it is linked to Wallerian-like degeneration and evokes poor prognosis, and not everybody agrees on an axonal scenario characterized by transitory dysfunction and prompt recovery as in AMAN with RCF or AMCBN. On the other hand, in GBS patients with anti-ganglioside antibodies and in the experimental models, although the pathogenic mechanism is mainly focused on the node, some demyelinating-like features such as detachment of myelin terminal loops and lengthening of the nodes (but never true segmental demyelination) have been reported. Moreover, RCF, on the basis of a single electrophysiological test, can be misdiagnosed as demyelinating CB, leading to a diagnosis of AIDP.

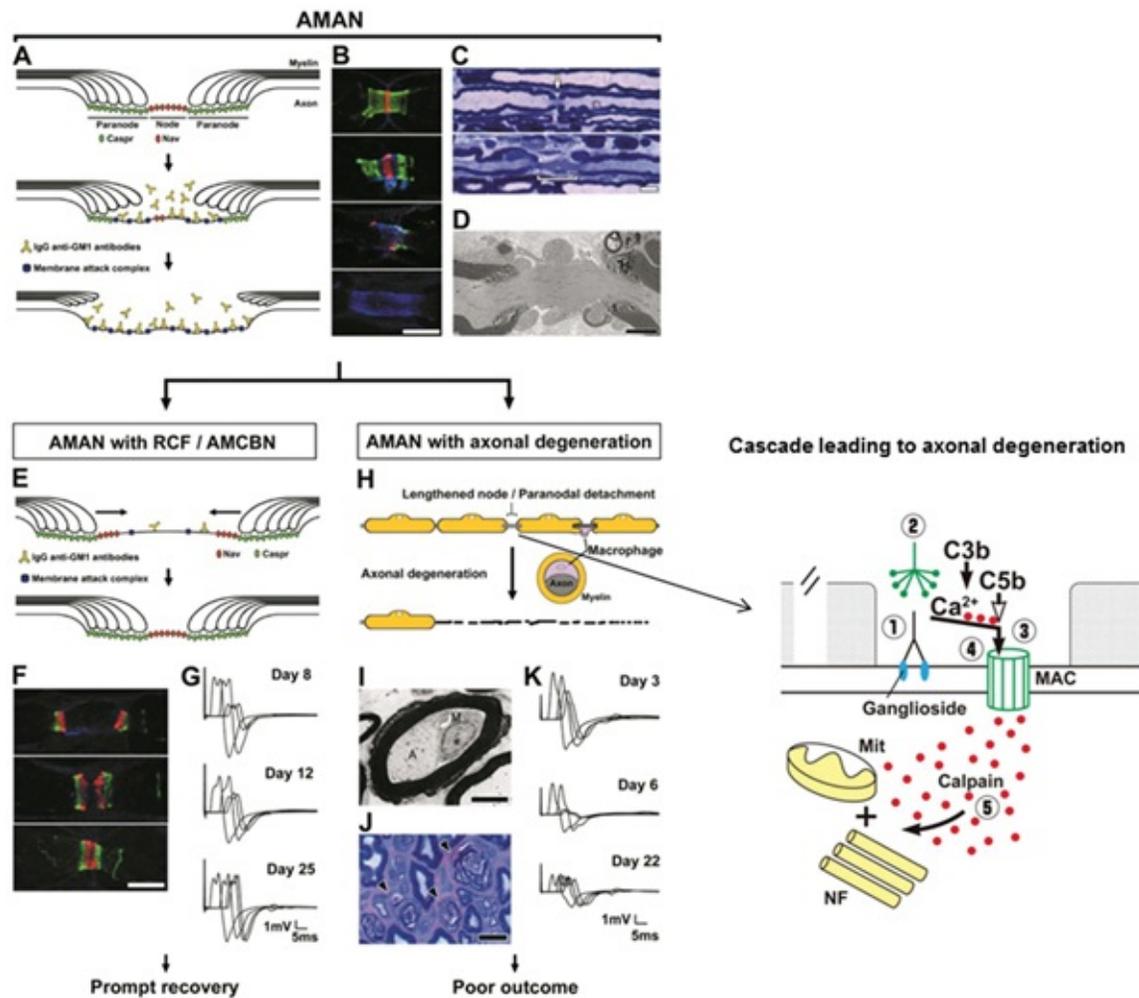


Figure 28.3 The immunopathologic cascade, the electrophysiologic and clinical correlates in acute motor axonal neuropathy (AMAN). (A–D) Acute nodal disruption. (A) Cartoon showing the normal node (top), the early (middle) and advanced (bottom) phase of nodal disruption. Caspr, contactin-associated protein; Na_v, voltage-gated sodium channel. (B) Immunostaining of ventral roots from normal (top) or AMAN rabbits with IgG anti-GM1 antibodies. Autoimmune attack (blue, membrane attack complex) occurs at the nodes first then extends to the paranodes. Clusters of nodal Na_v channels (red) or paranodal Caspr (green) are destroyed and eventually disappear. (C) Toluidine blue staining of ventral roots from AMAN rabbits. The arrow indicates a normal node (top). The bracket indicates an abnormally elongated node (bottom). (D) Electron microscopy showing abnormally lengthened node and paranodal myelin detachment in ventral roots from AMAN rabbit. At this point the cascade may follow 2 paths. (1) Left. Rapid repair of disrupted nodes as in AMAN with reversible conduction failure (RCF) or acute motor conduction block neuropathy (AMCBN) associated with prompt, favourable recovery (E–G). (E) Cartoon schematizing the recovery pattern of affected nodes. (F) Immunostaining of ventral roots from AMAN rabbits during recovery phase. Deposition of membrane attack complex is reduced. Clusters of Na_v channels (red) and Caspr (green) are formed on both sides of affected nodes, and appear to fuse together to form a new node of Ranvier. (G) Serial motor conduction of the ulnar nerve from an AMAN patient with high titre of IgG anti-GM1 antibodies. On Day 8 conduction block (CB) is present across the elbow: proximal compound muscle action potential (CMAP) amplitude is 48% reduced with slow (27 m/s) conduction velocity (CV). CB improves on day 12 and disappears

by day 25 with normalization of CV and without development of CMAP temporal dispersion. (2) Right. Progression to typical AMAN with axonal degeneration associated with poor outcome (H–K). (H) Cartoon schematizing the nodal disruption with abnormally elongated nodal gaps progressing to axonal degeneration. (I) Electron microscopy of ventral root from AMAN rabbit. A macrophage (M) is in the periaxonal space with an injured axon (A) in presence of intact myelin sheath. (J) Toluidine blue staining of sciatic nerve from AMAN rabbit. Arrowheads indicate the degenerated nerve fibres. (K) Serial motor conductions of the ulnar nerve from an AMAN patient with high titre of IgG anti-GM1 and -GD1a antibodies. On day 3 there is only a slight reduction of proximal CMAP amplitude across the elbow segment. On day 6 CB is present across the elbow segment (proximal CMAP amplitude is 78% reduced). At day 22 a reduction of all CMAP amplitudes is evident, indicating the evolution to axonal degeneration. The cartoon on the right details the possible events leading to axonal degeneration. Antibodies bind to gangliosides in the axolemma (1), the classical pathway of complement is activated (2), the terminal components of complement form the membrane attack complex (MAC) pore (3), Ca^{2+} enters through the MAC pores and accumulates in the axoplasm (4), activation of Ca^{2+} -dependent calpain (5) causing proteolytic cleavage of neurofilaments (NF), damage of mitochondria (Mit) and Wallerian degeneration. Finally macrophages move from nodal gaps into the periaxonal space to remove degenerating axons (H). Figures A, C, E, H and J used with permission from Uncini et al. 2013 [1]. Figure C was originally from Dr Koujiro Tohama (The Centre for Electron Microscopy and Bio-Imaging Research, Laboratory of Nano-Neuroanatomy, Iwate Medical University, Morioka, Japan). Figures B, D and F used with permission from [18]. Figures G and K used with permission from [10]. Figure I used with permission from [42].

To overcome these nosologic difficulties and avoid misclassification the new category of nodo-paranodopathies was proposed [1]. [Figure 28.4](#) summarizes the association of dysimmune neuropathies with antibodies to gangliosides and to axo-glial proteins and the evidence supporting the nodal and paranodal involvement.

Acute neuropathies with anti-ganglioside antibodies can be classified as nodopathies because of the common pathophysiologic mechanism and continuum. In the appropriate clinical setting the electrodiagnosis of an acute dysimmune nodopathy can be made by serial conduction studies documenting a promptly reversible CB or conduction slowing without development of excessive temporal dispersion, which is therefore defined RCF, or a progression from CB to axonal degeneration.

Regarding chronic neuropathies it has been debated whether multifocal motor neuropathy (MMN) is a demyelinating or an axonal disorder. MMN is characterized by persistent motor CB (with or without TD), presence of IgM to GM1 in about 50% of patients and response to intravenous immunoglobulin (IVIg) in up to 90% of patients [27]. In addition to CB, MMN is characterized by axonal degeneration [28]. Pathology studies in MMN are scarce and contradictory, with evidence of mild demyelination as well as of primary axonal degeneration [29,30]. Injection of human sera containing IgM anti-GM1 antibodies into the rat sciatic nerve induced CB, immunoglobulin deposition at the nodes of Ranvier, nodal widening and some paranodal demyelination [31]. Application of high-concentration anti-GM1 sera to rat single-myelinated axons after addition of complement reduced Na^+ currents sufficient to block action potential electrogenesis [32]. Results of nerve excitability studies suggest the Na^+/K^+ pump was blocked at the site of CB, causing permanent depolarization and continuous Na^+ influx [33]. Permanent axonal depolarization may induce increased Na^+ influx, intra-axonal Na^+ accumulation, reversal of the axolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger, intra-axonal Ca^{2+} accumulation, and Ca^{2+} -mediated axonal degeneration. All of the above findings suggest that MMN could be better classified as a chronic dysimmune nodo-paranodopathy ([Figure 28.4](#)).

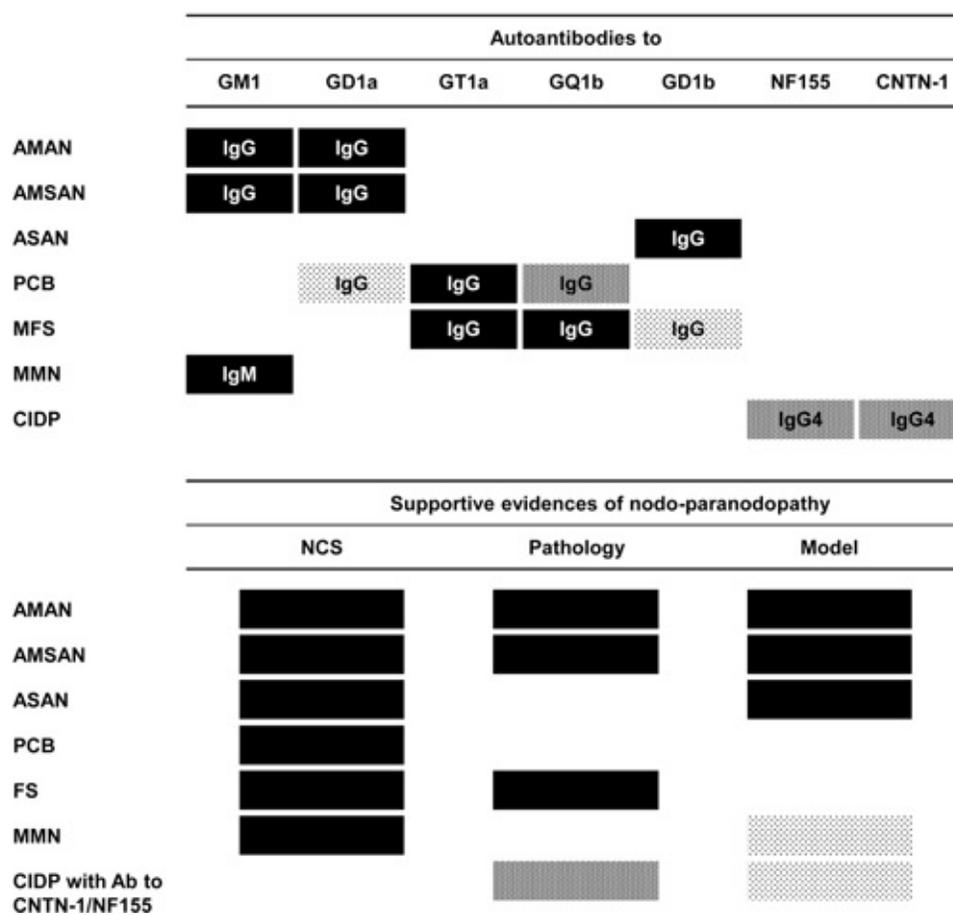


Figure 28.4 Dysimmune nodo-paranodopathies. The association of neuropathies with antibodies to gangliosides and axo-glial proteins and the evidences supporting the nodal and paranodal involvement are shown. Strength in association and evidence: black, strong; dark grey, medium; light grey, weak; blank, none. NCS indicates evidence of rapid reversible nerve conduction failure or persistent nerve conduction block. Pathology indicates evidence of lengthened nodes in human autopsy. Model indicates evidence of disruption of nodes in animal models by active immunization or by passive transfer of antibodies. AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; ASAN, acute sensory ataxic neuropathy; PCB, pharyngeal-cervical-brachial subtype of Guillain-Barré syndrome; MFS, Miller Fisher syndrome; MMN, multifocal motor neuropathy, CIDP chronic inflammatory demyelinating polyneuropathy. Modified from Uncini et al. 2013 [1].

Devaux JJ, et al. Nodal proteins are target antigens in Guillain-Barré syndrome. *Journal of the Peripheral Nervous System*, 2012; Querol L, et al. Antibodies to contactin-1 in chronic inflammatory demyelinating polyneuropathy. *Annals of Neurology*, 2013

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is thought to be an autoimmune disorder with heterogeneous clinical phenotypes. The proteins of the compact myelin have long been thought to be likely autoantigens because of segmental demyelination seen on pathological examination and because of the similarity with experimental allergic neuritis induced in rats by purified P0, P2 and PMP 22. Nonetheless, after many years of investigation there is little evidence for a pathogenic role of an autoantibody response to these proteins in the majority of CIDP patients [34].

An innovative research path was opened by Devaux and colleagues in 2012 reporting that IgG from 30% of CIDP patients bound at the nodal region of rat sciatic nerve recognizing, in some instances, contactin-1 (CNTN-1), neurofascin-186, gliomedin [35]. The following year Querol and colleagues showed that 6.5% of 45 CIDP patients had IgG to CNTN-1 or CNTN-1 and contactin- associated protein (Caspr) [36]. These patients shared a phenotype characterized by aggressive onset, motor predominance and poor response to IVIg. The same Spanish group reported 4 patients with antibodies to neurofascin

155, predominantly of the IgG4 isotype, presenting with severe distal weakness, disabling tremor in 3 patients and poor response to IVIg [37]. Anti-CNTN-1 antibodies, exclusively or predominantly of the IgG4 isotype, were found in 2.4% of 500 Japanese CIDP patients presenting with subacute onset and sensory ataxia, and in 8% of 53 German patients showing acute onset, prevalently motor neuropathy and a high occurrence of tremor [38,39]. The axonal cell adhesion molecules CNTN-1 and Caspr, and the glial neurofascin-155 form a ternary complex located in the paranode and is essential in the formation and stability of septate-like junctions and contributes to the impulse propagation in myelinated fibres (Figure 28.1). Interestingly IgG4 antibodies have a reduced capacity to activate complement and have been shown to be pathogenic via an ‘antigen blocking’ mechanism in which the antibody blocks critical functions of the target antigen. Studies of dermal myelinated fibres and sural biopsies of patients with anti-CNTN-1 antibody showed loss/destruction of paranodal Caspr and/or neurofascin immunoreactivity, elongated nodes and axonal damage but not demyelination [39]. The authors concluded that anti-CNTN-1 antibody-associated neuropathy “does not meet morphological criteria of demyelinating neuropathy and therefore might rather be termed a paranodopathy”. Electrophysiological studies of patients with antibodies to CNTN-1, Caspr, and neurofascin 155, show prolonged distal motor latencies and slowing of conduction in the ‘demyelinating’ range, CB and TD but also low amplitude distal CMAPs and spontaneous activity at electromyography indicative of significant coexistent axonal degeneration. Proofs of pathogenicity of these antibodies are still circumstantial. In an in vitro myelinated model, IgG4 anti-CNTN-1 from patients of the Spanish cohort prevent adhesive interaction between CNTN-1/Caspr and neurofascin-155 and lead to alteration of paranodes [40]. Antibodies against neurofascin exacerbate and prolong adoptive transfer experimental autoimmune neuritis and caused conduction defects when injected intraneurally [41]. So in summary, antibodies to CNTN-1, Caspr, or neurofascin155, are present in up to 12% of CIDP patients and seems to be associated with acute/subacute onset and poor response to IVIg. Because of the localization and function of the axo-glial protein and the lack of true segmental demyelination at pathology, the neuropathy with antibodies to CNTN-1, Caspr, or neurofascin 155 could be better classified as a chronic paranodopathy. Compared to acute nodopathy associated with anti-ganglioside antibodies this chronic paranodopathy seems to show the electrophysiological features of de-remyelination, even though segmental demyelination was not demonstrated, possibly because of the major involvement of the paranodes. Accurate sequential neurophysiological studies are necessary to investigate whether it will be possible to distinguish patients with antibodies to paranodal axo-glial proteins from other CIDP patients.

Conclusions

The nosological entity of nodo-paranodopathy we propose has the advantage of focussing on the site of primary nerve injury and avoids the confusing situation, as in neuropathies associated with anti-ganglioside antibodies, that in spite of the common site of nerve damage and pathophysiological mechanism, some patients might be classified as having a demyelinating and others as having an axonal neuropathy. In our opinion the nodo-paranodopathy category seems appropriate for various dysimmune acute and chronic neuropathies associated with antibodies to gangliosides and to paranodal axo-glial proteins, better systematizes the neuropathies characterized by an autoimmune attack targeting the nodal region, and integrates the classical classification into demyelinating and axonal.

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Nonconventional Electrophysiological Techniques

Judith Drenthen

Introduction

The development of electrophysiology started in the 18th century with Galvani's discovery of animal electricity. He showed that electrical stimulation of muscular tissue produces contraction and force. Although brilliant, his findings came too early for his time. His concept was received with considerable scepticism and it was not until the 19th century that his theory gained new enthusiasm. Then these findings were reproduced and various stimulation procedures were developed. In 1852, Hemplholtz was the first to measure nerve conduction in human subjects. In the next century the development of electrophysiological techniques gained momentum. During the first half of the 20th century the recording equipment improved. The first modern electromyography (EMG) machine was constructed by Jasper in 1942 at McGill University, Montreal, Canada, and in 1950 the first commercially available EMG system was introduced.

This technical progress and a better understanding of disease processes led to an increased use of electrical investigations and has led to our extensive knowledge of the human peripheral nervous system. Even in this day and age new techniques are being developed and old techniques are being rediscovered and adjusted for new purposes to study the various aspects of the human nerve and muscle. In this GBS100 monograph I try to give an impression of current and promising nonconventional electrophysiological techniques. It is not meant as a complete overview of all promising techniques.

Threshold Tracking Techniques in the Human Peripheral Nerve

One of the most important neurophysiologists of our time (of my time anyway) is Professor Hugh Bostock. His pioneering techniques have improved our understanding of the electrical properties of nerve membranes and their alterations in various diseases. One paragraph about his work is not doing justice to the enormous impact he has had on the neurophysiological world, and is not sufficient to explain his brilliant, albeit complex threshold tracking technique. However, in this short overview I will try to give an impression of the basic principles of this sophisticated technique.

In the 1990s, Professor Bostock developed a semiautomatic program called QTRAC [1]. With this program various nerve excitability tests assessing nerve membrane properties in vivo can be performed. It provides information about motor or sensory axons complementary to the information provided by conventional nerve conduction studies (NCS). Nerve excitability testing provides information on ion channels and the functions of energy-dependent pumps in the nodal and internodal membrane, and their changes during disease. In threshold tracking a resting threshold is compared to a threshold produced by changes in the nerve environment. First, the resting threshold has to be established. This is the threshold

that produces a predefined compound muscle action potential (CMAP) size (often 40% of maximum CMAP amplitude). Then nerve excitability is changed by different manoeuvres (by altering the nerve environment or by applying additional currents). The current that is then required to elicit the predefined CMAP amplitude will be determined automatically. For example, when axons are hyperpolarized, the test potential becomes smaller and the computer will increase stimulus intensity until the test potential has returned to its target size. In clinical practice, the nerve is stimulated using a computerized protocol and takes approximately 15 minutes. With this protocol several indices of axonal excitability, such as refractoriness, strength-duration properties, threshold electrotonus, supernormality and late subnormality can be measured. Multiple studies have been performed with this technique in a wide subset of neuromuscular disorders, providing a whole new insight into the physiology of those diseases.

Differences in Membrane Properties of Axonal and Demyelinating Guillain-Barré Syndromes

Although the threshold tracking technique is used in various neuromuscular disorders, so far only 1 study has been performed in Guillain-Barré syndrome (GBS) patients. In this study threshold tracking was used to measure axonal excitability in acute motor axonal neuropathy (AMAN) and acute inflammatory demyelinating polyneuropathy (AIDP) patients. Axonal excitability properties were different in AMAN patients than in those with AIDP. AMAN patients had a greater refractoriness during the acute phase of the disease that normalised during follow-up. The recovery cycle data suggest a critically reduced safety factor for impulse conduction in distal nerve terminals of AMAN patients [2].

The Electrophysiological Muscle Scan

In 2007, 2 publications were published more or less simultaneously about the clinical potential of a detailed stimulus response curve [3,4]. The stimulus response curve was originally developed for the excitability testing technique. Up until these 2 publications, the diagnostic value of this stimulus response curve itself was rare. When recorded with a sufficient stimulus number, this detailed curve has much to reveal that is of clinical interest. The 2 research groups joined together and explored the possibilities and optimal settings to record this technique. The technique was soon labelled the CMAP scan. The CMAP scan is noninvasive and is based on the successive activation of motor units (MUs) through transcutaneous electrical stimulation. Each MU has a different stimulus intensity (SI) at which it will be activated. A gradual increase in SI from threshold (the SI at which the MU with the lowest threshold is activated) to supramaximal values (the SI that elicits a maximum CMAP) will result in successive activation of all MUs in the muscle. Plotting the elicited CMAP amplitudes versus the corresponding SIs results in a curve.

If made with many stimuli and, hence, a high resolution, the CMAP scan provides information that is not (easily) available through conventional EMG methods. For example, it allows the identification and quantification of steps. Steps are clearly visible size differences in the CMAP amplitude between consecutive stimuli. They appear as abrupt jumps in the usually sigmoid curve and result from the firing of large, newly recruited MU potentials. They are a sign of reinnervation and/or MU loss. The presence and properties of steps differ significantly between normal subjects and patients with amyotrophic lateral sclerosis (ALS). Furthermore, this curve can be used to study basic excitability properties of peripheral nerves. In Miller Fisher patients, subclinical limb motor nerve dysfunction can be identified with this

technique [5]. Also, the CMAP scan displays great differences in excitability between axonal and demyelinating GBS patients, very early in its disease course.

Estimating Motor Unit Numbers from the CMAP Scan

The exact amount of MUs within the human muscles is unknown. With histopathological studies it is not possible to count the number of MUs. To evaluate changes of MU number during disease and recovery, the number of MUs have to be estimated. Various MU number estimation (MUNE) techniques have been proposed. Each method has its own shortcomings regarding reliability and reproducibility. Most methods are also very time consuming. The paper of Bostock [6] describes a new technique, based upon the CMAP scan. It is a very quick method and consists of fitting a recorded CMAP scan into a simulated CMAP scan. The model of the simulated CMAP scans consists of a wide range of MUs. Each MU contributing to the scan is described by 3 basic parameters: threshold, amplitude and spread of threshold. With this new method, MUNE values had a mean absolute error of 7%.

As with all modelling techniques, this technique is based on simplification of reality. Real scans do not necessarily behave like the idealized simulated scans. An important simplification of the model is the assumption that the contribution of each MU to the maximum CMAP amplitude is constant. Another simplification is that the spread of thresholds is assumed equal for all MUs and based on findings in healthy human MUs. For diseases such as ALS and GBS, these spread in thresholds are unknown. Despite these simplifications, this technique is very pragmatic. It is very easy to perform, produces reasonably accurate MUNE values and takes only a few minutes.

Motor Unit Number Estimation Using High-Density Surface Electromyography

A more time-consuming, but elegant method to estimate the number of MUs is the multiple point stimulation technique with high-density surface EMG (HDsEMG) [7]. MUNE is based upon the division of the maximal CMAP amplitude by an estimate of the mean MU potential (MUP) size [8]. This mean MUP is calculated by averaging a number of individual MUPs that have been sampled using one of a variety of approaches. In MUNE with HDsEMG, the nerve is stimulated and the MU responses are recorded with an array of 126 densely spaced electrodes positioned over the muscle. Using such an array provides spatiotemporal profiles ('fingerprints') of individual MUs, which facilitates the detection of MU potentials and increases the number of MUs that can be sampled compared to conventional single-electrode recordings [7,9]. Hence, this increases the accuracy of the MUNE. MUNE can be used to detect axonal loss, ranging from mild to severe. The more conventional electrophysiological methods are often not sensitive enough to detect mild to moderate axonal loss, especially when reinnervation has occurred. With this technique it has been demonstrated that residual fatigue after GBS is related to axonal loss (lower MUNE), while conventional techniques have failed to show objective abnormalities [10].

Motor Unit Tracking with High-Density Surface EMG

Most neuromuscular disorders affect MUs. This explains the diagnostic relevance of electrophysiological tests such as needle EMG and NCS. A disadvantage of these conventional electrophysiological techniques is that for the most part they rely on samples of MUs. Comparison of properties of MU samples obtained

in various stages of a disease yield only indirect evidence of changes occurring in individual MUs. Following individual MUs over time (tracking) can overcome this problem and provides insight into the functional and morphological properties of these MUs. It allows investigation of how these MUs are influenced over time by neuromuscular disorders or treatment.

Similar to the MUNE technique described in the paragraph above, MU tracking uses an array of 126 densely spaced electrodes positioned over the muscle [11]. Since each MU has a distinct 'fingerprint', this allows for detection of these individual MUs in different sessions, which are months or even years apart. To 'track' MUs and identify the same MU in different sessions, it is essential that the high-density electrodes are placed in the same position. To adjust for potential translational and rotational displacements, automated corrections are possible and are described in a paper by Gligorijević and colleagues [12]. The development of this technique is an example of how collaboration between various fields (doctors, physiologists and engineers) can lead to new techniques.

Electrophysiological Signs of Permanent Axonal Loss in a Follow-Up Study of Patients with Guillain-Barré Syndrome

Permanent axonal loss is possibly responsible for long-term motor impairment in various GBS forms [13]. Motor axonal loss is often compensated by reinnervation and is hard to identify with conventional electrophysiological techniques. The macroelectromyography (macro-EMG) signal can, in contrast to conventional EMG, be used as a measure of the MU size including the number of fibres [14]. Thus, it can evaluate reinnervation. In macro-EMG, the recording electrode is the cannula of a modified single fibre electromyography electrode. By means of spike-triggered averaging, the contribution from all muscle fibres in a MU is extracted. The resulting signal reflects the number and size of muscle fibres in one MU and is called macro MU potential (macro-MUP). In the current paper, [13] it was shown that the macro-MUP amplitudes of GBS patients were clearly larger compared to healthy controls. Furthermore, the macro-MUP amplitudes were also more increased in GBS patients with a residual neuropathy than in those without. With this technique, reinnervation can be objectified and quantified.

Triple-Stimulation Technique Improves the Diagnosis of Chronic Inflammatory Demyelinating Polyradiculoneuropathy

In demyelinating neuropathies, conventional NCS can be consistent with axonal neuropathies and can lead to the misdiagnosis of a potentially treatable neuropathy. The electrodiagnostic features of acquired demyelination, such as the presence of conduction blocks and focal temporal dispersion, are sometimes not detected due to the occasionally very proximal location of these features. The transcranial magnetic stimulation (TMS) technique, using collision (the triple stimulation technique (TST)) is a quantitative method for measuring the fraction of spinal motor neurons that can be brought to discharge. In healthy persons this approaches 100%. The presence of very proximal conduction blocks, between root entry and Erb's point, can be assessed with the TST. Attarian and colleagues demonstrate the additional value of using TST in the diagnosis of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) [15]. In 4 of the 14 CIDP patients they studied, conventional NCS showed an 'axonal-like' CIDP, without evidence of demyelination. Sural nerve biopsy in these 4 patients showed demyelination. With TST, very proximal conduction blocks in all 4 patients were detected. Also, in studies of patients with multifocal motor neuropathy, one-third of the conduction blocks were located in the very proximal nerve segments,

which cannot be accessed by conventional NCS. GBS is sometimes difficult to diagnose, especially the paraparetic form. In these cases finding demyelination might aid the diagnosis. TST might be useful to detect proximal demyelination.

Cauda Equina Conduction Time in Guillain-Barré Syndrome

As described in the paragraph above, proximal nerve segments are not easily accessible with conventional electrophysiological techniques. This is especially the case for the nerves in the lower extremities. The most proximal stimulation site for the most common investigated nerves in the legs, i.e. the peroneal and tibial nerve, is the knee pit. In the early phase of GBS, the disappearance of f-waves is frequently the only abnormal finding with conventional NCS. However, the f-wave technique has no clear localizing value. Matsumoto and colleagues describe a method to measure the cauda equina conduction time (CECT) [16]. With magnetic stimulation, both the distal and proximal cauda could be stimulated. Reproducible CMAPs were recorded. By subtracting the CMAP latency to S1-level stimulation from that to L1-level stimulation, the CECT could be obtained. Sixteen GBS patients (9 AIDP and 7 AMAN) were examined with conventional NCS and CECT. All axonal patients had a normal CECT. Ninety percent of the demyelinating patients had abnormal CECT, even when the distal conduction velocity was normal. When there is a difficulty in diagnosing GBS, especially in patients with predominantly leg symptoms (the paraparetic form), this technique may be useful.

Contact Heat Evoked Potentials as a Useful Means in Patients with Guillain-Barré Syndrome

Most conventional and nonconventional electrophysiological techniques investigate the large myelinated nerve fibres. Zhang and colleagues describe a technique that detects impairment of the small myelinated fibres [17]. With contact heat evoked potentials (CHEPs) they studied the nociceptive pathway in GBS. Short, painful heat stimuli were applied on the leg and waist, and cortical responses were recorded. The cortical responses in GBS patients had longer latencies and lower amplitudes than those in healthy control subjects, indicating that the acute neuropathy not only involves the larger myelinated fibres, but also small nociceptive fibres.

Discussion

Our understanding of nerve physiology has greatly expanded since Galvani's time, especially in the last 30 years. New neurophysiological techniques have been developed and are now being used in daily practice. Yet, despite this progress, the underlying pathology in GBS is still not fully known. More advanced electrophysiological techniques, focussing on other properties of the peripheral nerve than with conventional techniques, might help elucidate the continuing enigma.

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30

The Development of Nerve Imaging in GBS and Inflammatory Neuropathies

Guido Stoll

Introduction

The Guillain-Barré (-Strohl) syndrome (GBS) represents an acute inflammatory polyradiculoneuropathy. It is widely held that immune cells such as T-cells and macrophages as well as autoantibodies, activated or produced during host defence against infections, erroneously attack peripheral myelin or axonal components with ensuing demyelination and/or axonal injury. GBS encompasses a number of different clinical manifestations with a predominant demyelinating or axonal phenotype. Electroneurography and electromyography are the classical means of assessing peripheral nerve function and allow distinction between demyelinating and axonal nerve damage in GBS. By electrophysiology, however, one can only assess nerve dysfunction and grossly define lesion patterns, because morphological details are lacking. The diagnostic workup of disorders of the central nervous system regularly employs imaging techniques, in particular magnetic resonance imaging (MRI), while in peripheral nerve disorders imaging has been restricted to conditions in which a mass lesion compressing nerves or roots was expected on clinical grounds. This restraint approach has changed. In 1993, Filler and colleagues presented the first ‘image neurogram’ showing a human nerve graft by commercial MRI [1]. They coined the term ‘magnetic resonance neurography’ (MRN). During the last 20 years peripheral nerve imaging developed into an innovative scientific subspecialty with growing clinical impact. In this chapter I review advances in depicting nerve damage using MRI with focus on inflammatory neuropathies, and provide insights into current experimental approaches to directly visualize nerve inflammation.

Magnetic Resonance Neurography: Clinical Applications

MRN can be performed with routine 1.5 or 3T MR machines. It relies on the application of special MR pulse sequences to distinguish peripheral nerves from the surrounding soft tissue. Technical details are described elsewhere [1,2]. Briefly, fat-saturated, heavily T2-w (T2*-w) sequences are applied for detection of pathological signal alterations in nerves. In addition, unenhanced T1-w MRI scans are taken for anatomical orientation, and, finally, gadolinium (Gd)-DTPA-enhanced and fat-suppressed T1-w images are required to distinguish pathological nerve signals from vessels which also appear bright on T2-w sequences. For correct interpretation of the imaging data it is mandatory to apply these sequences with the same slice thickness and anatomical orientation. MRN mainly exploits alterations of the T2-signal of peripheral nerves upon injury. Intact nerves cannot be discriminated on T2-w MRI because they

are isointense to the surrounding tissue. The intrinsic nerve signal, however, profoundly changes upon nerve injury [1,2]: nerve segments with axonal damage undergoing Wallerian degeneration (WD) become hyperintense on T2-W MRI, and this hyperintensity disappears after nerve regeneration. T2-hyperintensities, however, are not specific for axonal injury. Nerves undergoing demyelination also display T2-signal increases which, in contrast to nerves undergoing WD, are patchy and focal. Thus, MRN can disclose areas of axonal damage as well as demyelination. As a note of caution, the nerve signal depends on the orientation of the nerve within the magnetic field, which may also lead to a hyperintense signal of normal nerves at a critical orientation of 55°, called the ‘magic angle effect’. This is relevant when assessing the integrity of the cervicobrachial and lumbosacral plexus often involved in inflammatory neuropathies.

The diagnosis of inflammatory neuropathies such as GBS and chronic inflammatory demyelinating polyneuropathy (CIDP) is usually based on the history of onset and course, clinical evaluation of neurological signs and characteristic electrophysiological findings which in typical cases reveal conduction block and/or slowing of nerve conduction. Thus, imaging is not required for the diagnosis, but may be extremely useful in the diagnostic workup of patients with suspected focal mononeuritis (see below). MRI in GBS patients revealed thickening and Gd-DTPA-enhancement of spinal nerve roots and the cauda equine. Gorson and colleagues [3] prospectively obtained Gd-DTPA-enhanced lumbosacral spine MRIs in 24 consecutive patients with acute GBS at an average of 13 days after onset. Twenty of 24 patients had some degree of cauda equine nerve root enhancement, indicating a disturbance of the blood-nerve barrier. Patients with prominent nerve-root enhancement had greater proximal weakness and were more likely to have back or leg pain and lower functional GBS scores than those with mild or no root enhancement [3]. Other studies showed that mostly anterior spinal nerve roots were affected, and that imaging abnormalities regressed upon clinical recovery. Similarly to GBS patients, CIDP patients show symmetrical nerve-root abnormalities on MRI, such as enlargement, T2-hyperintensities and contrast enhancement ([Figure 30.1a](#)). An MRI is sometimes required in GBS patients to exclude a spinal cord or lumbar-spinal mass lesion, especially in cases with the rare paraplegic variant ([Figure 30.1b](#)).

In contrast, focal nerve lesions often cause difficulties in the diagnostic workup and may lead to the suspicion of a peripheral nerve tumour [4]. An MRN may disclose additional lesions within other nerves and thereby support the diagnosis of a multifocal inflammatory neuropathy. In 2000, Van den Berg-Vos and colleagues coined the term ‘multifocal inflammatory demyelinating neuropathy’ based on MRN studies in patients suffering from a focal neuropathy of unknown cause [5]. Areas with a pathological MRI signal in these patients underwent a fascicular biopsy which revealed focal inflammation and nerve lesions responded to anti-inflammatory treatments. Thus, from a clinical standpoint MRI could dramatically improve the diagnostic yield if we were able to (A) specifically identify inflammatory foci (see below), and (B) cover larger parts of the PNS than with conventional MRI. In 2009, a technique termed ‘diffusion-weighted whole body imaging with suppression of body signal’ (DWIBS) was introduced, which allows selective visualization of peripheral nerves over long trajectories. This reconstruction magnetic neurography was recently applied to CIDP patients in a proof-of-concept study, and disclosed widespread symmetric and root-dominant nerve hypertrophy [6]. Interestingly, patients with the multifocal acquired demyelinating sensory and motor (MADSAM) variant of CIDP displayed multifocal fusiform hypertrophy only in some nerve trunks. This novel imaging technique may dramatically improve our diagnostic yield in inflammatory neuropathies, similar to another emerging technology, nerve ultrasound.

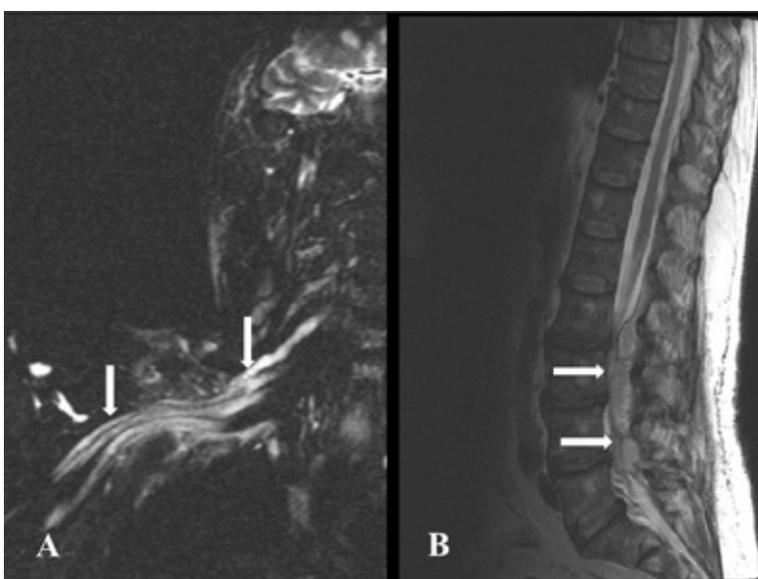


Figure 30.1 (A) MRI abnormalities in a CIDP patient. Note hyperintense (white), swollen nerve roots and plexus cervico-brachialis fibres (arrows) on T2*-w MRI. (B) Differential diagnosis of GBS: T2-w spinal MRI scan of a bacterial abscess compressing lumbar spinal nerve fibres (arrows) mimicking “paralytic” GBS. The patient had an increased CSF cell count of around 900, predominantly neutrophils.

Visualization of Nerve Inflammation and Breakdown of the Blood-Nerve Barrier

Nerve signal alterations as revealed by conventional MRN are nonspecific, and the extractable information is limited to the distribution of nerve lesions. In both GBS and CIDP, the pathophysiological hallmark is macrophage-mediated demyelination or, less frequently, axonal injury, but inflammation itself is not visible on conventional MRI. There is good evidence in inflammatory neuropathies that macrophages are attracted from the circulation to peripheral nerves and nerve roots by locally released chemokines, and upon nerve infiltration attack their targets by binding to the Fc-receptors of pathological autoantibodies deposited at myelin sheaths or paranodal axonal sites [7].

Over the last 15 years there have been intensive attempts to directly visualize inflammation in living organisms. This was made possible by the availability of novel, at present mainly experimental MR-contrast agents [2,8]. Currently, there are 2 MR-based approaches that hold promise for clinical use in the future, iron oxide particle-enhanced T1-and T2-w MRI, and perfluorocarbon (PFC) emulsion-enhanced $^1\text{H}/^{19}\text{F}$ MR spectroscopy (MRS). Iron oxide particles used for MRI as contrast agents consist of an iron oxide core of 4–8nm in superparamagnetic iron oxide (SPIO) particles or a smaller core in ultrasmall superparamagnetic iron oxide (USPIO) particles, both of which are surrounded by a variable coating affecting biological properties such as half-life within the blood. In tissues, iron oxide particles shorten both the T1 and T2 relaxation time. Mostly, SPIO particles have been used to study inflammation in experimental animals in the PNS. When SPIO particles are injected systemically into the circulation of mice or rats, they are avidly phagocytosed by monocytes/macrophages, before free SPIO particles are cleared by the reticuloendothelial system. Since this occurs rapidly, within 30 to 60 minutes, most remaining SPIO particles in the circulation are cell- (macrophage) bound thereafter, and blood pool effects can be neglected. This is in contrast to free USPIO particles which have an extended half-life of around 24 hours. When SPIO-laden macrophages are attracted to tissues during inflammation, they become visible due to a signal loss, appearing as hypointensity on T2 and T2*-w MR images.

In a seminal MRI study, Bendszus and Stoll injected SPIO particles into rats at various time points

after sciatic nerve crush and could follow macrophage infiltration in vivo [9]. Blood-derived macrophages first infiltrated the lesion site (figure 30.2). Thereafter, the entire distal stump undergoing WD became hypointense due to continuous accumulation of SPIO-laden macrophages. This process abruptly ceased around day 10 after nerve crush. Importantly, macrophage recruitment as shown by SPIO-enhanced MRI strongly corresponded to the well-known expression pattern of the chemokine macrophage chemoattractant protein-1 (MCP-1) produced by Schwann cells during WD. Delayed application of SPIO particles beyond day 10 after nerve crush no longer led to signal loss despite the persistence of numerous ED-1 positive macrophages in the degenerating nerve segment (see below). In the next step, we applied this technology to rats in adoptive transfer experimental autoimmune neuritis (AT-EAN), an animal model for human GBS. SPIO-enhanced MRI revealed focal signal loss of the cauda equine indicating macrophage infiltration already at day 3 after T-cell transfer, which was at a preclinical stage. Signal loss had already peaked at day 4 when first clinical signs had developed, but rapidly ceased thereafter at the peak of clinical EAN. Similar to the behaviour of ‘nerve-based’ macrophages in late WD, spinal nerves at a more advanced stage of AT-EAN, at days 6 and beyond, displayed no more signal loss upon systemic application of SPIO particles despite histological evidence of dense macrophage infiltrates.

Two major conclusions emerged from these studies: (A) SPIO-enhanced peripheral nerve MRI covers active phases of macrophage infiltration, but does not depict the proportion of the already ‘nerve-bound’ macrophages that entered from the circulation prior to contrast application, and (B) macrophage invasion in both WD and EAN occurs during a narrow time window. Thus, it is feasible to selectively visualize the recruitment of immune cells, mainly macrophages, to the PNS by SPIO-enhanced MRI. In contrast to monocytes/macrophages, spontaneous uptake of SPIO or USPIO particles by other immune cells such as T-cells is limited, but they can be preloaded in vitro with the aid of transfection agents, re-injected into the circulation, and then followed in vivo even in small numbers by T2-w and T2*-w MRI when entering tissues during inflammation. Iron-contrast-based ‘inflammation imaging’ is very sensitive, but hampered by some specificity issues: (A) small local haemorrhages can also cause signal loss and erroneously indicate ‘inflammation’, and (B) postphagocytic macrophages naturally contain significant intrinsic iron deposits which have to be taken into account at ultra-high field strengths.

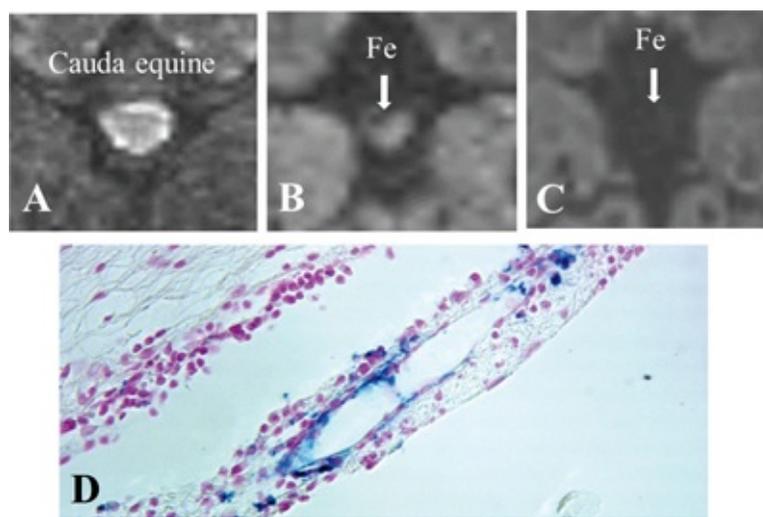


Figure 30.2 Imaging of macrophage infiltration in rats during adoptive transfer experimental autoimmune neuritis (AT-EAN), an animal model of GBS, by SPIO-enhanced MRI (technique see text). (A-C). Axial T2*-w MRI scans of the cauda equine in a normal rat (A), at 3 days (B), and 4 days (C) after T-cell transfer. All animals received SPIO-particles intravenously 24 hours before MRI. While the cauda equine still appears isointense in normal rats (A), signal loss due to infiltration of iron (SPIO)-laden macrophages into spinal nerves occurs focally in at day 3 prior to clinical disease (B) (Fe = iron, arrow), and culminates in complete signal loss at day 4 (C, arrow). (D) Histological examination of spinal nerves stained by Prussian blue confirms presence of iron-laden cells

(black dots) representing infiltrating blood-derived macrophages. Adapted with permission from Stoll et al., *Journal of Neuroimmunology* 149 (2004) 142–146.

Recently, ^{19}F -MRI emerged as a novel imaging modality for inflammation [8]. ^{19}F markers such as PFC compounds provide a unique signal in vivo due to the negligible ^{19}F background signal of the body. Similar to SPIO/USPIO particles, PFC nanoparticles are preferentially phagocytosed by monocytes/macrophages in the circulation and, thus, the fluorine signal in inflamed organs is mainly due to macrophage infiltration carrying intracellular PFC nanoparticles. In addition to ^{19}F images, ^1H images are acquired by MRS, which aid in placing the PFC-labelled cells into their anatomical context. Currently, there is only one study on $^1\text{H}/^{19}\text{F}$ MRS in the PNS. In a pilot study, we induced focal demyelination in sciatic nerves of rats by the chemical lysolecithin. At a certain concentration lysolecithin dissolves myelin sheaths while sparing the axons, and thereby induces a robust local inflammatory reaction with recruitment of hematogenous macrophages. We were able to visualize this macrophage infiltration by $^1\text{H}/^{19}\text{F}$ MRS in the rat, but compared to other organ systems like the heart and lung ^{19}F -MRI of neuroinflammation is still challenging, mainly because of sensitivity issues.

The central nervous system and peripheral nerves are secluded from the circulation by the blood-brain barrier (BBB) or blood nerve barrier (BNB), respectively. Gadolinium-DTPA is a approved MR contrast agent clinically used for detection of disturbances of the BBB and BNB since it does not pass the intact barriers. Although the BNB becomes leaky during WD, patients and experimental animals with acute axonal nerve lesions often do not show Gd-DTPA-enhancement. In contrast, as described above, nerve roots from GBS and CIDP patients often show Gd-DTPA-enhancement. Thus, it appears that Gd-DTPA-enhancement can be taken as a biomarker for inflammatory activity in the nervous system as widely anticipated in multiple sclerosis (MS) patients. However, there is strong evidence against this common assumption. Experimental studies in experimental autoimmune encephalomyelitis, an animal model of MS, and patients with MS which combined Gd-DTPA- or Gadofluorine- (Gf, an experimental agent) enhanced MRI with SPIO/USPIO-enhanced MRI revealed that areas with breakdown of the BBB (leakage of Gd-DTPA or Gf) and areas with acute macrophage infiltration (iron-particle induced hypointensities) often did not correspond. It appeared that leakage of the BBB/BNB for soluble factors such as contrast agents, and leukocyte infiltration were timely and locally discordant [10]. This notion is supported by numerous histological studies showing that immune cells mainly enter the nervous system by trans-endothelial migration, and not through the extracellular space due to destruction of tight junctions. Taken together, these studies emphasize the necessity to develop specific cellular MR contrast agents for clinical use that allow us to monitor inflammatory activity directly. A breakthrough in this field could help to improve the prognosis of GBS and CIDP patients by tailoring and monitoring stage-specific anti-inflammatory treatment strategies based on cellular MRI.

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BASIC SCIENCE

31

The Schwann Cell Biology Underlying GBS

P.J. Armati and E.K. Mathey

Introduction

Schwann cells, like the Guillain-Barré syndrome, have been victims of the lumpers and splitters phenomenon—the lumpers proclaim ‘A Schwann cell is a Schwann cell is a Schwann cell’, while the enlightened such as Emily, myself and all the other Schwann cell aficionados, consider it to be a wonder of the cellular world, complex, multifunctional and multiphenotypic. And so this cell has had an image problem, apart from the wonderful moment in 1838, when Schwann one evening after dinner with the botanist Schleiden went to Schwann’s laboratory, where he realised and described for the first time that animals were composed of cells—just as Schleiden had described for plants. He went on in the 1840s to define and describe cells aligned along the nerve fibres which bear his name to this day. However, it was Ranvier who was one of the first to acknowledge that Schwann cells are masters of multitasking and can do more than merely myelinate. In 1878 Ranvier mooted that the perisynaptic cells at the neuromuscular/tripartite synapse were in fact Schwann cells and not part of the muscle fibres [1].

So now to the next century and the defining of GBS in its many subtypes via a long and winding road to the ‘Rise of the Schwannopathies’. The light and electron microscope study by Wiśniewski, Terry, Whitaker, Cook and Dowling in 1969 of the ‘spinal nerve roots, spinal ganglia and sciatic nerve’ from a patient with GBS (then called Landry-Guillain-Barré syndrome but now, like Strohl, Landry has disappeared) [2] was pivotal. While the light microscopy showed no CNS abnormalities, in the EM ~15% of the sciatic nerve showed no evidence of axonal degeneration and for the first time clearly demonstrated severely damaged myelin lamellae, sometimes with adjacent normal myelin lamellae. They go on to describe macrophage infiltration through the Schwann cell basal lamina and invasion of the outer mesaxon of the damaged Schwann cell. So now, for maybe the first time, comes recognition that the compact myelin-forming Schwann cells are more than their compacted spiralling lamellae; that the Schwann cell itself can be the primary target pre-empting the rise of the Schwannopathies.

It is interesting that John Prineas in his perspicacious GBS paper of 1981 acknowledges that of GBS “the precise mechanism that leads macrophages to seek out and amputate a specialized region of the Schwann cell plasma membrane remains unexplained” [3]. It’s rather scary to think about ‘amputated’ Schwann cells when we now recognise their pivotal role in maintaining normal neural function of the peripheral nerve. They are no longer considered ‘insulation’ for the neuron but complex multifaceted cells that contribute to the functioning of the peripheral nervous system (PNS) in a variety of ways.

The Rise of Schwannopathies

The complexity of the Schwann cell was unveiled in the riveting lecture by Steve Scherer in 2001 at the Innsbruck Peripheral Nerve Society meeting, where he presented the drawing of the myelin-forming Schwann cell in all its architectural wonder, at the nodes of Ranvier, the uncompacted paranodal swirls with their microvilli stretching out to the axolemma, the Na_v channels clustering together, the juxtaparanode harbouring the K channels. These show so clearly the importance of the organisational basis of the Schwann cell/axonal relationship and the importance of maintaining this channel organisation for salutatory conduction (see [Figure 31.1](#)) [4]. This really nailed the concept that diseases such as GBS and chronic inflammatory demyelinating polyneuropathy (CIDP) are diverse syndromes, subsets of specific effects on not only the neurons but the conversation between the axon, cell body and Schwann cell compartments. Although the focus of neuropathologists and electrophysiologists has been on the damaged compact myelin regions of the Schwann cell and the associated significant slowing of conduction, it is also crucial to consider how maintenance of the axon/Schwann cell relationship underpins normal conduction. Because of the complex organisation of the node of Ranvier with its Schwann cell microvilli and tightly organised suites of molecules associated with the node, paranode and juxtaparanode, the role of the Schwann cell in all its forms and architecture is being examined for clues as to subtypes of GBS; especially the demyelinating subtype, acute inflammatory demyelinating polyneuropathy (AIDP).

That said, although AIDP may be considered to be a demyelinating disease, it also affects the so-called unmyelinated fibres such as the autonomic postganglionic axons. As Jack Griffin has pointed out, approximately 80% of peripheral nerve is made up of unmyelinated axons [5]. However, even these axons have a complex relationship with their ensheathing nonmyelinating Schwann cells, a fact that is often overlooked in many GBS-related publications. But this is still not their full story, as there are a diversity of Schwann cell phenotypes—satellite cells, nonmyelin-forming Schwann cells, myelin-forming Schwann cells and perisynaptic Schwann cells—all with the potential to contribute to the pathogenesis of GBS.

The Schwann Cell as a Target in AIDP/GBS

Due to the prominent demyelination observed in nerve biopsies of patients with AIDP, myelin proteins of the compact myelin region have long been regarded as the most likely targets of an autoimmune attack in the demyelinating form of GBS. However, the major myelin proteins have not proven to be significant targets. Hafer-Macko and colleagues [6] examined 3 autopsies taken 3, 8 and 9 days after disease onset in order to determine the role of complement in the pathogenesis of demyelination in these patients. Components of the complement pathway, including the activation marker C3d and the terminal activation complex C5b-9, were found deposited in the outer surface of the abaxonal Schwann cell plasmolemma. This finding led the authors to speculate that the target of the immune attack was an antigen located in this region of the Schwann cell and not in the compact myelin. These AIDP autopsies were part of a series of 14; the other 11 autopsies had an axonal subtype of GBS [7], where there was evidence of an antibody-mediated attack on the axolemma rather than on the Schwann cells.

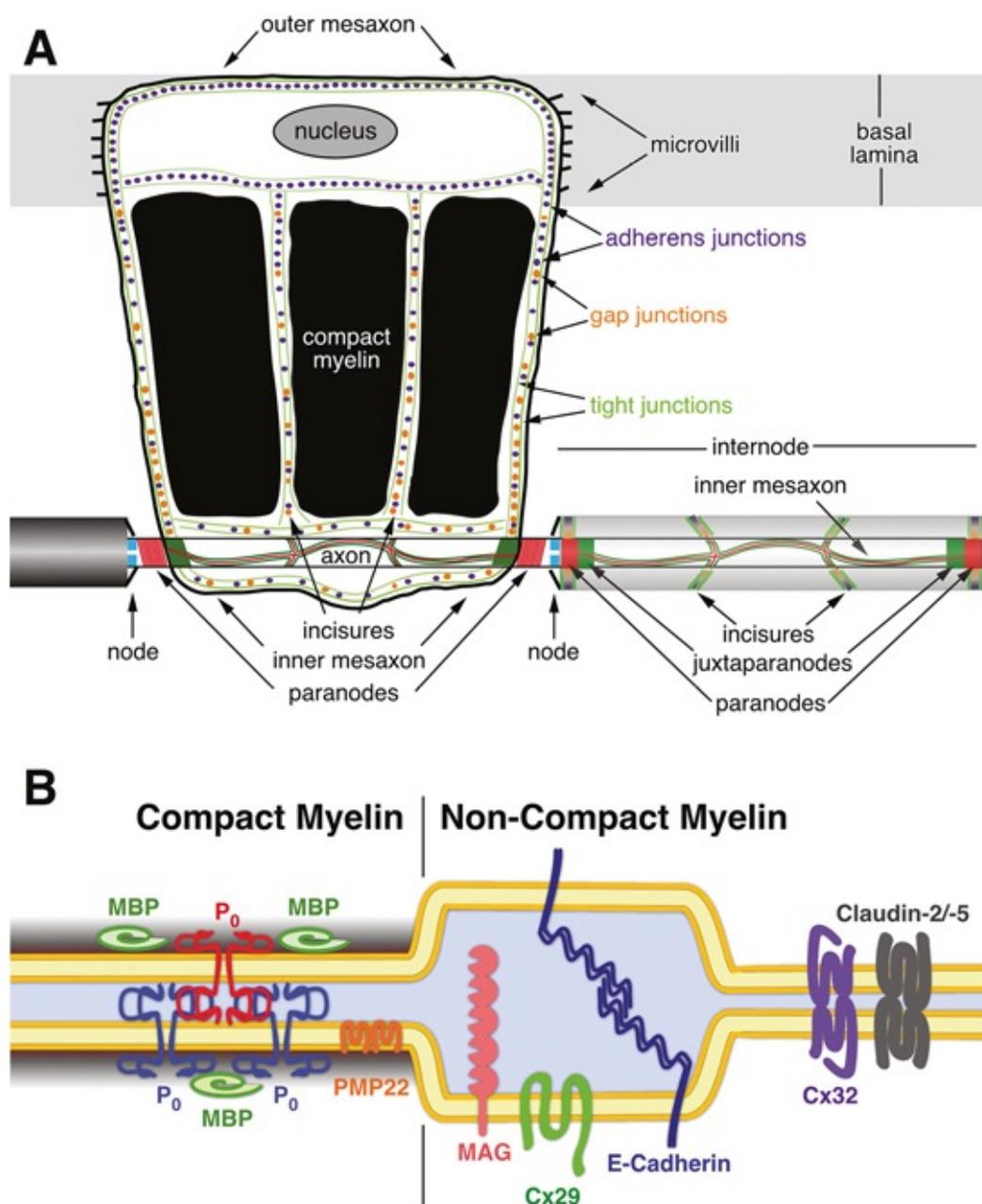


Figure 31.1 The organization of a myelinated axon.

(A) An 'unrolled' myelinating Schwann cell, revealing the regions that form compact and noncompact myelin. Tight junctions are depicted as 2 continuous (green) lines; these form a circumferential belt and are also found in incisures. Gap junctions are depicted as orange ovals; these are found between the rows of tight junctions and are more numerous in the inner aspects of incisures and paranodes. Adherens junctions are depicted as purple ovals; these are more numerous in the outer aspects of incisures and paranodes. The nodal, paranodal and juxtapanodal regions of the axonal membrane are coloured blue, red and green, respectively. (B) The proteins of compact and noncompact myelin. Compact myelin contains P₀, PMP22 and MBP; noncompact myelin contains E-cadherin, MAG, Cx32, Cx29 and claudins 1 and 5. Schwann cells can make as many as 100 spiral turns around an axonal length so that their longitudinal length far exceeds that of the axon they ensheathe. To put this in perspective, an unwrapped Schwann cell from an axon with a hypothetical diameter of 6mm would be 39m in length if unwrapped. Used with permission from Scherer and Arroyo [4].

Whereas the identities of the target antigens in the axonal form of GBS have been identified and rigorously described, the target of attack in AIDP is still unknown. However, further evidence of an autoantibody response directed towards the surface of the Schwann cell plasmolemma comes from a study using primary human Schwann cell cultures to screen GBS sera for IgG binding [8]. In this study, 24% of GBS sera bound to the distal tips of proliferating, nonmyelinating Schwann cells, indicating that there is a significant response against antigens not present in the compact myelin. The reason the target(s) of the

immune response remain elusive in AIDP is unclear, but it may be that for decades we have been barking up the wrong tree in looking for targets in the compact myelin. The fact that compact myelin is but one component of the complex cellular arrangement of the Schwann cell has often been overlooked. In reality, the perinodal loops, Schmidt-Lanterman incisures and transverse processes interdigitated between the compact myelin lamellae and the outer and inner mesaxon are all in continuity with each other and the compact myelin spiral. Immune-mediated injury to any of these areas has the potential to cause problems with maintenance of myelin integrity and possibly signal transduction.

This can be demonstrated by Willison's finding that in the Miller-Fisher variant of GBS the non-myelin-forming membranes of the perisynaptic Schwann cells [9] as well as the motor nerve terminal membranes are targeted. This shows that the pathophysiology is antibody-mediated, with both the Schwann cells and the terminals specifically targeted and damaged by the binding of the anti-GQ1b gangliosides and complement-mediated membrane attack complex formation. The dual targeting is a unique GBS neuropathology. This raises yet again the importance of the Schwann cell beyond its compact myelin-forming capacity. Thus the perisynaptic Schwann cells, in concert with the motor nerve terminals and the muscle fibres with their acetylcholine receptors, are now recognised as essential elements of the triumvirate forming tripartite synapse, formerly the neuromuscular junction.

More Than a Myelinator: Schwann Cells Provide Metabolic Support to the Longest Cells in History

Although Schwann cells are conspicuous for making myelin, they also support axons independent of myelination, and any perturbations in Schwann cell physiology has the potential to impact axonal function. In the PNS, axonal projections can extend up to 1.5m in a human, over 30m in a blue whale and as long as 40m in a supertyrannosaurus. Many soluble proteins vital for axonal form and function are synthesised in the cell body of the neuron and carried through the axon by slow axonal transport at the rate of ~ 0.2–10 mm/day. Some of these proteins could take years to reach the terminal of very long axons, making the supply lines for essential metabolites exceedingly slow. While it has long been postulated that Schwann cells provide essential metabolites for axons we are only now beginning to understand how. Beirowski and colleagues studied the impact of ablating the serine/threonine kinase LKB1, a pathway that has been associated with neurodegeneration only in Schwann cells [10]. Knocking out the LKB1 pathway in Schwann cells from birth led to progressive degeneration of both myelinated and unmyelinated axons, where small unmyelinated sensory fibres were amongst the worst affected. Deleting LKB1 from mature Schwann cells after they had completed myelination also led to axonal loss, while the myelin remained ostensibly normal. This study provides strong evidence that Schwann cells are essential to axonal wellbeing and provide metabolic support independent of myelination.

Schwann Cells Turn Up in the Most Unexpected Places: Overlook Them at Your Peril; Or Alternatively, a Saga of Bad Table Manners

While the following paragraph has little or nothing to do with GBS, the tale of the Tasmanian devil (*Sarcophilus harrisii*) is a bizarre story that is worth telling, and who knows it may have relevance in the future. The Tasmanian devil, the world's largest marsupial carnivore, has the strongest bite per unit body mass of any extant mammal land predator. It survived as an apex predator for thousands of years without challenge, until it encountered an extremely rare, aggressive and transmissible cancer [11]. Now known

as the devil facial tumour disease (DFTD), its origin appears to have arisen when a single cell in a female devil transformed into a cancerous clonal cell line. This was then transmitted to another devil during a facial biting session (the bad dining manners of Tasmanian devils are legendary—see <https://www.youtube.com/watch?v=SU44KwIfBXM>).

DFTD is one of only 2 currently known transmissible tumours and the only one that is fatal. It has almost forced the Tasmanian devil population into extinction. Because the disease spread geographically over time, epidemiologists assumed that the cause was an infectious agent, but they could not identify a virus or bacterium. Karyotypic studies suggested that the infectious agent was in fact the tumour cells themselves, as tumour cells isolated from different devils had similar karyotypic rearrangements. In 2010 Murchison and colleagues used large-scale genetic analysis to confirm that the disease was caused by a clonally transmissible cancer of Schwann cell origin [11].

While not directly related to GBS, the tale of the Tasmanian devil and the Schwann cell emphasises the point that abnormal or damaged Schwann cells can have unexpected effects and should not be overlooked as a cause of neurological dysfunction in GBS.

“If you do not expect the unexpected, you will not find it; for it is hard to be sought out, and difficult”.

—HERACLITES (CA 535 BC–475 BC)

Or alternatively:

“To expect the unexpected shows a thoroughly modern intellect”.

—OSCAR WILDE, *AN IDEAL HUSBAND*

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32

The Node of Ranvier

Steven Scherer

Introduction

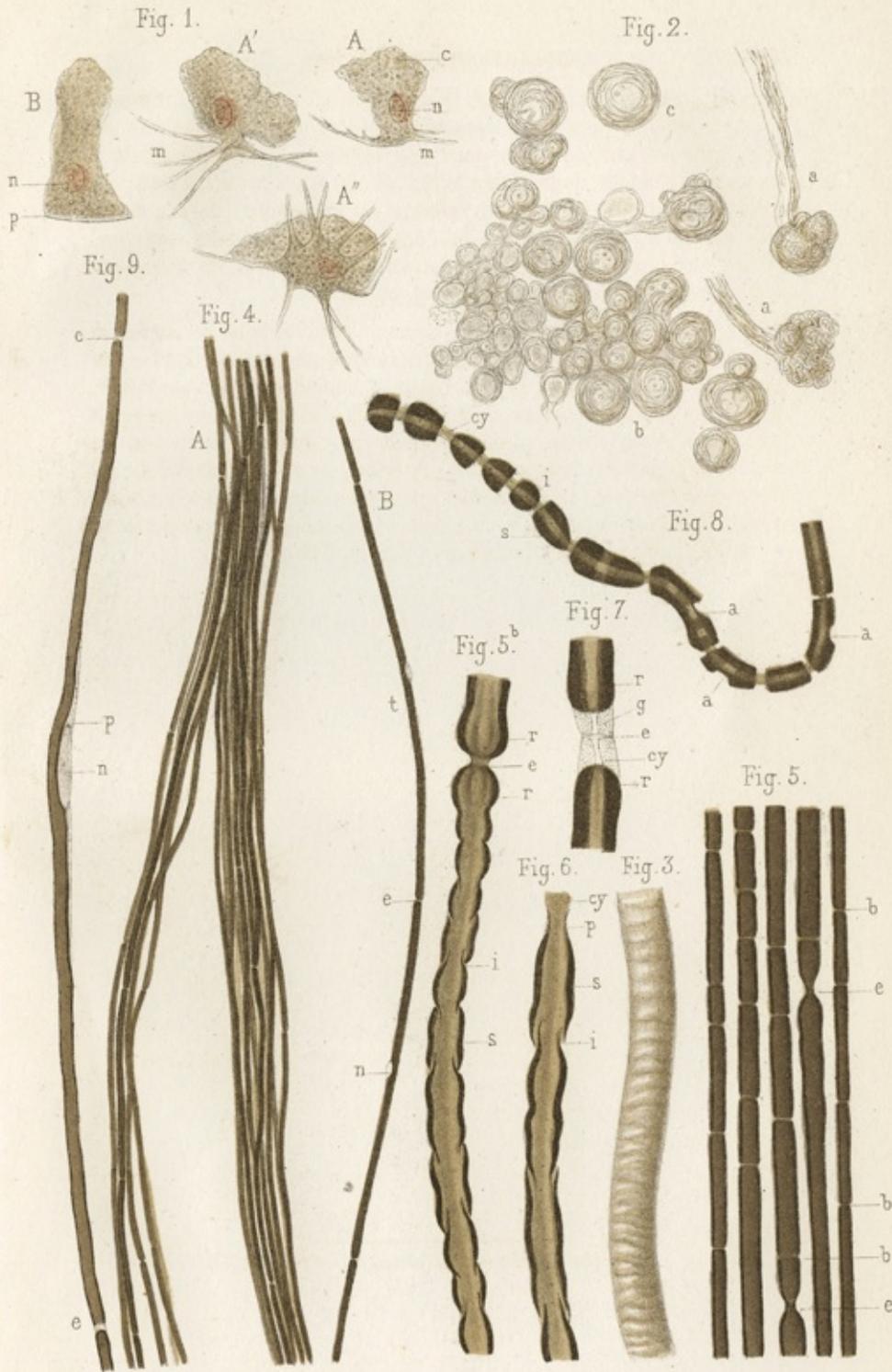
The node of Ranvier is the anatomical substrate for saltatory conduction. Just as interactions between myelinating glial cells and axons are required for the development of myelin sheaths, the molecular anatomy of nodes is based on specific molecular interactions between these 2 cell types.

1: Ranvier, Paris, 1876–1878

In 1878, Louis-Antoine Ranvier published “*Leçons sur l’Histologie du Systeme Nerveux*” which was based on a series of lectures he had given between 1876 and 1877 [1]. In that book, he reported the thin (~1 micron) periodic interruptions between adjacent myelin internodes in the peripheral nerves of frogs and mammals. He called these structures ‘étranglement annulaire’ (Figure 32.1), but subsequently they have been linked to his name. Whereas Ranvier had the prescient insight that myelin was a liquid covering of axons that serves protective, insulating and nutritive functions, his idea that nodes prevented the effects of gravity on the myelin sheaths has not been confirmed.

2: Ramón y Cajal, Madrid, 1899–1904

The greatest neuro-anatomist of all, Ramón y Cajal, used a repertoire of staining techniques to discern many features of the nodal region and masterfully illustrated them as shown in Figure 32.2. He wrote of the peripheral nervous system, “The myelin sheath is interrupted at the nodes of Ranvier, where a short length of axon always appears to be exposed, covered only by the Schwann membrane” [2]. This space is filled with a material that could be stained with silver nitrate that is then reduced by exposure to light, revealing the ‘discs of Ranvier’ (Figure 32.2A). The paranodes illustrated in Figure 32.2B showed “a double axonic bracelet ... the constitutive laminae of the medullary sheath end perpendicularly”, anticipating what would be revealed by electron microscopy 50 years later (Figure 32.4). Ramón y Cajal also recognized nodes of Ranvier in the central nervous system, a topic that would remain controversial for decades.



A. Karmanski ad nat. del. et lith.

Imp. Becquet, Paris.

F. Savy Editeur

Figure 32.1 Plate I, from "Leçons sur l'Histologie du Systeme Nerveux"[1].

Figures X.5–9 were made from sciatic nerves that were teased after fixation in osmic acid, which hardens the myelin sheaths and turns them black. Figures 4, 7 and 9 are from rabbits; Figures X.5, X.6 and X.8 are from frogs.

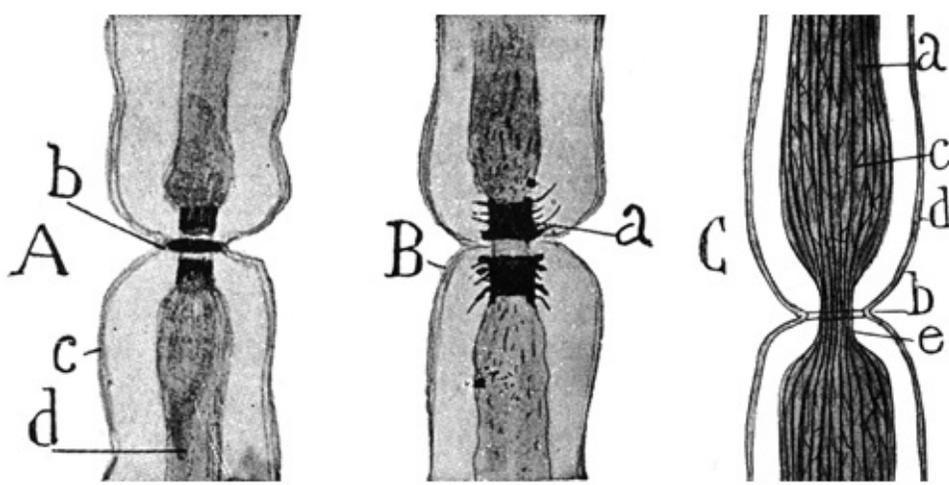


Figure 32.2 Ramón y Cajal's depiction of the nodal region.

AB: "Node of nerve fibres. *A*, axonic impregnation often found in diluted and quick-acting silver solutions; *B*, impregnation with silver, after fixation in formol-pyridine-manganese; *a*, spinous bracelets of Nageotte; *b*, disc of Ranvier; *d*, axon; *e*, Schwann's membrane." **C:** "Schematic drawing of the nerve fibre at the level of the node. *a*, fine oblique neurofibrils; *b*, cementing discs; *c*, longitudinal stout neurofibril; *d*, neurilemma; *e*, region of the axon which corresponds to the node." Figures and quotations are from [17] with permission of Oxford University Press.

3: Lillie, Chicago, 1925

In 1925 Ralph Stayner Lillie demonstrated that interrupted glass segments increase conduction of an iron wire immersed in nitric acid [3]. He introduced the term 'saltatory' conduction, and emphasized the analogy to myelinated axons: "Whether conditions analogous to those just described enter in the case of nerve and other transmitting structures in living organisms is difficult to determine experimentally, but seems not improbable. We observe, for example, that in the most rapidly conducting protoplasmic tracts known, the medullated nerves of vertebrates, the conducting element (axone) is enclosed by a tubular sheath of apparently high electrical resistance, the medullary sheath, which is constricted or interrupted at regular intervals. The medullated nerve transmits impulses at about 10 times the velocity of the nonmedullated nerve, in which, except for the absence of the segmented sheath, the structure is similar."

4: Huxley and Stämpfli, Cambridge and Berne, 1949

Erlanger and Gasser, pioneers of peripheral nerve electrophysiology (for which they shared the Nobel Prize in 1944), appreciated that nodes likely "regenerated" action potentials [4] "It seem much more reasonable to suppose that a nerve fiber conducts by means of a self-contained mechanism ..." This was directly demonstrated by Huxley and Stämpfli in 1949 [5], who isolated single myelinated axons from frog sciatic nerves and measured the amplitude of the current as a function of position along the internode (Figure 32.3). They found that the amplitude was highest at nodes, and that "positive current began to enter the axis cylinder before the potential change had reached its maximum. This relation is impossible in a system of resistances and capacities and is shown to be a necessary characteristic of the points which maintain decrementless conduction in a cable-like structure. It is concluded that the process which gives rise to the action potential takes place at the nodes of Ranvier, confirming the theory of saltatory conduction."

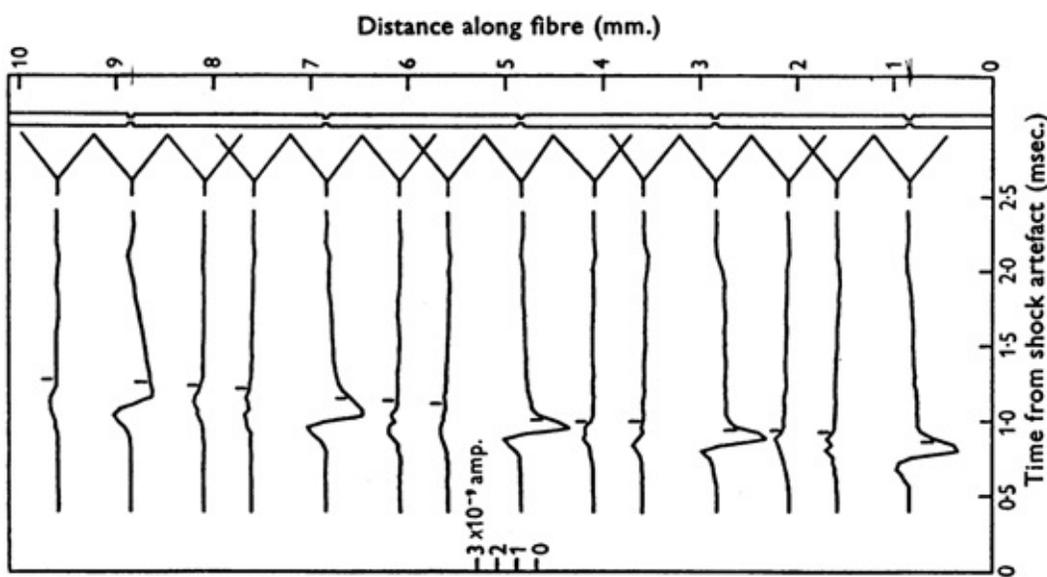


Figure 32.3 Membrane currents of a myelinated fibre.

“Each curve shows the difference between the longitudinal currents at 2 points 0.75 mm. apart on the fibre. The positions of those 2 points relative to the nodes is indicated on the diagrammatic fibre on the right. The vertical mark above each graph shows the time when the change in membrane potential reached its peak at that position on the fibre.” Outward current is plotted leftwards. Reproduced with permission from [5].

5: Electron microscopists, from around the world, 1955–1990

The resolution of the electron microscope was required to reveal the relationships of glial cells to axons, including the structure of the nodal region. From humble beginnings in the 1950s, and through the efforts of many workers who refined the preparation of specimens and the microscopes themselves, a detailed ultrastructure of the nodal region was developed over 30 years. [Figure 32.4](#) is a beautiful depiction of this work. The structure of CNS nodes are quite similar to that of PNS nodes, with the major exception that glial processes do not contact the nodal axolemma nearly as extensively as do Schwann cell microvilli.

6: Levinson lab, Denver, 2000

Beginning in the 1980s, the molecular architecture of nodes of Ranvier has been built, molecule-by-molecule, with antibodies that recognized its molecular constituents. The intramembranous particles ($1200/\text{m}^2$) in the nodal axolemma shown in [Figure 32.4](#) are mostly voltage-gated Na^+ channels (Na_v channels), which are essential for the propagation of action potentials. Mature channels are composed of one α and 2 β subunits; a single α subunit forms the actual channel. Of the $\text{Na}_v1.1$ - $\text{Na}_v1.9$ subunits gene family, $\text{Na}_v1.6$ is the main one expressed in mature CNS and PNS nodes [6]. Recessive mutations in the gene encoding $\text{Na}_v1.6$ lead to paralysis and death in mice, demonstrating that motor axons require this Na_v channel.

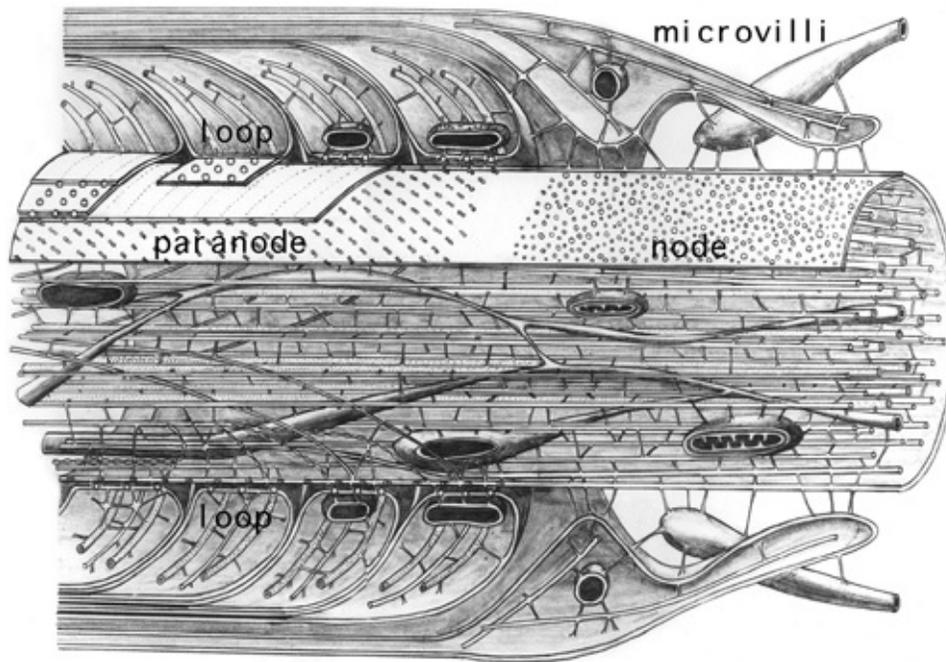


Figure 32.4 Depiction of the ultrastructure of the node and paranode.

Note that the nodal axolemma has many large particles, some of which are voltage-gated Na^+ channels, and transcellular bridges connect the Schwann cell microvilli the nodal axolemma. Rows of particles in axolemma are in register with the rows of particles in the paranodal loops, forming septate-like junctions. From [16], with permission of Kluwer Academic Press.

7: Bennett lab, Durham, 1990s

In the 1990s, Bennett and colleagues identified isoforms (270 and 480 kDa) of ankyrin_G, that were localized to nodes of Ranvier and axon initial segments [7]. This group also discovered that two cell adhesion molecules, NF186 and Nr-CAM, were localized to nodes and axon initial segments by their intracellular interactions with ankyrin_G an adaptor protein that links these intrinsic membrane proteins to the spectrin cytoskeleton. Specific interactions with ankyrin_G have been subsequently identified for other molecules that are localized to nodes ([Figure 32.5](#)).

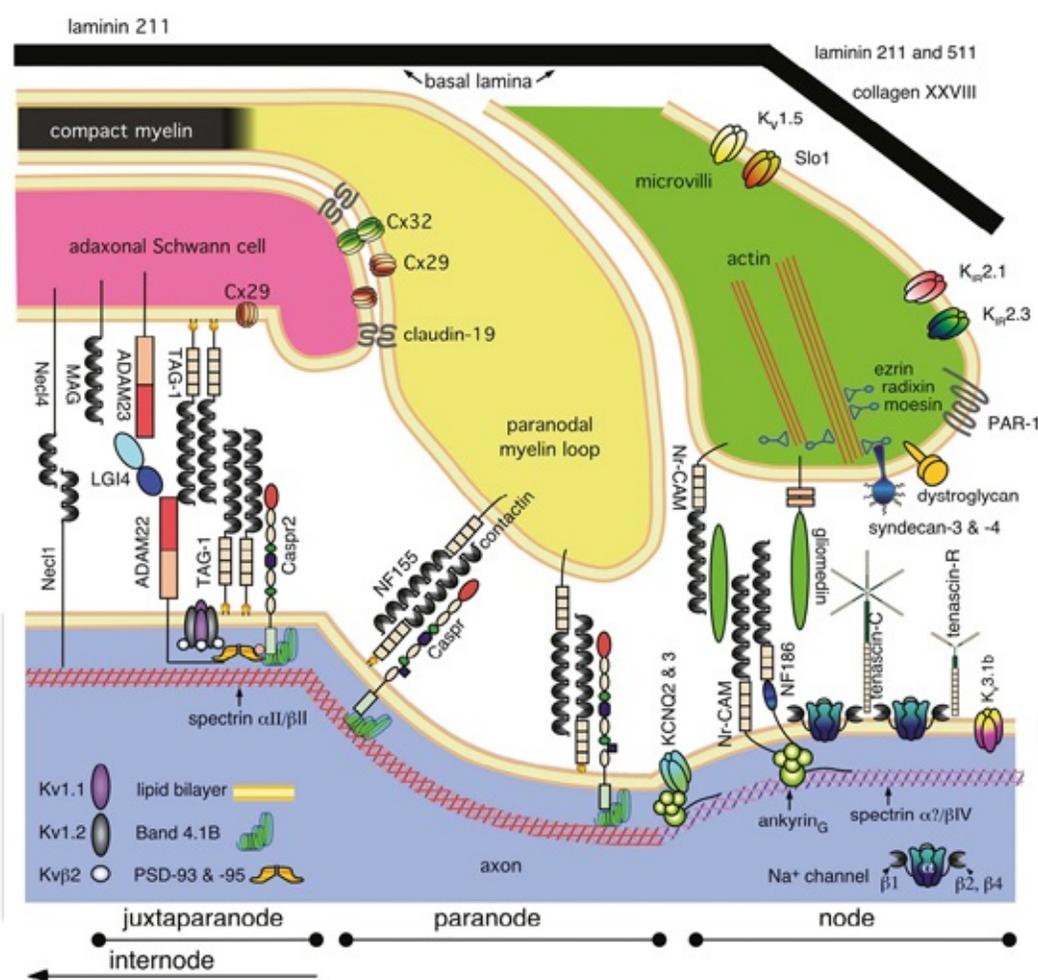


Figure 32.5 Molecular organization of the nodal region.

This schematic drawing depicts the molecular organization of nodes, paranodes, and juxtapanodes. At nodes, ankyrin_G binds to KCNQ23 channels and Na_v channels β subunits, as well as Na_v channels α subunits, tenascin-R, and tenascin-C, and the spectrin cytoskeleton. Nr-CAM, NF186, and β subunits may interact in *trans* with CAMs on the Schwann cell microvilli. At paranodes, Caspr and contactin heterodimers interact in *trans* with NF155. At juxtapanodes, TAG-1 dimers interact homophilically in *trans*. Axonal TAG-1 forms a complex with Caspr2, tetramers of Kv1.1/Kv1.2 channels, and PSD-93, PSD-95. Protein 4.1B links the cytoplasmic tail of Caspr and Caspr2 to the spectrin cytoskeleton. Homotypic gap junctions comprised Cx32 link the paranodal membranes of the myelin sheath.

8: Peles lab, Tel Aviv, 2005–2010

The Peles lab [8] identified gliomedin as a binding partner for NF186 and Nr-CAM, showed that gliomedin is expressed by myelinating Schwann cells and is localized to nodes, and is required to form nodes of Ranvier in myelinating co-cultures. Subsequently, this group showed that gliomedin and Nr-CAM are required to cluster voltage-gated Na⁺ channels at hemi-nodes, which then fuse to form mature nodes of Ranvier [9]. Because gliomedin is scarcely expressed in the CNS, other mechanisms must form nodes of Ranvier in the CNS.

9: Brophy lab and Peles, Edinburgh and Redwood City, 1997–2000

At paranodes, the lateral edge of the myelin sheath spirals around the axon, forming the axoglial junctions that contain septate-like junctions (Figure 32.5). Septate-like junctions limit the diffusion of large molecules and separate the juxtapanodal Kv1.1/Kv1.2 K⁺ channels from the node of Ranvier. The molecular composition of paranodes began with the discovery that Caspr interacts with contactin, a GPI-

linked protein [10], that Caspr was localized to paranodes [11], and that contactin/Caspr heteromers bind *in trans* to NF155, a glial isoform of neurofascin that is located at the paranodal loops [12]. The genetic deletion of contactin, Caspr, or NF155 results in the failure to form septate-like junctions, and is associated with neurological deficits.

10: Scherer and Paul, Philadelphia and Boston, 1995

There are “reflexive”/“autotypic” junctions between the paranodal loops themselves— including tight junctions, gap junctions, and adherens junctions (Figure 32.5). Tight junctions (formed by claudin-11 in the CNS and claudin-19 in the PNS) join the layers of the myelin sheath together. Gap junctions formed by Cx32 appear to be crucial in allowing the diffusion of ions and small molecules through the layers of the myelin sheath [13]; loss of these channels may be the reasons that mutations in *GJB1*, the gene that encodes Cx32, cause an inherited neuropathy.

Next up at the nodes

Nodal antigens are the likely targets for a variety of acquired peripheral neuropathies. Gangliosides have been long implicated as immunological targets of antibodies in multi-focal motor neuropathy and various form of Guillain-Barré syndrome [14]. More recently, antibodies against nodal (NF186, Nr-CAM, and gliomedin), paranodal (contactin, NF155), and juxtaparanodal (TAG-1/contactin-2 and Caspr2) have been found in small subsets of patients with chronic inflammatory demyelinating neuropathy and Guillain-Barré syndrome [15], and Caspr2 is the main target antigen in acquired neuromyotonia. It will be interesting to determine how these antibodies cause their associated diseases, and whether there are more specific therapies.

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Pain and GBS: The Basic and Clinical Science

David L.H. Bennett

Introduction

Given the grievous consequences of motor dysfunction, this has understandably taken precedence in GBS research over and above the understanding of sensory loss and pain. It has however been long understood that persistent pain is an important and common sequel of GBS with major deleterious impact on quality of life. In the initial sequence of papers I will review clinical progress in defining the nature of pain in GBS and its pathological correlates, and subsequently focus on progress in understanding the pathophysiology of this pain. There has been a recent convergence between the pain and immunology fields in recognising that immune mechanisms have an important part to play in the development and persistence of neuropathic pain whatever the cause. These innate and adaptive immune mechanisms not only impinge on neurons but also have a major impact on myelinating and non-myelinating glia. I will discuss not only what we have learnt about the specifics of pain aetiology in GBS but also how this has gone hand in hand with our wider understanding of the many factors which drive neuropathic pain following nerve injury.

The Recognition and Description of Pain in GBS

Haymaker WE and Kernohan JW. The Landry-Guillain-Barré syndrome; a clinicopathologic report of 50 fatal cases and a critique of the literature. *Medicine*, 1949

Of course the first question in relation to pain and GBS is how common is it and what are the characteristics of the pain? In fact pain was noted in the initial cases described by Guillain, Barré and Strohl and in subsequent case reports/series. However, it was not until the publication of this paper by Haymaker and Kernohan that a more systematic approach was employed in elucidating sensory dysfunction in GBS [1]. Many of their comments chime with our experience of pain suffered by GBS patients in the 21st century. They reviewed a clinicopathological series of 50 cases from the American Army Institute of Pathology. In 50% of cases, sensory symptoms, pain, paraesthesia and numbness in various combinations were the presenting symptoms preceding weakness. Paraesthesia usually affected the hands and feet. Overall pain was reported in 56% of cases, which is a lower frequency than in modern series perhaps due to fact that this was a retrospective case review. Pain was often described as initially affecting the lower trunk and legs but was also described in a minority of patients as affecting the joints and muscles. Usually pain developed early in the course of the illness and, although pain improved in the majority of cases, in a significant group it persisted. The authors note that the majority of patients had

demonstrable evidence of sensory loss, usually in a 'glove and stocking' distribution but in some cases also affecting the trunk. Not only is impaired sensibility described on examination (to touch, pinprick and vibration sense), but we also see descriptions of sensory abnormalities which we now associate with neuropathic pain, such as hyperalgesia and allodynia [2]. There is some comparison of sensory dysfunction with pathology in that those patients with prominent sensory loss had a greater inflammatory change within the dorsal roots. The authors also noted variable changes within dorsal root ganglia (DRG) with injury to DRG cells clearly present in some cases, as demonstrated by the presence of chromatolysis. This paper therefore firmly established sensory dysfunction and pain as key features of GBS.

Ropper AH and Shahani BT. Pain in Guillain-Barré syndrome. *Archives of Neurology*, 1984

Ropper and Shahani published the first GBS cohort specifically focussing on pain and its characteristics [3]. They reported 29 patients with GBS who were assessed in the acute phase of the illness (within 15 days of onset of weakness) making comparison with clinical, electrophysiological and pathological features. The cohort was described as being mainly typical GBS with 2 of the 29 having the Miller Fisher syndrome (MFS). Fifty-five percent of patients had pain early in the course of GBS and 72% of patients reported significant pain (which in the vast majority was described as moderate or severe) at some point during the course of their illness. The most common locations were the upper legs; back pain was often transient; other affected areas were flanks and shoulders, with the hands and feet being less commonly affected. There was very little correlation between clinical signs, electrophysiological findings or the presence of inflammation within the DRG and the presence of pain. The lack of correlation between the presence of pain and sensory loss make the authors conclude that is unlikely to be arising as a consequence of nerve injury, suggesting that it is more likely to be muscular in nature. However, it should be noted that subsequent studies will show that although there is likely to be a musculoskeletal component pain in GBS there is clearly also a neuropathic component.

Moulin DE, et al. Pain in Guillain-Barré syndrome. *Neurology*, 1997

Moulin prospectively followed a cohort of 59 GBS patients between admission and 24 weeks [4]. There was a high rate of pain which was reported in 89% of patients during the course of their illness. The pain was moderate to severe in intensity (mean pain rating on visual analogue scale at admission of 4.7). The severity of the pain is borne out by the fact that three-quarters of patients required opioid analgesics. The most common pain distribution, consistent with the findings of Ropper and colleagues, was the lower back and upper legs, although a significant group reported pain in the extremities. One means of explaining such a distribution would be dorsal root inflammation, which is commonly reported in pathological series of GBS (see Haymaker and Kernohan above) resulting in lumbar back pain and pain in the upper thighs. In addition, inflammation may ultimately lead to secondary axon injury resulting in sensory loss and pain in the extremities. Subsequently, Ruts and colleagues described a large longitudinal cohort of 156 GBS patients with follow-up to 1 year, at which point 38% of patients reported significant pain [5]. These initial case series did not find a simple relationship between pain incidence/severity and injury to sensory neurons. However, the use of skin biopsy to directly visualise sensory fibres would throw light on this issue.

Pan CL, et al. Cutaneous innervation in Guillain-Barré syndrome: pathology and clinical correlations. *Brain*, 2003

In the 1990s it became clear that the free nerve endings of C-fibres and thin myelinated A-delta fibres could be visualised and quantified within the epidermis by immunostaining for the pan-neuronal marker PGP-9.5. These represent the terminals of nociceptors and thermoceptors, and this technique is now widely used in both research and clinical practice. This has been a major advance given that the function of these fibres is not interrogated with standard electrophysiological testing. Pan and colleagues [6] applied this technique to a group of GBS patients, showing that there was a significant reduction in intraepidermal nerve fibre density (IENFD) in the distal leg as compared to matched controls. There was a correlation between the reduction in IENFD and raised thermal thresholds in GBS patients. There was a correlation between IENFD and disability; however, the authors reported that IENFD was the same in those patients with and without neuropathic pain. It was not clear, however, exactly what tool was used for the assessment of neuropathic pain in this study, and there was no assessment of pain intensity reported which would have enabled a direct correlation with IENFD. These issues were dealt with at a functional level by Martinez and colleagues [7], who demonstrated that there was a significant relationship between the degree of small fibre dysfunction as assessed by quantitative sensory testing to measure thermal thresholds and the presence of neuropathic pain. The subsequent paper went on to thoroughly assess the relationship between neuropathic pain and cutaneous innervation.

Ruts L, et al. Unmyelinated and myelinated skin nerve damage in Guillain-Barré syndrome: correlation with pain and recovery. *Pain*, 2012

In a cohort of 32 GBS patients these authors showed a significant reduction in IENFD in the lumbar region in 61% of patients, and at the distal leg in 60% of patients in the acute phase of GBS (first 3 weeks) [8]. One important implication is that injury to small fibres is not length dependent given the significant reduction in IENFD in the lumbar region. Additionally, even some patients with pure motor forms of GBS or MFS had a significant reduction in IENFD. Patients were followed for up to 6 months and IENFD remained low; only a minority showed recovery of IENFD. In the acute phase those patients with neuropathic pain were more likely to have a reduction in IENFD at the distal leg and there was an inverse correlation between IENFD and pain intensity. Lumbar IENFD during the acute phase of GBS also correlated with poorer GBS disability at 6 months. The authors also studied myelinated cutaneous dermal fibres and demonstrated that GBS is associated with fragmentation of myelin and disorganisation of the nodal complex. This was only performed in a subset of patients and so statements on the degree of myelin/nodal injury and pain or functional status could not be made.

This series of papers have provided important insight into the clinical characteristics of pain in GBS. It is common, severe and in a significant group of patients persistent even at 1 year and affects both proximal regions such as lower back as well as the extremities. With pathological correlations we can even make some broad hypotheses on causation—severity is related to degree of injury to the sensory nervous system, there is prominent inflammation in GBS and myelin loss which is one potential factor in pain aetiology, and there is direct evidence that the loss of C-fibre terminals inversely correlates to pain severity. Subsequently I will go on to consider which studies have provided mechanistic insight into the pathogenesis of neuropathic pain in GBS at both the molecular and cellular level.

The Aetiology of Pain in GBS

Wall PD and Gutnick M. Properties of afferent nerve impulses originating from a neuroma. *Nature*, 1974

This paper relates to a model of painful traumatic neuropathy; however, it was seminal in moving forward our understanding of peripheral neuropathic pain [9]. We now take for granted the fact that sensory neurons develop ectopic or spontaneous activity following nerve injury; this paper was the first direct demonstration of these phenomena. Prior to this report it had been shown that cutting a nerve would lead to an acute ‘injury discharge’ within seconds, which would then subside; however, there was no knowledge as to what would happen to the electrophysiological properties of primary afferents in the subsequent days and weeks, which was the topic of this paper.

The authors ligated the sciatic nerve to generate a neuroma and then recorded from individual units within fine filaments of the teased dorsal root at up to 40 days post-injury. They noted ongoing spontaneous activity in myelinated afferents terminating in the neuroma. The fact that application of lidocaine to the neuroma could block this ongoing activity was provided as evidence that the impulses arose from the neuroma itself; subsequent studies have shown that ectopic activity can also arise at the level of the cell body within the DRG [10]. The sensory neurons also adopted a novel mechanosensitivity. In the conclusion the authors presciently argue that this ongoing activity would be an important pharmacological target for novel analgesics. A recent study using peripheral nerve blocks in man suggests that such ongoing spontaneous activity is required for the maintenance of neuropathic pain, and spontaneous activity in myelinated and unmyelinated afferents has been noted in many different types of neuropathy, including following demyelination (see below).

Wallace VC, et al. Focal lysolecithin-induced demyelination of peripheral afferents results in neuropathic pain behavior that is attenuated by cannabinoids. *J Neurosci*, 2003

Historically in preclinical models of neuropathic pain the major emphasis has been on traumatic injury to axons in the rodent (usually involving partial axotomy of the sciatic nerve or its branches). An important question for the demyelinating variants of GBS is whether primary demyelination in which the myelin sheath is removed while the axon remains intact can result in neuropathic pain. Wallace and colleagues showed clearly in their work that this is in fact the case; they also went on to provide mechanistic understanding of pain aetiology [11]. They used topical application of the chemical lysolecithin to the mouse sciatic or saphenous nerve in order to induce primary demyelination. On behavioural testing within a week, this resulted in the development of both thermal and mechanical hypersensitivity, which lasted for the subsequent 2 weeks, a time course which would parallel the period of remyelination.

Electrophysiological recordings at the peak of these behavioural changes demonstrated the presence of ectopic ongoing activity. Such ectopic activity is a virtually invariant feature of peripheral neuropathic pain and is a major target for drug discovery. Following primary demyelination this ongoing activity is likely to be a consequence of altered expression/trafficking of voltage-gated ion channels in the axolemma of sensory axons as a consequence of the altered interaction with Schwann cells.

The authors show that primary demyelination results in increased expression of the voltage-gated sodium channel $Na_v1.3$, which is normally only expressed during development and is absent in adulthood. Others had previously demonstrated that demyelination of axons leads to radical changes in ion channel distribution: for instance, voltage-gated sodium channels, which would normally be confined to the node of Ranvier, now spread along the axolemma and could act as ectopic ‘generators’. This study was significantly extended when it was shown that pain-related hypersensitivity was not restricted to demyelination produced by chemical means but was also observed in an animal model of inflammatory neuropathy ‘experimental auto-immune neuritis’ [12].

Xiao WH, et al. Electrophysiological characteristics of primary afferent fibers after systemic administration of anti-GD2 ganglioside antibody. *Pain*, 1997

Gangliosides either individually or in complexes have been implicated as targets for autoantibodies. This paper provides a mechanistic link between ganglioside autoantibodies and the development of neuropathic pain [13]. The 'B' series gangliosides (such as GD2) in particular are known to be expressed by sensory neurons at the surface of the cell body and along the axolemma. An example of the direct effects of antiganglioside antibodies is that a monoclonal antibody directed against GD2 used therapeutically in the treatment of neuroblastoma was noted to cause pain and mechanical allodynia as side effects.

To understand the pathogenic effects of anti-GD2 antibodies, Xiao and colleagues systemically administered anti-GD2 antibody to rats. These antibodies resulted in the rapid development of mechanical hypersensitivity in the rat which mirrored the development of mechanical allodynia in patients administered these antibodies. On electrophysiological recording (performed on the same day as the behavioural assessment) both C-fibres and A-delta sensory fibres developed spontaneous activity. In addition, their threshold to mechanical stimuli dropped. In summary, antibodies to the GD2 ganglioside known to be expressed by sensory neurons could acutely sensitise primary afferents leading to spontaneous activity, reduced mechanical thresholds and the development of spontaneous pain and mechanical allodynia. This is therefore proof of concept of the potential effects of antiganglioside antibodies in GBS.

Zhang ZY, et al. Improved outcome of EAN, an animal model of GBS, through amelioration of peripheral and central inflammation by minocycline. *J Cell Mol Med*, 2009

Experimental allergic neuritis is an animal model in which an acute, inflammatory demyelinating neuropathy develops and is used as a model of the AIDP variant of GBS. A number of groups (including Moalem-Taylor and colleagues [12]), have recently shown that the development of experimental allergic neuritis (EAN) is associated with the development of pain-related hypersensitivity. In this paper Zhang and colleagues study the effects of minocycline—a second-generation tetracycline with anti-inflammatory properties—in this model [14]. EAN was induced by a neuritogenic peptide of P2. EAN results in the recruitment of inflammatory infiltrate to peripheral nerve and increased inflammatory cytokine (TNF- α) expression. This inflammatory environment is likely to sensitise primary afferents, and indeed individual cytokines such as TNF can produce spontaneous activity and sensitise primary afferent nociceptors. Although EAN results in peripheral nerve injury, maladaptive plasticity within the CNS is also likely to have a role in neuropathic pain development. Indeed, as has also been noted following traumatic nerve injury, microglia within the spinal cord transform to a pro-inflammatory phenotype [15] and express the purinoceptor P2RX4. These pro-inflammatory (P2RX4 +ve) microglia within the dorsal horn release brain-derived neurotrophic factor (BDNF), which sensitises nociceptive signalling. Minocycline treatment reduces the severity of EAN as assessed by motor function and ameliorates all of the inflammatory changes described above, resulting in reduced pain-related hypersensitivity. This produces an important illustration of how pro-inflammatory process engaged by EAN (and, by inference, in GBS) can act to sensitise the nociceptive system. Disease-modifying therapy in GBS is likely to reduce the incidence/persistence of neuropathic pain associated with GBS but how should we approach analgesic therapy?

This recent paper on behalf of the Neuropathic Pain Special Interest Group (NeuPSIG) of the International Association for the Study of Pain (IASP) is a comprehensive, systematic review and meta-analysis of pharmacotherapy for neuropathic pain [16]. There have been a number of small studies of analgesic therapy in GBS; however, a recent Cochrane review pointed out that the quality of the evidence was poor and it was not possible to draw definitive conclusions [17]. One of the emerging themes in neuropathic pain is that pathophysiological mechanisms (for instance ectopic activity in primary afferents or sensitisation within the CNS) are not aetiology specific and in general pharmacological agents show efficacy across a range of causes of neuropathic pain. In fact, an aspiration for the future is that we should use somatosensory phenotyping rather than aetiology to stratify patients and target treatment. The authors of this paper point out that their analysis and guidance should be applicable to a wide range of neuropathic causes (and we hope this includes GBS). One caveat is that most large randomised controlled trials for neuropathic pain are performed on patients with painful diabetic neuropathy or post-herpetic neuralgia. Based on efficacy and tolerability, recommended first-line agents for neuropathic pain are the gabapentinoids, tricyclic antidepressants (TCAs) or serotonin/noradrenaline reuptake inhibitors. In the acute phase, care would need to be taken with the use of TCAs if there were autonomic involvement. The second-line agents which were recommended included lidocaine plasters and high-dose capsaicin patches; however, the regions of neuropathic pain may well be too extensive for topical therapy in GBS. Third-line agents include strong opiates. This systematic review also emphasises that, although combination analgesic therapy is intellectually appealing, the trial results have been mixed. The hope for the future is further well-powered studies of combination therapy and a more stratified approach to see if this can predict treatment response.

Summary and Conclusions

It is now well recognised that pain is an important and disabling consequence of GBS. There may be a musculoskeletal component, but a key element is neuropathic pain due to damage to sensory afferents, and a number of studies have shown a relationship between the degree of injury to small nerve fibres within the epidermis and pain severity. The aetiology of neuropathic pain is likely to be multifactorial, including inflammation triggered by humoral and cell-mediated immunity as well as the consequences of injury to sensory axons and dysfunction of ion channels resulting in ectopic activity. Interestingly, there is some experimental evidence that antiganglioside antibodies (anti-GD2) can trigger spontaneous activity in sensory neurons. Currently the best treatment algorithms will use generic guidelines on the management of neuropathic pain such as those recently developed by NeuPSIG; however, future trials are needed to test analgesic efficacy specifically in GBS. A key aspiration of the neuropathic pain field is to use sensory profiling in order to stratify patients, with the hope that such stratification will reveal particular underlying pathophysiology and hence predict treatment response. Given the pain drivers which are proposed in GBS, it would be advised that analgesic trials in GBS employ such an approach.

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Complement in Guillain-Barré Syndrome: From Bench to Bedside

Amy Davidson and Susan K. Halstead

Introduction

Having previously joined Professor Hugh Willison's group at different times, we were jointly approached to write a chapter based on complement and its role in Guillain-Barré syndrome. Given our different backgrounds, we aim take you through scientific research (Sue Halstead) into a clinical setting (Amy Davidson), reflecting the translational nature of our work.

Due to the personal journeys this chapter represents it is not intended to be a comprehensive review of the literature in this field. First, Susan highlights 5 papers which influenced her as a PhD student, and later as a postdoctoral scientist aiming to glean new insights and strategies for complement therapeutics, with the hope that these could prove influential in the clinic. This is followed by 5 papers selected by Amy, a clinical fellow who took up the gauntlet and is now recruiting GBS patients for a clinical trial of eculizumab, the world's first clinically approved, antibody-derived complement therapeutic.

The Science of Complement

Having graduated with a B.Sc. (Hons) in neuroscience, I joined the Willison group in 2000 to embark on 3-year journey to gain my Ph.D., funded by the Guillain-Barré support group in the UK. With only a very basic appreciation of the various branches of immunology, I found this to be a steep learning curve, as my remit was firmly focused on dissecting out the role of the complement cascade in the pathogenesis of GBS. At the time of joining the group, we worked very closely with our friends in Leiden (Jaap Plomp and group). This collaboration had proved very successful, having already established an *ex vivo* mouse model of GBS which was described as producing an 'alpha-latrotoxin-like effect', resulting in extensive destruction of the presynaptic neuromuscular membrane. As this model was complement-dependent, I was initially set the task of determining the critical components of complement resulting in damage, with the long term goal of developing an *in vivo* mouse model.

Over the last 15 years, I've read many papers that greatly assisted me in my quest, and in the following section I would like to highlight 5 papers that I've found both helpful and encouraging.

Morgan BP. The role of complement in neurological and neuropsychiatric diseases. *Expert Review of Clinical Immunology*, 2015

When first approached to contribute to this publication, I knew without doubt that one of Professor Morgan's publications had to be featured in my list of notable papers. The only problem was which one to select. As a prolific author of numerous high impact journals and publications, Morgan is a true world expert in complement, who has always been extremely generous with both his time and reagents. Amongst his many publications I have selected his recent review regarding the evidence for a pathogenic role for complement in a host of neurological and neuropsychiatric diseases [1]. The reason I picked this over one of his original research papers is that I find whenever I'm presented with new subject matter, it is vitally important to place it in context. In this article both PNS and CNS diseases are discussed and evidence is presented implicating complement as driving key pathological events. Disease types include autoimmune (including GBS), infections, neurodegenerative, acute injuries and neuropsychiatric. The importance of understanding the disease process in these diseases has become increasingly important of the last few years, as new regulators of complement have emerged which target different points of the complement cascade, making it feasible in the future to attenuate complement-driven pathology.

Ong GL and Mattes MJ. Mouse strains with typical mammalian levels of complement activity. *Journal of Immunological Methods*, 1989

For research scientists, it is fundamental to have an accurate and reproducible model system in which to study disease pathology. Despite the existence of several valuable animal models of GBS, to date no model fully encompasses all the pathological events seen in humans. But why is this? Although there are numerous explanations for the differences seen, my personal research focus has been on complement and why I was unable to detect endogenous complement at the mouse neuromuscular junction, when abundant mouse antibody deposits were present on the presynaptic membranes after passive immunisation. My naive understanding of the mouse immune system led me in search of an explanation, and I came across this publication which shed light on this conundrum.

In this article Ong and Mattes acknowledge that "common laboratory mouse strains have very low complement levels relative to humans, rats, guinea pigs, rabbits and other mammals, which limits the value of the mouse as an experimental model" [2]. To address this issue, this paper sought to measure the complement activity of 43 strains of mice, using both antibody-coated erythrocytes and human tumour cell targets, thereby measuring classical pathway activation. Only 8 of the 43 mouse strains tested had levels of complement comparable to other mammals, and it was interesting to learn that 4 of these had only very recently been derived from a wild mouse population. Of course, the mice I had been using in my research didn't make this short list of strains with effective complement activity! In search of an explanation for this phenomenon, the authors measured the individual components of the classical pathway and reported elevated levels of C3, C5, C6 and C7 (with no difference in other component levels) in mice with greater complement activity.

Because this article provided a valuable insight into the issue of mouse complement, we were confident that this relatively weak performance of complement in our mice could be circumvented by utilising complement-regulator knockout mice, and this takes me nicely on to my next paper.

Lin F, et al. Markedly enhanced susceptibility to experimental autoimmune myasthenia gravis in the absence of decay-accelerating factor protection. *Journal of Clinical Investigation*, 2002

In 2003 I was very fortunate to spend a week in the laboratory of Professor M. Edward Medof and Dr

Feng Lin at Case Western Reserve University in Ohio. This visit resulted from reading this article, which drew many similarities with my own model system, and I was keen to see if the impressive results that they had documented in their decay accelerating factor knockout (DAF KO) mouse model of myasthenia gravis could be replicated for GBS [3].

In their model of myasthenia gravis, Lin and colleagues passively induced mice with rat monoclonal antibodies targeting nicotinic acetylcholine receptors on the post-synaptic neuromuscular membrane. They reported that, compared to wild type mice, knocking out the gene for the complement regulator DAF, which accelerates the breakdown of both the classical and alternative pathway derived C3 and C5 convertases, resulted in mice with exacerbated disease pathology. This was measured both physiologically and at the ultrastructural level, in addition to detecting a greater frequency of NMJ colocalising with the intermediate complement component C3b. This clearly demonstrated a key role for this complement regulator at the post-synaptic membrane, but I wanted to see if the presynaptic membrane would be equally governed by this regulator in our disease paradigm. Disappointingly, preliminary results didn't reveal an acute model of GBS in these mice. However, this presented us with a new tactic to bypass the inherent issues of low complement activity in mice and site-specific complement regulator activity in mice.

Pangburn MK and Müller-Eberhard HJ. Complement C3 convertase: cell surface restriction of β 1H control and generation of restriction on neuraminidase-treated cells. *Proceedings of the National Academy of Sciences of the United States of America*, 1978

Following on the theme of complement regulators, I have selected another paper highlighting the dominant activity of the complement regulator β 1H (Factor H) in a sialic acid rich-membrane environment.

In this paper the authors identify a role for sialic acid in influencing alternative pathway activation of complement [4]. While the alternative pathway is not directly triggered by antibody fixation, it can serve to amplify the complement cascade subsequent to classical pathway activation. In this article treating cells with neuraminidase (which cleaves sialic acid residues from cells) converted a non-activator into an activator of alternative complement. It is now understood that the fluid phase complement regulator, Factor H (β 1H), readily binds to sialic acid and other polyanionic molecules, thereby protecting cells from complement by decay accelerating activity of the alternative pathway C3 convertase and cofactor activity for Factor I mediated C3b cleavage. While this principal suggests a relative protective environment of ganglioside-rich neuronal membranes to complement activation, whereby alternative pathway amplification is attenuated under normal conditions, it also highlights one of the many problems associated with generating a reliable complement-dependent model at this site.

Hillmen P, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *The New England Journal of Medicine*, 2004

As discussed in the Morgan paper above, complement plays a key role in driving inflammation and cell damage in many neurological diseases. Pharmaceutical companies are aware of the great need to modulate or inhibit complement activity, yet until recently there has been a void of clinical complement therapeutics. It was in 2007 that the FDA approved the use of eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH) and to date it has been approved for use in 50 other countries. Eculizumab is a recombinant humanised monoclonal antibody which binds to complement component C5, thereby specifically inhibiting progression of the complement cascade to the terminal lytic

pore, membrane attack complex and the release of the potent anaphylatoxin C5a.

In this paper the authors describe the trial use of eculizumab over a 12-week period for the clinical treatment of a small group of patients with PNH, a rare genetic disorder arising from the somatic mutation of the PIG-A gene in pluripotent hematopoietic stem cells, which encodes a protein essential for the synthesis of glycosylphosphatidylinositol (GP1, a lipid component which anchors a variety of proteins in the plasma membrane) [5]. Two of these GPI-anchored proteins that are absent in PNH patients are the complement regulators DAF and CD59, which leaves patient erythrocytes highly vulnerable to intravascular hemolysis, venous thrombosis and hemoglobinuria. Biochemical and clinical monitoring of the patients in this trial revealed a remarkable improvement in haemolytic indicators. The requirement for blood transfusion was reduced, hemoglobinuria was reduced by 96% and quality of life indicators also improved significantly.

Back in 2003 I was first introduced to Alexion Pharmaceuticals at the European Complement Network in Trieste, Italy. The therapeutic power of eculizumab was very apparent and we were delighted when they agreed to let me test its effectiveness in our humanised mouse model of GBS. This was the beginning of our collaboration with Alexion Pharmaceuticals which has now flourished into an ongoing clinical trial of eculizumab in GBS. I am extremely proud to have contributed towards this goal of developing new therapeutics, and I wait with great anticipation for the outcome of this study.

Complement in the Clinic

One of my favourite quotes of all time is from Sir Isaac Newton—“If I have seen further than others, it is by standing upon the shoulders of giants”—and I feel it is a fantastic image of research in general, and of our progress in understanding complex conditions such as GBS. Whilst I have no pretensions to my own significance, contributing to this chapter allows me to reflect on the path that has shaped my own role in GBS research.

Selecting papers from the legion that have shaped our understanding of the role of complement in GBS is nearly impossible, so forgive any perceived folly and allow me to indulge in a rather personal journey, chronicling key moments in my understanding.

Koski CL, et al. Activation of terminal components of complement in patients with Guillain-Barré syndrome and other demyelinating neuropathies. *The Journal of Clinical Investigation*, 1987

This paper was my very first introduction to the topic of complement and GBS. Little did I realise that I would go on to be intimately familiar with it, and frequently quote it, when discussing my project.

Koski and colleagues' use of ELISA, using antibodies to C9 expressed neoantigens, on sera from both patients with GBS and controls showed the presences of SC5b-9, or the complement-induced membrane attack complex (MAC), in those with GBS, but none in the normal control group [6]. Their work on kinetic studies for complement-fixing antibodies showed a correlation between decreasing levels of circulating antibody and SC5b-9, and an improvement in muscular strength. Immunostaining of peripheral nerve segments detected the presence of C9, which was focal and segmental in nature. This drew a temporal association between antibody production and MAC formation in patients with GBS.

Griffin JW, et al. Pathology of the motor-sensory axonal Guillain Barré syndrome. *Annals*

of *Neurology*, 1996; and Hafer-Macko C, et al. Acute motor axonal neuropathy: an antibody mediated attack on axolemma. *Annals of Neurology*, 1996

With my appetite whetted, I delved further into the work of Griffin, Hafer-Macko and their colleagues during the 1990s, who characterised pathological findings in GBS and formed much of the basis our modern understanding of the AMAN variant [7]. These 2 papers explore the immunopathology of axonal GBS through autopsy pathological samples. The former demonstrates Wallerian-like degeneration in affected nerves, showing macrophage infiltration in the peri-axonal space, and uses its discussion to postulate links between severity of nodal damage and activation of complement in the nodal region. The Hafer-Macko paper describes nodal complement deposition, and highlights the differences in patterns between what we see in AIDP and AMAN [8]. Together these papers began to unpick the relationship between complement and AMAN, allowing me to get a further handle on not only the complex relationships between complement and GBS, but also on the complex relationships between different subtypes of GBS.

McGonigal R, et al. Anti-GD1a antibodies activate complement and calpain to injure distal motor nodes of Ranvier in mice. *Brain*, 2010

Jumping forward somewhat, to a time when pathogenic anti-ganglioside antibodies thought to drive axonal GBS were characterised, McGonigal and colleagues' paper not only demonstrated the deposition of membrane attack complexes at nodes of Ranvier, but also offered a functional analysis, using electrophysiological assessments to show lack of current flow once the MAC had bound, and conversely showing that inhibiting complement activation could also be protective to the nodes of Ranvier, which of course was significant to me as I was hoping to prove something akin to this on a slightly different population [9]!

Halstead SK, et al. Eculizumab prevents anti-ganglioside antibody mediated neuropathy in a murine model. *Brain*, 2008

This paper is, for me, a cornerstone for the use of C5 inhibitors in the treatment of GBS. Sue Halstead and colleagues used a novel murine model of Miller Fisher variant GBS, created by intraperitoneal injection of anti-GQ1b antibody and normal human serum, to show the antibody-driven, complement-derived respiratory paralysis could be abrogated by intravenous injections of C5 inhibitor eculizumab [10]. The in vitro work performed in parallel showed application of Eculizumab completely prevented MAC deposition at neuromuscular junctions, compared to controls using a 'dummy' mAb, and damage to the perisynaptic Schwann cells was abolished. This evidence was pivotal in the move to use eculizumab in a clinical trial setting, in a patient cohort with severe GBS.

What I have touched on here is very much the tip of the iceberg, but for me encapsulates the beginnings of a journey into understanding some of the complex immunopathology of GBS. As a clinician by trade I also am drawn to translational research, where I can see a story moving from 'bench to bedside', which is very much the case for complement and GBS. We are now at a point where, for the first time ever, people have received doses of eculizumab for their GBS, and time will tell if we can replicate the fantastic responses we have seen in our animal models. What I find most intriguing about this is the thought of reading this chapter again in 10 years' time. Will complement inhibition be the norm in treating GBS? Or will it be relegated to annals of history? I await with bated breath what the future will hold.

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As with all good scientific research, our research was not performed in isolation—it has truly been a collaborative affair. We would like to acknowledge the contributions of our past and present colleagues based at the University of Glasgow and the Greater Glasgow NHS trust and our key collaborators across the world: Professor Paul Morgan, Dr Jaap Plomp and Alexion Pharmaceuticals. In particular we (the authors) wish to acknowledge our appreciation and gratitude to our mentor, Professor Hugh Willison, who has a tireless patience and a relentless ability to gain inspiration and fresh insight from the most negative of results.

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The Role of T Cells in Guillain-Barré Syndrome

Christopher Linington

Introduction

Adoptive transfer studies demonstrate that the minimal requirement to initiate inflammation, demyelination and axonal loss in the peripheral nervous system (PNS) is a tissue-specific CD4⁺ T-cell response. This observation marked my first foray into neuroimmunology and prompted speculation that a similar mechanism contributed to the pathogenesis of Guillain-Barré syndrome (GBS). This concept is now largely discounted with respect to GBS, but increasing evidence indicates the T-cell compartment plays important roles in other diseases affecting the PNS, in particular Charcot-Marie-Tooth disease (CMT) and chronic inflammatory demyelinating polyneuropathy (CIDP). My personal and somewhat eclectic Top 10 attempts to place these studies in their historical context, developing a theme that T cell-mediated autoaggression synergises with other effector mechanisms to exacerbate tissue damage across a broad swath of PNS disorders.

Boston 1955: Experimental Autoimmune Encephalomyelitis (EAN)

Adams and Waksman developed the first model of experimental allergic neuritis (EAN) in which inflammatory demyelination was restricted to the PNS by immunising rabbits with sciatic nerve homogenates emulsified in Freund's complete adjuvant [1]. They noted that the structure and cellular composition of these lesions resembled that seen in some—but not all—cases of GBS, leading them to state these were “sufficiently impressive to justify a further exploration of the possibility that acute ‘infectious’ polyneuritis may have an immunologic basis”. Moreover, as similar lesions could be induced by immunizing rabbits with CNS tissue homogenates, they concluded “there must clearly exist in the rabbit's peripheral nervous system an antigen which is absent in its central nervous system.” This statement set the scene for future studies on EAN and its relationship to GBS, but 30 years passed before the efforts of researchers across the globe identified this ‘neuritogen’ and its mode of action.

Evidence is lacking, however, that humoral antibody to nervous tissue antigens is in any way related to the disease process; passive transfer of either disease with serum from sensitised animals has proved impossible.

Boston 1962: EAN, a Cell-Mediated Disease

The development of EAN as a model replicating several of the clinical and pathological features of GBS

in man prompted an intense effort to determine the nature of the immune response responsible tissue damage in the PNS. Initial interest focused on the possible role of antibodies, but passive transfer of EAN with serum from sensitised animals was ineffective. This led Åström and Waksman to consider the possibility it was mediated by a cellular, delayed hypersensitivity response within the PNS [2]. The ability of lymph node cells derived from a sensitized animal to induce hypersensitivity responses in normal recipients had been established some 40 years earlier by Landsteiner and Chase, so it was a logical step to extend this concept to the pathogenesis of tissue specific ‘allergic’ diseases such as EAN. In this paper Åström and Waksman describe the ability of lymph node cells isolated from donors immunised with bovine sciatic nerve homogenate in adjuvant to induce in naïve recipients a disease that was indistinguishable from actively induced EAN. This study was performed in New Zealand rabbits, and perhaps not surprisingly, in view of our current understanding of immunology, disease penetrance and severity were low. Nonetheless, cells from donors immunised with kidney homogenates or adjuvant alone, or killed cells from animals immunised with nervous tissue, were unable to induce EAN, establishing the dominant role of a cellular immune response in disease induction.

London, 1979: P2 Protein: The First Defined Neuritogen

Beyond the fact EAN was cell mediated [2] further progress towards understanding its pathogenesis had to await identification of the ‘neuritogenic’ component of PNS myelin first posited to exist by Adams and Waksman [1]. This was finally achieved in 1979 by Kadlubowski and Hughes, who demonstrated purified bovine P2 protein was highly neuritogenic in the Lewis rat, thereby opening the way towards elucidating the mechanistic basis of EAN [3]. The authors’ success was dependent largely on their selection of an appropriate combination of antigen source and species, but they also note “clinical disease produced by the pure neuritogen was slightly less severe than that induced by whole myelin”, an observation that pointed towards the existence other neuritogens in PNS myelin [4]. A complication that may in part account for the failure of subsequent attempts to identify a clear association between P2 protein-specific immunity and disease activity in human disease.

Würzburg, 1984: Adoptive Transfer of EAN by P2-Specific CD4⁺ T Cells

Demonstrating that the P2 protein-specific T-cell response was the minimal requirement to induce EAN in the Lewis rat marked my entry into the world of neuroimmunology [5]. I was fortunate enough to join Hartmut Wekerle at the Max-Planck Clinical Research Group for Multiple Sclerosis and developed the first adoptive transfer model of T cell-mediated EAN in the rat. We found intravenous transfer of as few as 5×10^4 freshly activated P2-specific T cells was sufficient to induce mild but consistent clinical signs of disease in naïve Lewis rats. Clinical disease in these animals was associated with focal infiltrates of CD4⁺ T cells and macrophages, breakdown of the blood-nerve barrier and primary demyelination. However, the intensity of this inflammatory response increased in proportion with the dose of T cells transferred, reaching a threshold beyond which axonal degeneration was the primary pathologic effect which resulted in widespread secondary demyelination that in many cases extended into the dorsal columns of the spinal cord. This study established that the minimal requirement to initiate EAN in a naïve, immunocompetent host was a neuritogenic CD4⁺ T-cell response, and it initiated a number of studies exploring effector mechanisms that were ultimately responsible for loss of function and tissue damage. These are now known to be largely macrophage-dependent and can be exacerbated by antibody-

dependent mechanisms [6], raising speculation about a similar combination of effector mechanisms which might drive disease progression in CIDP.

Chicago, 2001: Spontaneous Murine Autoimmune Peripheral Polyneuropathy (SAPP)

As documented elsewhere in this volume, the last decade of the 20th century saw an increasing awareness that autoantibody-dependent pathomechanisms were involved in many peripheral neuropathies, and not surprisingly this was paralleled by a concurrent decline in interest in T cell-dependent mechanisms. That was until the serendipitous and completely unexpected finding that the B7-2 blockade in the NOD mouse initiates a spontaneous autoimmune peripheral polyneuropathy (SAPP) which reproduces many of the clinical, electrophysiological and pathological features of CIDP [7]. The mechanistic basis by which ablation of the CD28/B7-2 co-stimulatory pathway unleashed this previously cryptic neuritogenic CD4⁺ T-cell response in the context of this particular genetic background remains unclear, but it rekindled interest in the possibility that the CD4⁺ T-cell compartment contributes to the aetiology of CIDP, and possibly even to some variants of GBS.

San Francisco, 2009: P0-Specific CD4⁺ Th1 Effector T Cells Mediate SAAP in NOD Mice

Some 8 years after the initial description of SAPP [7], Bluestone's group identified myelin P0 protein as the major target for the neuritogenic T-cell response [4]. Using P0-specific T-cell hybridomas derived from T cells infiltrating the PNS of affected mice, the authors then generated a P0 T-cell receptor transgenic mouse line (POT) to explore the development, regulation and pathogenicity of P0-specific T cells in vivo. This revealed that P0-specific T cells can survive thymic selection to populate the periphery, but their neuritogenic potential in immunocompetent wild type mice is then held in check, primarily by CD4⁺ Foxp3⁺ regulatory T cells. This regulatory checkpoint is lost when POT mice are bred onto a recombination-activating gene (RAG) deficient background, resulting in a fulminant early-onset neuropathy mediated by P0-specific CD4⁺ IFN γ ⁺ Th1 effector T cells. The phenotype of this effector T-cell response is in agreement with other studies, indicating development of SAPP is IFN γ dependent, and by extrapolation suggests this T-cell subset might contribute to the immunopathogenesis of CIDP.

London, 2009: CIDP Is Associated with a Functional Defect in Regulatory T Cells

The low prevalence of CIDP makes it difficult to obtain sufficiently large patient cohorts to identify disease-associated changes in immune cell function. However, several research groups took on this challenge, including Hughes and colleagues, who provide evidence that CIDP is associated with a functional defect in regulatory CD4⁺CD25^{high} T cells [8]. However, this defect does not occur in isolation as immunophenotyping revealed circulating monocytes were increased whilst NK cell numbers were decreased between patients and controls. In contrast, there were no significant differences in the frequency of circulating B cells, CD4⁺ and CD8⁺ T-cell subsets or crucially CD4⁺ Foxp3⁺ or CD4⁺CD25^{high}Foxp3⁺ cells. The authors were cautious in interpreting their findings and state that further

studies will certainly require larger numbers of patients. Nonetheless, their results clearly indicate CIDP is associated with a defect in Treg function, an observation that has parallels to the SAPP in the mouse [4,7].

Sydney, 2014: Synergy between T-Cell and Antibody-Dependent Mechanisms in EAN

Neuritogenic CD4⁺ Th1 T cell responses play an essential role in the pathogenesis of EAN [5] and SAPP [4,7], and many may be persuaded this is also the case in CIDP [8]. However, while these studies highlight the ability of CD4⁺ T cells to trigger inflammatory disease activity in the PNS, they do not rule out involvement of other immune effector mechanisms, in particular autoantibody- and CD8⁺ T cell-mediated effects. The former is particularly relevant in CIDP, which exhibits multiple clinical phenotypes and in some cases responds to plasma exchange. However, if antibodies are involved in these cases, how do pathologically significant amounts gain access to the PNS? In this report Mathey's group demonstrate the inflammatory response induced by a low dose of neuritogenic CD4⁺ T cells is sufficient to disrupt BNB integrity enough to facilitate access of pathogenic neurofascin-specific antibody into the PNS and exacerbate clinical disease [6]. Extrapolating this concept to human neuropathies raises a simple but rather important question: what are the threshold values for antigen-specific T cells and/or autoantibodies to induce a clinically relevant effect in the PNS? This is important as progressively more sensitive cell-based assays are being introduced to detect 'potentially' pathogenic autoantibody responses in clinical samples.

Montreal, 2014: At Last Something about CD8⁺ T Cells

EAN [5] and SAPP [4,7] established a pre-eminent role for CD4⁺ T cells in the pathogenesis of inflammatory demyelinating peripheral neuropathies, but as stated by Salomon in 2001 these results do not rule out the possibility pathogenic CD8⁺ T cells may play a role in the aetiology of these diseases after longer periods of time. Thirteen years later evidence is now available that this is indeed the case, as demonstrated by the development of a spontaneous autoimmune peripheral polyneuropathy (SAPP) in L31 transgenic mice bred onto a CD4^{-/-} background [9]. These mice develop a range of motor and sensory deficits that are associated with large numbers of macrophages and CD8⁺ T cells infiltrating the PNS accompanied by demyelination and varying degrees of axonal loss. This model indicates that disruption of immune homeostasis due to constitutive overexpression of B7.2 can trigger a profound CD4⁺ T cell-independent inflammatory response in the PNS. However, data are not yet available to formally demonstrate or confirm tissue damage, and associated clinical deficits in this model are mediated directly by components of the CD8⁺ T-cell repertoire.

Würzburg, 2006: T Cells in Inheritable Neuropathies

The rather eclectic collection of papers discussed above map the development of the concept that adaptive T-cell responses play significant roles in the pathogenesis of acquired inflammatory demyelinating diseases of the PNS, but it should not be forgotten that there is a small but parallel literature implicating T-cell immunity in the pathogenesis of demyelination in mouse models of Charcot-

Marie-Tooth (CMT) disease [10]. In these genetically determined neuropathies, demyelination and axonal injury are associated with CD8⁺ T cells infiltrating the PNS, an observation that led Martini's group to cross these mutants onto a RAG-1-deficient background to determine whether this response had any functional significance [10]. This proved to be the case as tissue damage was significantly ameliorated in the double mutants, demonstrating for the first time the involvement of the immune system in the pathogenesis of an inherited neuropathy.

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Towards Understanding Endoneurial Inflammation and Nerve Injury in GBS

Kazim A. Sheikh

Introduction

We begin by strongly commending Hugh Willison and John Goodfellow's initiative of GBS100 Top 10 monographs, particularly its clever, unconventional/bohemian format. This format allows free association of one's own work with that of most influential and brilliant works in the field; provides another chance to give new meaning and context to one's works; gives unfettered opportunities to generate hypotheses (conjecture) and conclusions (opinions); and importantly unburdens the fear of rejection from the highly overrated process of peer review. We take the liberty of formulating this chapter in conformity with the free-spirited intent of the editors (Hugh and John).

Understanding the Final Common Pathogenetic Pathway(s) of Endoneurial Inflammation That Mediate Nerve Injury (Myelin and Axonal)

Guillain-Barré syndrome (GBS) encompasses a group of related neuropathic disorders, considered autoimmune in nature, that commonly share endoneurial inflammation (cellular and noncellular) as a key feature. That a synergism of cellular and humoral immune elements is involved in the pathogenesis of these disorders is a commonly favoured hypothesis but not accepted universally. That antigen specificity and nature of adaptive autoimmune responses, especially T-cell responses, are not well defined, particularly for the commonest form of GBS, acute inflammatory demyelinating polyradiculoneuropathy (AIDP); this is considered a critical gap in our knowledge by some. There is strong evidence for the role of specific anti-glycan or ganglioside antibodies (AGAs) in the pathogenesis of axonal and Fisher forms of GBS. Adaptive autoimmunity uses the powerful effector functions of cells of the innate immune system, including monocytes/macrophages to induce target tissue inflammation and injury in autoimmune disorders. The pathologic studies in demyelinating and axonal GBS indicate a central role for macrophage populations, which are the key components of innate immune system and endoneurial inflammation. Macrophage-mediated myelin stripping and nodal and periaxonal macrophage-mediated attacks on axons are pathognomonic features of AIDP and axonal GBS, respectively. Perhaps classic immunology paradigms overemphasize the role of recognizing the specific antigens and adaptive immune responses in autoimmune disorders. Studying inflammation, independent of antigen and adaptive immune

response specificity, can be a fruitful endeavour, as borne out in the area of multiple sclerosis. A major challenge in the context of GBS is to determine whether shared common pathways of innate immunity that constitute endoneurial inflammation and mediate nerve (myelin and axonal) injury exist in individual inflammatory neuropathies grouped under GBS and what their key components are. The current discussion narrowly focuses on the role of inflammation and/or inflammatory milieu, downstream of known or unknown adaptive nerve-specific immune responses, in mediating nerve injury in GBS, highlighting some of our work in this context. The discussion is skewed towards macrophage inflammation in the endoneurium, as we anticipate other contributors of this volume will cover other cellular and noncellular (such as complement) elements of endoneurial inflammation. With this background in mind, our Top 10 monographs in English follow.

Waksman BH and Adams RD. Allergic neuritis: Experimental disease in rabbits induced by the injection of peripheral nervous tissue and adjuvants. *Journal of Experimental Medicine*, 1955

Waksman and Adams' landmark paper is highly likely to feature in several monographs in the current collection [1]. The authors provide fundamental and comprehensive experimental animal data that support the concept of inflammatory neuropathic disease. In this study, the experimental allergic neuritis (EAN) was generated in rabbits by immunizing with homologous and heterologous sciatic nerves. These animals developed clinical disease approximately 2 weeks after immunization. There was elevation of CSF protein without pleocytosis. Notably, spinal roots, dorsal root ganglion, and peripheral nerves developed endoneurial histiocytic/monocytic and lymphocytic inflammation and nerve fibre demyelination with variable secondary axonal injury. Importantly, the authors emphasize the close relationship of histiocytes with demyelination, including the presence of myelin debris in post-phagocytic monocytes. Based on these clinical, CSF and pathologic findings, Waksman and Adams postulated that this disease model resembles the clinical conditions grouped under Landry's paralysis, GBS and acute infectious polyneuritis, and represents one of the earliest models of inflammatory disease of peripheral nerves. In the context of the current discussion, this is one of the earliest studies noting endoneurial histiocytic/monocytic cell populations as immune effectors mediating myelin injury and clearance. This monumental study has influenced innumerable researchers in the area of GBS and led to an enormous amount of clinical and experimental work over the following half century.

Subsequent studies in EAN have confirmed the critical role of lymphocytes in inducing nerve injury in adoptive EAN models [2]. Early ultrastructural studies on EAN nerves by Lampert emphasized the role of mononuclear cells in demyelination [3]. He reported that mononuclear cells traverse the Schwann cell sheath, penetrate the outer mesaxon, push the Schwann cells aside, contact the myelin sheath, and strip and dissolve myelin sheath. These studies imply mononuclear cellular contact-dependent inflammatory demyelination. In contemporaneous ultrastructural studies, Ballin and Thomas reported early disruption of contacts formed by myelin terminal loops and paranodal axolemma at the nodes of Ranvier after inflammatory cells invaded endoneurium in EAN [4]. These studies reported myelin vesicular changes and postulated that some of the demyelination is 'chemical', implying the endoneurial presence of contact-independent soluble mediators of inflammation that induce myelin injury.

Asbury, et al. The inflammatory lesion in idiopathic polyneuritis: its role in pathogenesis. *Medicine*, 1969

Another hugely influential study from Ray Adams' group, a direct extension of their experimental work in EAN, reported the universal presence of lymphocytic inflammation, in autopsied peripheral nerves of a MGH cohort of 19 GBS patients, at all stages of the disease [5]. Analogous to EAN, primary demyelination, including early nodal changes, and secondary axonal injury was found in almost all cases. The authors opined "the role of macrophages in EAN and in idiopathic polyneuritis is uncertain. Macrophages in both diseases are particularly prominent at sites of extensive myelin breakdown and contain fragments of degenerating myelin. Whether they have some primary role in initiating myelin breakdown or act purely to clean up myelin destroyed by lymphocytes is not known."

Whether demyelination and T-cell inflammation are universal pathologic features of all patients grouped under GBS was unclear at that time. Haymaker and colleagues, prior to the publication of the Asbury study, reported that T-cell inflammation was not a constant early pathologic feature in their GBS cases with demyelinating pathology [6]. In the mid 1990s, Hafer-Macko and colleagues also reported demyelinating GBS cases with sparse endoneurial T-cell inflammation [7]. Overall, these clinical studies suggest heterogeneity in T-cell inflammation and pathogenesis of demyelinating GBS.

In a related series of experimental studies, Saida and colleagues showed that inflammatory demyelinating neuropathy can be induced in experimental animals without prominent T-cell inflammation [8,9,10]. In their studies, rabbits immunized with galactocerborside (GalC) developed anti-GalC antibody-mediated demyelinating neuropathy, which was complement dependent. Notably, macrophage recruitment and macrophage-mediated myelin phagocytosis was prominent in these studies as well.

In sum, T-cell inflammation could significantly vary in demyelinating GBS and both T cells and autoantibodies can induce overlapping pathological inflammatory demyelinating neuropathic features in EAN models. Macrophages fare prominently in pathological descriptions of demyelinating GBS and EAN models independent of T-cell inflammation.

Heininger K, et al. The role of macrophages in experimental autoimmune neuritis induced by a P2-specific T-cell line. *Annals of Neurology*, 1988

Heininger and colleagues' important study showed that macrophages are essential to endoneurial inflammation and nerve fibre damage in adoptive EAN [11]. A number of studies prior to this publication had shown the effector role of macrophages in active EAN [12]. Active EAN with myelin or myelin protein immunization include an induction phase of the autoimmune response to myelin antigens, during which macrophages play an important role as antigen-presenting cells to the lymphocytes (adaptive immune arm). The AT-EAN paradigm precludes the antigen-presenting role of macrophages as a mechanism of protection in this model. This Heininger study supports the hypothesis that adoptively transferred T cells interact with macrophages at amplification and effector phases in this paradigm, and thus that macrophages are key components of endoneurial inflammation that mediate myelin/nerve fibre injury. This and other EAN studies by the same group support the notion that macrophages induce myelin injury by soluble effectors and myelin phagocytosis (cellular contact-independent and -dependent, respectively) [13,14].

Spies JM, et al. Intra-neural activated T cells cause focal breakdown of the blood-nerve barrier. *Brain*, 1995

Spies and colleagues' influential study demonstrates that local inflammatory milieu is important in mediating antibody-induced demyelination [15]. Myelin protein P2 reactive T cells were injected

intraneurally and rabbit EAN serum containing anti-GalC activity was administered systemically (i.p.), which led to focal demyelination detected by electrophysiology and pathology. Similar changes in endoneurial milieu could be obtained with activated T cells specific for non-neural antigens, again emphasizing the role of local inflammatory milieu, including the breakdown of the blood-nerve barrier (BNB) as critical determinants of nerve fibre demyelination/injury. This study showed synergism between cellular and humoral autoimmune responses to produce inflammatory demyelination in peripheral nerves.

Feasby TE, et al. An acute axonal form of Guillain-Barré polyneuropathy. *Brain*, 1986

Introducing the concept of axonal GBS in the absence of T-cell inflammation, Feasby and colleagues describe 7 patients with acute neuropathic illness with motor predominant phenotype (similar to Landry's cases) and axonal electrophysiology and pathology [16]. The detailed pathological studies on autopsied materials in one case showed primary axonal degeneration without significant demyelination or lymphocytic inflammation. Predominant motor axonal involvement was confirmed by electrophysiology (inexcitable motor nerves) and anterior root and phrenic (motor) nerve pathology. Notably, this study directly correlated the severity of axonal injury with poor recovery. The authors opined that one mechanism for inexcitable motor nerves is an increase in the threshold of excitation of the nerve fibres due to undefined changes in Schwann cells or axonal membranes, particularly along the nodal and paranodal regions of the nerve fibre. Subsequent experimental studies support this notion of conduction failure along the nodal axolemma [17].

Yuki N, et al. Acute axonal polyneuropathy associated with anti-GM1 antibodies following *Campylobacter* enteritis. *Neurology*, 1990

Nobuhiro Yuki and colleagues reported, for the first time, a triad of acute axonal motor neuropathy, preceding *Campylobacter* infection, and IgG anti-GM1 antibodies [18]. This small but pivotal study (based on 2 cases) wielded huge influence in this area of research. The triad recognized in this observational study forms the basis of the now well-accepted hypothesis of molecular mimicry as the pathogenetic mechanism for axonal and Fisher variants of GBS. A large number of experimental studies have established that *Campylobacter jejuni* lipooligosaccharides carry ganglioside-like moieties mimicking peripheral nerve gangliosides, that these lipooligosaccharides can induce AGAs in experimental animals, and that AGAs can produce axonal injury, mimicking the pathology of axonal GBS, in preclinical models. Recognition of specific AGAs in association with axonal GBS has paved the way for the experimental studies linking these autoantibodies with axonal injury.

Griffin JW, et al. Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barré syndrome. *Journal of Neurocytology*, 1996

Jack Griffin and colleagues have performed a seminal series of studies to define the pathology of axonal GBS [19]. This ultrastructural study examined the peripheral nervous system (PNS) of 7 cases afflicted with the acute motor axonal neuropathy variant of GBS. The study focused on identifying early changes and establishing the sequence of those changes. By electron microscopy the earliest and mildest changes consisted of lengthening of the node of Ranvier with distortion of the paranodal myelin, and in some instances breakdown of the outermost myelin terminal loops. At this stage many nodes had overlying macrophages which extended their processes through the Schwann cell basal lamina covering the node

and apposed the axolemma. At later time points macrophage processes extended beneath the myelin terminal loops and the whole macrophage entered the periaxonal space at the paranode. Macrophage processes dissected the axon from the adaxonal Schwann cell plasmalemma and the macrophages advanced into the internodal periaxonal space, where they typically surrounded a condensed-appearing axon. This association of macrophage and axons appeared to be stable for some time, and the axons subsequently underwent Wallerian-like degeneration. The internodal myelin sheath and the abaxonal Schwann cell cytoplasm remained normal. The pathologic sequence emerging from these studies is that early in the course there are only mild changes at the nodes of Ranvier, sufficient to induce paralysis; it is only later that the axons degenerate. This study suggests that macrophages are critical to the pathogenesis and injury and dysfunction of the nodal axolemma initially and of the internodal motor axons subsequently. Moreover, the nodal and axonal injury likely depends upon macrophage contact with the axons/axolemma (contact-dependent).

In separate studies, the same group has demonstrated the deposition of IgG and C3d (membrane bound cleaved product of C3) at the nodes of Ranvier initially and at paranodal and internodal axolemma at later time points after the onset of the disease [20]. Whether early complement component(s) deposits at structural specializations along myelinated axons originate from circulation or adjacent microglial/macrophage cells in the endoneurium and/or perinodal and periaxonal spaces is not established. It has been shown previously that macrophage populations can synthesize and secrete various complement components. It is believed that complement activation products may provide chemotactic cues for macrophage recruitment at the nodes and periaxonal location. In summary, these human studies on pathologic materials from axonal GBS cases implicate macrophages as effector cells mediating nodal and axonal injury.

Yuki N, et al. Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. *Proceedings of the National Academy of Sciences*, 2004

In this study Yuki and colleagues report an animal model of post-infectious autoimmune neuropathy induced by immunization of rabbits with *C. jejuni* lipooligosaccharide-bearing GM1-like moieties [21]. These animals developed IgG anti-GM1 antibodies, which were associated with paralytic disease and peripheral neuropathy with overlapping pathological features with axonal GBS, including periaxonal macrophages characteristic of the human disorder. These studies provide strong support for the concept that axonal GBS are post-infectious disorders in which humoral/antibody directed against carbohydrate antigens shared by peripheral nerve fibres and infectious agents induce axonal neuropathy. Whether autoantibodies against gangliosides are by themselves sufficient to induce nerve damage or require other effectors of the innate immune system was not established in this study. The presence of periaxonal macrophages in this experimental model again emphasized that axonal injury may depend on contact between axons and macrophages.

Zhang et al. Fcγ receptor-mediated inflammation inhibits axon regeneration. *PLoS ONE*, 2014

The background for the study by Zhang and colleagues is that our translational work initially focused narrowly on establishing reproducible passive transfer animal models of AGA-mediated nerve injury in the context of axonal GBS [22]. Reproducing human pathology in mouse models has been daunting. Early

on we learned that passive transfer of AGAs in small laboratory animals did not produce neuropathic disease. This led us to study the pathogenic effects of AGAs in a nerve crush model, as nerve stumps distal to the crush site have an inflammatory milieu due to recruitment of macrophages and virtually no BNB integrity. We showed that passive transfer of AGAs (experimental and human) impair nerve repair and severely inhibit axon regeneration [23,24]. The primary intent of these studies was to show that passive transfer of AGAs has deleterious effects on nerve fibres and this antibody-mediated nerve fibre injury is dependent upon endoneurial inflammatory milieu (including recruited macrophages). An implication of these findings was that they echo the clinical association of AGAs and poor recovery in GBS.

The goal of the Zhang and colleagues study was a careful dissection of a nerve crush model using various mutant and transgenic mice with an altered expression of specific FcγRs and macrophage/microglia populations and nerve transplant strategy, to demonstrate that the presence of AGAs in the injured mammalian peripheral nerves switch the proregenerative inflammatory environment to growth inhibitory milieu by engaging specific activating FcγRs on recruited monocyte-derived macrophages to cause severe inhibition of axon regeneration. A fundamental principle learned from this series of nerve crush studies was that inflammatory milieu, primarily consisting of activated FcγR-bearing macrophage/microglia, are critical mediators of Ab-mediated nerve injury. These data demonstrate that the passive transfer of AGAs can induce neuropathic injury, but endoneurial inflammation, particularly macrophages bearing Fcγ receptors, are necessary for antibody-mediated pathogenicity.

He et al. Anti-ganglioside antibodies induce nodal and axonal injury via fcγ receptor-mediated inflammation. *Journal of Neuroscience*, 2015

The 'inflammatory milieu' hypothesis stemming from the crush model discussed above was tested in a new AGA-mediated passive transfer mouse model induced by L5 spinal nerve transection (L5SNT; modified Chung's model) to study AGAs effects on intact nerve fibres [25]. L5SNT causes the degeneration of a small proportion of fibres that constitute the sciatic nerve and its branches, but more importantly sets up an inflammatory milieu in the endoneurium. Our studies indicate that, in this mouse model, AGAs induce sequential nodal (early) and then axonal (late) injury of intact myelinated nerve fibres, recapitulating pathologic features of human disease. Notably, macrophages were seen adjacent to widened nodes of Ranvier at early time points. Importantly, our studies showed that immune complex formation, macrophage/microglia and the activating FcγRs were involved in the AGA-mediated nodal and axonal injury in this model. These studies provide further experimental evidence of the role of macrophages in AGAs-mediated nodal and axonal injury in axonal GBS.

Discussion

The majority of clinical and experimental studies included in this discussion highlight the importance of endoneurial inflammation in mediating myelin and axonal injury in AIDP and axonal forms of GBS. Many aspects of endoneurial inflammation are shared between demyelinating and axonal GBS and their animal models, and macrophage inflammation appears to be common to clinical disease and disease models. In sum, inflammatory cells in the endoneurium, particularly macrophage recruitment, is associated with early injury to the nodes of Ranvier in both demyelinating and axonal variants of the disease. Although macrophages in the vicinity of the nodes are well documented in axonal cases, whether the nodal injury is

macrophage contact-dependent and/or -independent and the the precise molecular mechanisms of nodal injury in this situation are not well defined for either axonal or demyelinating cases of GBS. Monocyte-derived macrophages appear to be important effectors of nerve injury in experimental studies of EAN and anti-ganglioside antibody-mediated nerve injury models. T-cell inflammation orchestrates endoneurial inflammation and macrophage recruitment in EAN and probably in AIDP cases with T-cell inflammation. How macrophages mediate Schwann cell/myelin injury is not completely understood. The pathological studies favour both chemical (cell contact-independent) and phagocytic (cell contact-dependent) macrophage-mediated injury to myelin compartment in demyelinating variants of GBS, but mechanisms and molecular actors involved in, perhaps, the dichotomous injury to Schwann cells/myelin are not completely understood.

How endoneurial inflammation and macrophage recruitment is accomplished in AIDP cases without prominent T-cell inflammation and axonal variants of GBS is a key question, which to a large part remains unaddressed. Could endoneurial glia (including microglia) be activated by soluble signals diffusing from systemic immune compartment and subsequently set up endoneurial inflammation including macrophage recruitment in these situations? Our studies in Chung's (L5SNT) model show that partial nerve injury generates endoneurial signals that recruit macrophages from circulation and set up inflammatory milieu in the nerve. Moreover, our experimental studies in the context of anti-ganglioside antibodies and axonal injury indicate that activating FcγRs on macrophage populations are key molecular effectors mediating nerve injury. Whether the macrophage and activating FcγRs interactions with immune complexes formed on nerve fibres are random or other molecular signals abet in this process (such as complement activation products) acting as chemoattractants for directing the macrophages within endoneurium to specific sites along the nerve fibres remains to be established. Moreover, the kinetics and evolving phenotype(s) (pro-inflammatory, anti-inflammatory or in between these polarized states) of macrophage/microglial cells in the endoneurial compartment of intact and injured/diseased nerves are not well defined. The molecular effectors of nerve fibre injury downstream of macrophage-immune complex interactions are also not elucidated. It appears more than one inflammatory pathway at the motor nerve terminal and in the endoneurium can produce similar pathologic reactions (nodal and axonal injury) in experimental models, as indicated by a series of elegant studies with AGAs and complement [26,27,28]. It is likely that there is close coordination and crosstalk between noncellular and cellular elements of endoneurial inflammation to produce specific patterns of pathologic injury in GBS. Current therapies in GBS, for the most part modulate components of immune system in the systemic immune compartment. We believe understanding the endoneurial inflammation particularly the pathobiology of macrophages within this nerve compartment is a promising avenue likely to identify molecular effector pathways that could be targets of new drug development for GBS and beyond. Indeed novel strategies to deliver such therapies selectively to endoneurial compartment would be a requisite and a challenge.

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The Motor Nerve Terminal as a Site of Anti-Ganglioside Antibody Attack in Guillain-Barré Syndrome

J.J. Plomp and H.J. Willison

“If I have seen further it is by standing on the shoulders of giants.”

Sir Isaac Newton, letter to Robert Hooke, 5th February 1675.

Introduction

Everything we think about is predicated on the discoveries of those who thought long before us. When we (the authors) started investigating the possibility that Guillain-Barré syndrome (GBS) associated anti-ganglioside antibodies could injure the motor nerve terminal, we marshalled our thoughts around existing studies that might support such an idea, of which there were many. We gathered techniques and reagents that had been previously developed and used to study neuromuscular synaptic function in experimental mouse preparations, and applied them to investigate our hypotheses. In doing so we quickly realised that pretty much everything we thought about had already been thought about by someone before us, and we were simply inching an old idea forward or nudging it sideways towards our application. When Jaap started quoting that we should not aim to drag “oude koeien uit de sloot” (old cows out of the ditch) we both knew that truly new knowledge was a rare beast, mostly well beyond our grasp. The purpose of this memoir is to highlight some of the pre-existing ideas, reagents and methods described within our ‘Top 10’ papers that helped us to investigate this problem, rather than describe the findings and conclusions that we reached; these can be accessed in great detail elsewhere [1,2]. Choosing these ‘Top 10’ papers does not undermine the importance of many hundreds of others that form part of this field of exploration, including the wonderful work more directly on GBS that has seen the field move forward in extraordinary ways over the last 100 years. In particular we acknowledge the large number of outstanding papers on the motor nerve terminal in GBS conducted by other researchers that lie out with the scope of this memoir.

Svennerholm L. Chromatographic separation of human brain gangliosides. *Journal of Neurochemistry*, 1963

The structural analysis of gangliosides (sialylated glycosphingolipids first discovered in the 1930s in the brain by the German biochemist, Ernst Klenk) and their classification has been a key resource for GBS

researchers who in general are not drawn from the carbohydrate chemistry community. Working one's way through ganglioside nomenclature and committing the structures to memory in a clinically meaningful way is greatly aided by the logical layout of the members of the complex and large ganglioside family described by Lars Svennerholm, one of the founding fathers of ganglioside biochemistry [3]. His classification, based on the migration pattern seen on thin layer chromatography, is still widely used today, and the underlying synthesizing enzymatic pathways leading to this neuronal 'sialome' are now well-known. Svennerholm worked in an incredibly rich community of ganglioside biologists to whom our GBS field owes a huge debt [4].

Fatt P, Katz B. An analysis of the end-plate potential recorded with an intracellular electrode, *The Journal of Physiology*, 1951

The ability to record and analyse the synaptic signals, i.e. miniature endplate potentials, at neuromuscular junctions is a cornerstone of synaptic neuroscience and has allowed for the discovery of many universal synaptic principles. The neuromuscular junction offers a perfect *ex vivo* system, and Bernard Katz used this system to work out key aspects of the quantal theory of neurotransmission [5] for which he was awarded the Nobel Prize for Physiology or Medicine in 1970 (jointly with Ulf von Euler and Julius Axelrod). Our studies on the electrophysiological effects of anti-ganglioside antibodies at neuromuscular junctions, first performed with the Miller Fisher syndrome sera in Oxford, and subsequently in Leiden, relied heavily on the classical technical and theoretical knowledge of this process provided by Katz. The measurements nowadays are obviously much more accurate and efficient due to modern digital electrophysiological equipment and data acquisition and analysis software.

Simpson LL, Rapport MM. Ganglioside inactivation of botulinum toxin. *Journal of Neurochemistry*, 1971

Three pieces of knowledge have been crucial for directing our studies: (A) that clostridial botulinum and tetanus neurotoxins exert their paralytic effects by first binding to the presynaptic membrane at neuromuscular junctions; (B) that these toxins bind complex gangliosides [6]; and (C) that gangliosides are present in high density at presynaptic membranes of neuromuscular junctions to which circulating factors have ready access because these synapses are localized outside the blood-nerve barrier. This led us to the hypothesis that autoantibodies against the relevant gangliosides, from the Miller Fisher variant but also other GBS cases, should also bind and exert effects at the motor nerve terminal. Proving this to be the case was a lot more complicated than developing the idea. Similarly, the development of knowledge about botulinum neurotoxin binding to cholinergic nerve terminals was a longstanding process that has gradually evolved since the 1960s and still remains an interesting area for research today, particularly with the booming use of botulinum neurotoxins in medical and cosmetic applications. Specific ganglioside binding sites have now been identified on the neurotoxin molecule, as well as a mechanism of secondary binding to synaptic vesicles proteins when they become exposed to the extracellular environment during neuroexocytosis. Contemporaneous studies on cholera toxin B subunit binding to GM1 ganglioside, and its use for localisation of GM1 including nerve terminals and nodes of Ranvier, also provided us and others working on GBS with crucial insights into the predicted sites of action of anti-GM1 antibodies.

Chang CC. Looking back on the discovery of α -Bungarotoxin. *Journal of Biomedical Science*, 1999

Continuing on the toxin theme, the use of a wide range of neurotoxins has been germane to electrophysiological studies on both neuromuscular synapse and nodal function over many decades. Professor Chang from National Taiwan University, Taipei, clearly merits recognition for his discovery, reported in the early 1960s (with CY Lee), that a fraction in the venom of the banded krait, *Bungarus multicinctus*, irreversibly blocked nicotinic ACh receptors [7]. The reference we include, rather than reverting to the primary literature (which at the time was rejected by the *Journal of Physiology!*), comprises a delightfully insightful and very funny retrospective account of Professor Chang's scientific adventures that we recommend as a must-read for all interested in this area. The fraction, called α -Bungarotoxin, has become perhaps the most applied pharmacological tool in neuromuscular junction research, and indeed has been vitally important for our work. Rarely has a day past in which fluorophore-labelled α -Bungarotoxin hasn't been applied to illuminate ACh receptors to enable easy identification of neuromuscular junctions in preparations used for our immunostaining studies. Professor Chang was also highly instrumental in understanding the mechanism of action of μ -Conotoxin-GIIIB and the application of this toxin as an important pharmacological tool in the electrophysiological recording of synaptic signals at neuromuscular junctions. Due to its selective blocking action on skeletal muscle type voltage-gated Na^+ channels ($\text{Na}_v1.4$), μ -Conotoxin-GIIIB prevents muscle fibre action potentials and thus contraction. This allows for undisturbed microelectrode recording of endplate potentials.

Frontali N, Ceccarelli B, Gorio A, Mauro A, Siekevitz P, Tzeng MC, Hurlbut WP. Purification from black widow spider venom of a protein factor causing the depletion of synaptic vesicles at neuromuscular junctions. *Journal of Cell Biology*, 1976

The recurring theme of toxins must include reference to the electrophysiological and morphological effects of α -Latrotoxin, a neurotoxic component of the venom of the black widow spider venom. Frontali and colleagues [8] purified a protein fraction from the venom that was highly toxic for mice and showed that this factor was responsible for the depletion of synaptic vesicles from motor nerve terminals, associated with a tremendous increase in the frequency of miniature endplate potentials, shown before by others with the whole venom. Later studies showed that one of the toxin's mechanisms of action is to form a tetrameric pore that causes uncontrolled presynaptic influx of Ca^{2+} , stimulating neuroexocytosis. The similarity between the effects of this α -Latrotoxin and the action of anti-ganglioside autoantibody containing the Miller Fisher syndrome sera (and later, acute motor axonal neuropathy sera as well as corresponding anti-ganglioside monoclonal antibodies generated by us) in the presence of complement activation is remarkable. It allowed us to build up a model of presynaptic membrane injury resulting from pore formation with aberrant Ca^{2+} influx. The electron micrographs of nerve terminals are virtually identical between the 2 conditions, i.e., swollen terminals devoid of synaptic vesicles. Similarly, the electrophysiological phenotypes are identical, i.e. the 'explosion' of miniature endplate potentials for tens of minutes (corresponding with the release of >100,000 ACh quanta), followed by complete block of ACh release.

Podack ER, Tschopp J. Membrane attack by complement. *Molecular Immunology*, 1984

Where antibodies bind, complement follows. This is a long-held maxim that applies to GBS as much as other autoantibody-mediated disorders. Clearly this especially refers to the complement-fixing immunoglobulin isotypes and subclasses, notably IgM and IgG1-3. Elucidating the role of complement in humans and animal models of GBS has occupied researchers for over 20 years. In parallel, the basic biology of the complement system has been elucidated in great detail and there is no better place to focus attention than on the formation of membrane attack complex [9]. This is a self-assembling transmembrane complex that becomes deposited in plasma membranes targeted by complement-fixing autoantibodies. In an autoimmune situation, at nerve membranes in particular, it appears to be highly toxic, disturbing the ionic balance between intra- and extracellular compartments and maintenance of the resting membrane potential. In this review paper, Podack and Tschopp presented beautiful ultrastructural images and a model of this process that greatly helped us understand the relationship between membrane attack complex and the pore-forming α -Latrotoxin. In our studies, immunostaining clearly showed the presence of membrane attack complex at mouse motor nerve terminals in nerve-muscle preparations that had been treated with anti-ganglioside antibodies and added human serum as a source of complement.

Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, 1975

We are only now beginning to understand why anti-glycolipid antibody-containing sera collected from clinically affected GBS cases may not be the best source of antibodies for investigating neurotoxic effects in GBS. Very early on in our joint studies we invested considerable time in isolating monoclonal antibodies reactive with neural gangliosides, also relying heavily on the GM2/GD2 synthase mice referred to below as an excellent immunisation vehicle. Köhler and Milstein's brilliant contribution to medical science in the form of cell fusion for hybridomas ranks alongside PCR as a fundamental tool of daily lab activity [10]. Along with Niels Jerne, Köhler and Milstein were awarded a Nobel Prize in 1984 for the discovery of this principle of antibody production. We have learnt a huge amount about GBS by trawling through panels of our anti-ganglioside monoclonal antibodies trying to understand why some have effects whereas others do not. The ability to accurately apply fixed concentrations of known monoclonal antibodies with known ganglioside binding patterns in unlimited supply, compared with scarce human sera with less well-defined properties, has been a cornerstone of our lab activities. Indeed, without these monoclonal antibodies we would not have succeeded in further understanding our subject area beyond the simplest of points. So hats off to hybridoma technology as a remarkable game changer for our field!

Takamiya K, Yamamoto A, Furukawa K, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proceedings of the National Academy of Sciences of the United States of America*, 1996

The development of transgenic mice that lack glycosyltransferases involved in ganglioside biosynthesis has allowed researchers to uncover hitherto unknown functions of gangliosides. A preplanned meeting

with Koichi and Keiko Furukawa at the Gordon Research Conference on Glycolipid and Sphingolipid Biology in 2000 provided the opportunity for us to use the GM2/GD2 synthase knockout mice they had recently developed that lacked complex gangliosides [11]. No other single animal tool has been more important to us than these mice. Similar studies have been conducted by other GBS researchers using mice generated contemporaneously by the Proia group at NIH. GM2/GD2 synthase knockout mice develop sensory and motor-coordination deficiencies upon aging. Despite prior suggestions that complex gangliosides were key components of the presynaptic apparatus, it turned out that gangliosides are remarkably redundant in supporting neurotransmitter release at the neuromuscular junction. Even total ganglioside ablation (obtained by crossing GM2/GD2 synthase KO mice with GD3 synthase knockout mice and treating their nerve-muscle preparations with neuraminidase to destroy the only remaining ganglioside GM3) resulted in only mild changes in ACh release from the motor nerve terminal. This indicates that gangliosides are not absolutely required for neurotransmitter release. Nevertheless, the ability to manipulate ganglioside content and levels in presynaptic membranes provided key insights into the binding and subsequent action of anti-ganglioside antibodies.

Lloyd KO, Gordon CM, Thampoe IJ, DiBenedetto C. Cell surface accessibility of individual gangliosides in malignant melanoma cells to antibodies is influenced by the total ganglioside composition of the cells. *Cancer Research*, 1992

Monoclonal antibodies targeted against tumour cell-specific antigens have long been considered to be magic bullets for therapy development. Whilst the GBS field has been working out how to stop anti-ganglioside antibodies from killing neural tissue, cancer scientists have been working on the opposite problem—how to coax anti-ganglioside antibodies into becoming effective killers. The use of anti-GM3 and GD3 antibodies to kill melanoma and neuroblastoma cells (which express relatively high GM3 and GD3 levels at their membrane) has been a long-studied problem [12]. We were often surprised by how difficult it was to get anti-GD3 antibodies to bind GD3-rich nerve membranes, and the answer clearly lies in the similar problem encountered by Lloyd and colleagues in a large series of papers published in this area. Reading this and related work crystallised our ideas on the principle that one type of ganglioside can sterically hinder the binding of antibodies to another type in the plane of the plasma membrane, thus limiting nerve injury in models of GBS. This is perhaps best illustrated in the work we did on anti-GM1 antibodies, showing that some of these can only exert neuropathophysiological effects at motor nerve terminals if the presynaptic membrane had been treated with sialidase to remove (apparently sterically hindering) neighbouring gangliosides such as GD1a.

Ritchie TC, Fabian RH, Choate JV, Coulter JD. Axonal transport of monoclonal antibodies. *The Journal of Neuroscience*, 1986

The presynaptic membrane of motor nerve terminals is not static, but exists in a highly dynamic state due to the continuous addition and removal of membrane surface due to the processes of synaptic vesicle exocytosis and endocytosis, respectively. One issue that always intrigued us has been the fate of antibody landing on this surface. How long is it held there and does this provide sufficient time for activation of the complement cascade? The papers by Fabian and colleagues provided constant interest whilst we were studying this field, making us realise that the motor nerve terminal was a highly dynamic structure with

very rapid rates of membrane turnover [13]. Once a ligand, in our case a GBS-associated anti-ganglioside autoantibody, in another case perhaps a neurotoxin or virus, has landed on the presynaptic membrane, how quickly is it endocytosed? Is it then recycled to the plasma membrane or destined for a retrograde trafficking pathway? This remains an area of great interest, almost 30 years after this beautiful paper was published, and a constant source of inspiration that also undermines any idle preconceptions one might hold.

Conclusions

Research on the effects of anti-ganglioside antibodies at the mouse motor nerve terminal has engaged us in collaborative work for 15 years, during which time we published 25 papers and reviews in what for us has been a totally engaging and interesting aspect of GBS research. Perhaps most importantly, we each also gained a highly trusted and lifelong friend. We are the first to acknowledge the limitations of our experiments and have regularly been challenged, quite rightly, about the clinical relevance of the nerve terminal as a site of injury in GBS patients and its variants. Now is not the time to argue a case for or against. Perhaps more simply it is the time to quote the immortal lines from ‘The Adventure of Silver Blaze’, penned by Sir Arthur Conan Doyle, one of the most popular stories from the Sherlock Holmes canon:

Detective Gregory “Is there any other point to which you would wish to draw my attention?”

Holmes: “To the curious incident of the dog in the night-time.”

Detective Gregory: “The dog did nothing in the night-time.”

Holmes: “That was the curious incident.”

Paraphrased for the current context, hypothesis-driven scientific enquiry perhaps provides more information when the results are negative, than when they are positive.

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We and the motor nerve terminal-GBS field are permanently indebted to the shared PhD students and postdoctoral researchers who conducted motor nerve terminal and related experiments over a 15-year period and collaborated together across the North Sea, including numerous exchange visits. On our joint publications, either based in Leiden or Glasgow, these include Carl Goodyear, Roland Bullens, Graham O’Hanlon, (a younger) Bart Jacobs, Susan Halstead, John Goodfellow, Tyrone Bowes, Masaaki Odaka, Ian Morrison, Femke Zitman, Kay Greenshields, Simon Rinaldi, Alistair Easton, Simon Fewou and Angelika Rupp. We wish to thank the following funding organizations for continuous support of our research projects in the form of grants and PhD studentships on anti-ganglioside autoantibodies: ‘Prinses Beatrix Spierfonds’; The Wellcome Trust; Guillain-Barré Syndrome Support Group UK (now GAIN); Medical Research Council; Pathological Society of Great Britain and Ireland; Patrick Berthoud Charitable Trust.

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Glycolipids in GBS: From Basic Science to Clinical Interface

Gavin R. Meehan

Introduction

In the 100 years since GBS was first described, GBS research has advanced significantly from basic electrophysiology studies to the identification of the pathological pathways involved in the onset of the disease. Arguably, one of the most important discoveries in the history of GBS was the association of the condition with anti-ganglioside antibodies. These antibodies are able to elicit injury by targeting gangliosides in the peripheral nerves and have become a major focus of current research—but what of the gangliosides themselves?

Since their discovery in the late 1930s, these cell surface receptors have been shown to be essential components of not only the nervous system, but also of almost all cells and tissues of the body. This Top 10, therefore, focuses on establishing the major milestones in ganglioside research, with a particular emphasis on their impact on the study of GBS.

Let's Start at the Very Beginning: Ernst Klenk and Gunnar Blix

The discovery of gangliosides is often attributed to a German scientist named Ernst Klenk, but their initial description was more of a collaborative process and took place over a number of years. Klenk was the first researcher to describe gangliosides, which he isolated from the organs of patients with Niemann-Pick and Tay-Sachs disease [1], but his work was reinforced by that of a Swedish researcher named Gunnar Blix, who was the first person to identify sialic acid [2].

Klenk initially called his unknown lipid 'Substanz X' and found that it formed black humin when it underwent acid hydrolysis and produced a purple colour when heated with Bial's reagent. Blix, following on from his sialic acid discovery, also isolated a compound from bovine brain that he believed to be 'Substanz X', but he also noted the similarities between this compound and sialic acid [3].

As a result Blix compared both compounds using Bial's and Ehrlich's reagents and found that they produced the same colours. Based upon this observation, he postulated that sialic acid was a major component of 'Substanz X', but Klenk disagreed. He had isolated a similar compound, termed 'neuraminic acid', which he believed to be a major component of the lipid [4].

As such, Klenk developed a method for quantifying the distribution of neuraminic acid in different brain sections using orcinol and found that it was only present in the grey matter, where it was particularly enriched in the cerebral cortex [5]. This observation led to the adoption of the name 'gangliosides' and

thus a new lipid was founded.

The rivalry between Klenk and Blix persisted for several years as they investigated the structure of gangliosides. Ultimately, both competitors were proven right, as Klenk demonstrated that N-acylated neuraminic acid was a major component of gangliosides, whilst Blix showed that all acylated neuraminic acids were sialic acids [6]. Regardless of their competitiveness, both scientists made important contributions to ganglioside research, which helped set the topic on the right path.

What Is in a Name?

Following on from the fiery beginnings of gangliosides, our focus shifts to one of the lipids' more important aspects, its nomenclature. As more gangliosides were discovered it became apparent that a unified naming system was required to simplify their identification in the literature. This task was taken on by one of the lipids' earliest proponents, Lars Svennerholm, who in 1964 developed a beautifully simplistic naming strategy that is still in use today [7].

This simplified system employs the use of a code, where 'G' denotes a ganglioside, whilst the use of a prefix represents the number of sialic acids, such as M for mono, D for di, T for tri and Q for quad. This is followed by a number, which is determined by the migration of the lipids on TLC and is related to the size of the oligosaccharide chain. Finally, a lowercase letter denotes the isometric arrangement of the sialic acids on the headgroup; for example GQ1b [8].

GM1 and Cholera Toxin: A Toxic Relationship

Early researchers focussed on the biochemistry of gangliosides, but the 1997 paper by Hansson, Holmgren and Svennerholm was among the first to determine their specific location in the ultrastructure of the nerves [9]. This was achieved through the ingenious use of cholera toxin, which previous research by Holmgren, Lönnroth and Svennerholm had suggested bound to the ganglioside GM1 [10].

By using this toxin as a ganglioside marker, Hansson, Holmgren and Svennerholm were able to localise GM1 to the pre- and postsynaptic membranes of the synaptic terminals. Furthermore, through the use of sialidases, they were able to show that other complex gangliosides were also enriched in these locations.

This experiment led to the widespread adoption of cholera toxin as a ganglioside marker, which has proven so popular that it is still in use today. In addition, this method of localising gangliosides formed the basis for the electron microscopy and immunofluorescence work that is currently carried out in GBS research laboratories.

Ganglioside Function: It's a Bit Complex

Despite the plethora of research that had been performed on gangliosides, their functions remained obscure and poorly defined until the generation of ganglioside knockout mice. It was only once these mice were created that the roles of complex gangliosides, particularly within the context of a whole organism, could be elucidated.

The knockout mice were generated by disrupting the GalNAc T gene, which prevented the formation of the enzyme responsible for the synthesis of all complex gangliosides [11]. Initial histological analysis of tissue from these mice indicated that the complex gangliosides did not have roles in organogenesis or morphogenesis. However, there was a reduction in neuronal conduction velocity, suggesting that

gangliosides had a role in normal neuronal function.

This was confirmed in a subsequent study, which analysed the behaviour of these mice [12]. It was found that the mice developed tremors and deficits in balance, strength and coordination at 12 weeks of age. In addition, they experienced Wallerian degeneration and myelination defects, which demonstrated the roles complex gangliosides play in normal neuronal maintenance.

These mice were the first example of the necessity of gangliosides and indicated how disruption could lead to the symptoms experienced by GBS patients. As a result, these mice act as an essential tool in many GBS research laboratories.

The Rise of the Anti-Ganglioside Antibody

The papers discussed thus far have focussed on the structures and characteristics of gangliosides, but it is their roles in disease that are of a particular interest to neuropathy researchers. In GBS, these roles were first explored in a paper from 1992 in which antibodies targeting the ganglioside, GQ1b, were discovered in the serum of 6 patients with Miller Fisher syndrome (MFS) [13]. The authors of this paper postulated that these antibodies may act as a marker for this disease, which was confirmed in subsequent research that explored the pathology of MFS.

It was discovered through biochemical analysis and immunofluorescence studies that GQ1b was enriched in the oculomotor and cranial nerves in humans [14,15]. The close association of these antibodies with ophthalmoplegia therefore led researchers to conclude that they were causing the symptoms by specifically binding and injuring the oculomotor nerve.

This study was the first to demonstrate a cause-and-effect mechanism in GBS and led to the search for anti-ganglioside antibodies in a host of other GBS subtypes. In addition, the discovery of a biomarker led to the adoption of ganglioside enzyme-linked immunosorbent assays (ELISA) as a supportive clinical test in hospitals, which has aided in the diagnosis of GBS in thousands of patients worldwide.

Keeping Up the Standards

As the use of ELISAs increased as a diagnostic tool, it became apparent that discrepancies existed between different laboratories. Although both clear positive and negative results tended to remain consistent, intermediate signals could vary 25-fold, which raised questions about the validity of the results.

In an attempt to address this problem, Willison and colleagues produced a standardised ELISA in conjunction with the other laboratories that formed the European Inflammatory Neuropathy Cause and Treatment (INCAT) group [16]. Using this method, they were able to reduce the variation between laboratories; however, there was an internal laboratory error rate of 41%.

This suggested that further technical enhancements would be required to improve the standardised ELISA. It also suggested that even with the upgrade the new format was still highly variable. This was an important point, as it showed that the technique was not robust enough to be used for definitive diagnosis and could only act in a supportive role.

Further improvements in quality control, new techniques such as the combinatorial glycoarray [17], and the use of ganglioside complexes (see below) have addressed some of these issues, but further work is still required to further the use of gangliosides assays in diagnosing autoimmune neuropathies.

Imitation Is the Sincerest Form of Flattery

The connection between GBS and antecedent infections has been noted by clinicians since the 1950s but it was not until the discovery of anti-ganglioside antibodies that researchers were able to adequately explain this link. Based upon the similarities between the lipooligosaccharide (LOS) coats of bacteria and host gangliosides, they proposed that cross-reactive antibodies arose in GBS that targeted both structures, in a process known as molecular mimicry [18].

It was not until 2004, however, that this hypothesis was proven in an in vivo model of GBS. Yuki and colleagues immunised rabbits with LOS from *Campylobacter jejuni* and found that the animals developed anti-GM1 IgG antibodies, which produced acute flaccid paralysis [19]. In addition, they demonstrated that immunising mice with LOS resulted in the production of anti-GM1 IgG antibodies that were capable of binding to human peripheral nerve.

This paper was the first to definitively show a pathological mechanism for the onset of paralysis and proved that the antibodies detected in patient sera were relevant to the induction of GBS. This also supported the research being performed by other groups and substantiated the use of monoclonal antibodies in GBS models.

A Little More Complication, a Little More Action

Following the discovery of anti-GQ1b antibodies in MFS, researchers began screening other GBS patients for anti-ganglioside antibodies. Whilst a number of these were found against a variety of targets, they were rarely found in as high numbers as in MFS. In an attempt to overcome this problem and improve antibody detection, a Japanese research group screened patient sera against complexes composed of 2 different gangliosides [20].

They found that a number of patients had antibodies which bound to GD1a:GT1b complexes without binding to the single-constituent gangliosides. The authors proposed that these antibodies differed from regular anti-ganglioside antibodies in that they likely bound to clustered epitopes formed by components of both gangliosides rather than to an epitope expressed by just one.

This was a completely novel concept that opened up new avenues in GBS research. Antibodies specifically targeting ganglioside complexes were found in other autoimmune neuropathies and were linked to different clinical outcomes [21,22,23]. In addition, antibody detection increased in patient sera which improved the performance of clinical assays.

This discovery also changed researcher's viewpoints of how gangliosides exist in the membrane. Rather than being expressed in standalone formations, gangliosides are likely to form complexes with neighbouring lipids and proteins, which will affect their presentation to the immune system. The use of ganglioside complexes therefore takes the complexity of the membrane into account for the first time, which has shaped the direction GBS research has taken in recent years.

Come Out, Come Out, Wherever You Are

Although anti-ganglioside antibodies were thought to be responsible for the pathology observed in GBS, researchers had noted that their abilities to bind their respective target antigens could be highly variable. The reason for this variability was explored by Greenshields and colleagues, who found that the binding epitopes of GM1 could be shielded from antibody access by the presence of neighbouring gangliosides [24].

This was shown in particular with GD1a, which, due to its terminal sialic acid, prevented the exposure of certain epitopes on the GM1 molecule. When the tissue was treated with neuraminidase this sialic acid could be removed, which liberated the GM1 binding epitope and allowed binding to take place.

This novel work demonstrated that a gangliosides local microenvironment affected which antibodies could bind and which tissues were vulnerable to immune mediated injury. This had huge implications in assessing the sites of injury in different autoimmune neuropathies and determining the relevance of clinical data in disease pathogenesis. The work derived from this research is therefore ongoing.

Conclusion

Gangliosides are an essential component of life and have important roles in neuroregulation, signal transduction, and maintenance and repair of the nervous system. As a result they are abundantly expressed throughout the body, but unfortunately this high expression makes them particularly vulnerable to the immune mediated attack seen in GBS.

Although this Top 10 may only offer a snapshot of the importance of gangliosides, it covers the major milestones in their history and their relevance in GBS. As our knowledge into their roles progresses I'm sure more discoveries will be added to this list, because, as the old adage goes, the best is yet to come.

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IVIg Pharmacokinetics and Mechanisms of Action in GBS

Willem Jan R. Fokkink

Introduction

Ever since intravenous immunoglobulin (IVIg) proved to have an elusive immune modulatory effect in many inflammatory diseases, there has been an ongoing rat race (quite literally, given the many animal studies) to find the mechanism of action. IVIg has pleiotropic effects on the activated immune system, and every year more and more potential mechanisms are added to the list. One may wonder if all these reported effects of IVIg actually hold true in all the numerous disorders it is prescribed for, and in every single patient. In spite of this continuous quest to decipher the pharmacodynamics of IVIg, its pharmacokinetics receive little attention. While we do not know how exactly IVIg works in the many indications it is administered in, the common denominator is the need for a high dose. The current dosing strategy, of 2 grams per kilogram bodyweight over 5 days, is derived from Imbach and colleagues study in 1981 [1], and has been given, unaltered, to all patients ever since. Many questions regarding the dosing still remain unanswered: Is a peak in IgG levels necessary for its effect? Or is it better to have sustained high levels? Where does IVIg in the body go? Should we dose higher or lower? Should we base our dosage on something other than bodyweight? Answering these basic questions regarding the dosing, distribution and metabolism of IVIg is prudent to fine-tuning this therapy. So where do we stand today regarding the pharmacokinetics of IVIg?

Intravenous Immunoglobulin

IVIg is not a typical pharmaceutical drug, but a preparation of pooled human immunoglobulin G (IgG) obtained from thousands of blood donors. Depending on the product, IVIg mostly consists of IgG (> 95%), with the addition of sodium and/or stabilizers, and trace amounts of other molecules [2]. Since treatment of GBS with IVIg proved equal to plasma exchange [3], the products underwent several modifications. Currently most products are in liquid form, which has the advantages of being storable at room temperature, a longer shelf-life, an increased infusion rate and possibly fewer adverse events [4,5]. The latter is in part achieved by the reduction of the dimeric fraction in IVIg [6]. Interestingly, this fraction was also thought to be the 'active ingredient' for IVIg's anti-inflammatory effects [7]. (For more postulated mechanisms of action, see [Table 39.1.](#))

| What | How | Who | Who in the field of GBS |
|--|---|--|---|
| Anti-idiotypic anti-bodies / Natural (auto) antibodies | Antibodies directed against pathogenic antibodies | Sultan et al., <i>Lancet</i> , 1984. | van Doorn et al., <i>Neurology</i> , 1988. Yuki Miyagi, <i>J Neurol Sci</i> , 1996. Malik et al., <i>Ann Neurol</i> , 1996. Buchwald et al., <i>Ann Neurol</i> , 2002. |
| B-cell modulation | Modulation of proliferation, differentiation, antibody production, and induction of apoptosis | Stohl, <i>Clin Exp Immunol</i> , 1985. | Mori et al., <i>PLoS ONE</i> , 2008. |
| Complement inhibition | Downregulating and inhibition on various levels of the complement cascade | Hauptmann, <i>Presse Med</i> , 1983. | Zhang et al., <i>Brain</i> , 2004. |
| Dendritic cell modulation | Modulation of differentiation, maturation, and induction of a tolerogenic phenotype | Bayry et al., <i>Blood</i> , 2003. | Press et al., <i>J Neuroimmunol</i> , 2005. |
| Fc receptor binding/ blockade | Blocking, binding and modulation of Fc receptors (both activating and inhibiting) | Fehr et al., <i>N Engl J Med</i> , 1982. | Vedeler et al., <i>Neurology</i> , 2000 <i>J Neuroimmunol</i> , 2001. van der Pol et al., <i>Neurology</i> , 2000. Van Sorge et al, <i>J Neuroimmunol</i> , 2005. |
| Glycosylation of IgG/IVIg | Carbohydrates on IgG induce an anti-inflammatory immune response | Kaneko et al., <i>Science</i> , 2006. | Fokkink et al., <i>J Proteome Res</i> , 2014. |
| Modulation of cytokines | Up/down regulation of pro-/anti-inflammatory cytokines | Kuhnert et al., <i>Clin Immunol Immunopathol</i> , 1990. | Créange et al., <i>JNNP</i> , 1998. Tekgül et al., <i>Turk J Pediatr</i> , 1998. Sharief et al., <i>Neurology</i> , 1999. Press et al., <i>J Neuroimmunol</i> 2001. |
| Remyelination | Induction of remyelination | Van Engelen <i>J Neurol Neurosurg Psychiatry</i> , 1994. | van Schaik et al., <i>Mult Scler</i> , 1997. |
| Saturation of IgG recycling | Competition for the recycling receptor leading to accelerated clearance of (auto)antibodies | Yu Lennon, <i>N Engl J Med</i> , 1999. | Kuitwaard et al., <i>Ann Neurol</i> , 2009. |
| Treg induction | Expansion of T-regulatory cells | Ephrem et al., <i>Blood</i> , 2008. | Maddur et al., <i>Immunol Res</i> , 2014. |

An overview of proposed mechanisms of action of IVIg for the Guillain-Barré syndrome. The column "who" reports the first findings the column "who in the field of GBS" reports the studies wherein the specific mechanism was studied in direct link to IVIg and GBS. Since this overview is limited to a subjective Top 10, some studies and/or proposed mechanisms of action are missing.

In general, all products are considered equal [8,9,10], and often only patient tolerability plays a role in selecting a particular product [11]. However, sufficient data on clinical efficacy of different IVIg products are lacking [12], and products are known to differ from each other in various aspects. Regarding IVIg as just a generic product is therefore an oversimplification; choosing a product that is readily available (or least expensive) can very well impact efficacy and/or responsiveness to treatment [13]. One of the striking differences between the marketed products at a first glance is the half-life, ranging from ~23 to ~40 days on average, and in individual patients and products it can even range from ~10 to ~90 days. Results are influenced by the study design (dosing, interval, sampling time points etc.), but they also indicate the wide range of clearances of IVIg. The importance of this variation is emphasized in GBS by the finding that increases in IgG levels 2 weeks following the start of a 5-day course impacts clinical

outcomes [14]. Patients that seem to clear the IVIg faster were worse off; however, an explanation for this large variation in pharmacokinetics is lacking. Identifying these patients, preferably before the start of treatment, could be of great benefit. An important limitation in these pharmacokinetic studies is the inability to distinguish exogenous IgG (via IVIg) from endogenous IgG, making it difficult to do a proper IVIg pharmacokinetic study.

The Unique Pharmacokinetics of IgG

Pioneering work on the pharmacokinetics (PK) of IgG was completed in the 1960s, culminating in a hallmark paper by Waldmann and colleagues in 1970 [15,16]. In this 110-plus page body of work, iodine-radiolabeled IgG was administered to patients with a neoplastic cancerous disease, allowing separation of the administered IgG from the total serum pool. After administration of a small amount of radiolabeled IgG subclass (1–4) in patients with either normal or increased serum IgG levels, radio decay and immunoglobulin levels were measured over a period of 2 weeks. Several key observations were made during this study. Firstly, the extraordinary half-life of IgG and its subclasses (~21 days, except for IgG3 ~7 days) was observed, which is still textbook knowledge. Secondly, the observation of the concentration dependence of IgG was established: the higher the total serum level the faster the catabolism, the shorter the half-life (i.e., first-order kinetics). Both the long half-life and concentration dependent clearance are unique and unlike any other serum protein (the only exception is albumin, showing comparable features). An explanation for these peculiar observations, and at the same time, transfer of humoral immunity from mother to neonate, was already devised: the Brambell receptor [17,18]. If not saturated by high amounts of IgG, this hypothetical receptor would selectively bind and protect IgG molecules from degradation. It took a further 2 decades before the ‘neonatal Fc receptor’ (FcRn) was unambiguously identified [19], and even longer for all concepts of IgG catabolism and transport to be unified and attributed to this one receptor [20,21,22,23].

Next to the catabolism, distribution in the body is equally important. IVIg as a therapy is far removed from the concept of Ehrlich’s magic bullet—frankly we just overload the humeral immune system, rather than having a specific target, without knowing whether the IVIg actually reaches the site of inflammation at all (and does that even matter, in light of all possible working mechanisms)? Subsequent radiolabeling studies found that the majority of intravenous-administered IgG first accumulates in the skeletal muscles and the liver. Thereafter, despite being nonspecific, the radio-traceable polyvalent IgG migrates to sites of inflammation within 24 hours [24,25]. While no inflammatory neuropathy patients were tested in these studies, an educated guess would be that in the case of ongoing inflammation at the peripheral nerves or nerve roots in GBS, IVIg will actually reach it (and for instance; interfere with complement deposition).

Current knowledge on IVIg pharmacokinetics is mostly derived from immune deficiencies (low levels of IgG) and discussed in an excellent review by Bonilla [26]. However, GBS patients have normal, and some perhaps even slightly elevated IgG levels [27]. Therefore, it is not hard to imagine that introducing truckloads of IgG directly into the venous pool, increasing blood viscosity and causing a high peak in IgG [28] alters pharmacokinetics compared to patients receiving replacement therapy. Bearing in mind the concentration-dependent catabolism of IgG, the half-life of supraphysiologic quantities of IgG is greatly diminished [29]. This mechanism of accelerated (auto) antibody clearance, via the saturation of FcRn, is one of the more popular mechanisms of action of IVIg as immune-modulator.

IVIg as Immune Modulator

Concurrent to all the work done on the catabolism of IgG, a different revolution occurred with immunoglobulins as treatment, either as pooled polyvalent immunoglobulins or eventually as monoclonal antibody (mAb). The former was successfully used as replacement therapy in an immune-deficient patient lacking IgG in 1952 [30]. Next to replacement therapy, an additional beneficial factor in fresh-frozen plasma was suspected by several authors studying immune thrombocytopenia (ITP), typically a post-infectious, acute but self-limiting, predominantly antibody mediated disease against glycoproteins on platelets. As early as 1933 it was noted that blood transfusions could induce disease remission in acute ITP, whilst stressing the necessity of repeated transfusions [31]. Almost 20 years later it was first speculated (to my knowledge) that some factors in the transfused blood, other than platelets, might be responsible for the beneficial effect (an increase in platelets) [32]. Closing down on this elusive factor was carried out by work from Mila Pierce and colleagues, when she contemplated that the transient beneficial effect in a patient (lasting 20–23 days) correlates with blood infusions and that fresh-frozen plasma (FFP, devoid of platelets) could also elicit this response [33]. The authors discontinued corticosteroid therapy and found that FFP gave a dose-dependent beneficial effect, leading to the first speculation and research performed regarding the mode of action of this factor in FFP. The authors found and settled on an effect on megakaryocytes (Note: Thirty years later Saito and colleagues showed this to be at least partly true, with IVIg entering the bone marrow and these cells [34]).

But IVIg's current claim to fame came with successful treatment with intravenous immunoglobulin (IVIg) of patients with ITP by Imbach and colleagues, causing a surge of interest in high-dose immunomodulatory functions [1]. Based on previous observations, the decision was made to treat a patient refractory to all other treatments with a course of IVIg [35]. The proposed working mechanism was the requirement of a large amount of IgG to overload and block the reticuloendothelial system by its catabolism. The treatment with 0.4 g/kg of bodyweight (BW) a day was deduced from the use of IVIg as replacement therapy in an ITP patient with hypogammaglobulinemia, wherein Imbach and colleagues found the first effects of an increase in platelets.

In contrast, the choice to administer the IVIg over 5 days, resulting in the high-immunomodulatory-dose of 2 gr/kg BW (for GBS considered as standard), is more enigmatic (and might very well have been just out of convenience: Monday–Friday). Nevertheless, this regimen was subsequently tried in many different autoimmune diseases. The field of neurology quickly followed and successful treatment of chronic inflammatory demyelinating polyneuropathy (CIDP) with FFP, and subsequently attaining the same results with IVIg (identifying IgG as the crucial factor in FFP) was reported [36]. Later on the need for a high-dose IVIg regimen was also established in CIDP [37]. Given the initial findings in CIDP, it did not take long before the first experiences with IVIg and GBS were noted [38,39]. Unambiguous clinical efficacy of IVIg in GBS was for the first time demonstrated by the Dutch Guillain-Barré study group [3], when it was found to be equally effective to plasmapheresis.

Rigid Regimen

Alas, since that major breakthrough and despite meticulous efforts to improve therapy for GBS, we still treat with IVIg in that same, high-dose, 2 gr/kg BW, 5-day regimen. Dose-dependent anti-inflammatory effects were reported in in vitro studies early on [40,41], and also in animal models [42,43]. Yes, good comparative clinical studies of different regimens are lacking, but there is evidence from other fields that the high dose is indeed necessary for an immunomodulatory effect. Whilst low dosing might yield some effect in ITP, there is a correlation with a better effect and higher response rate with increased doses [44,45]. Furthermore, for ITP, IgG levels within the normal range values (~6 g/L—16 g/L) seem

inadequate to halt the disease [46].

Another disease treated with IVIg is Kawasaki's disease (KD), suspected to be post-infectious in nature, acute but self-limiting, with an important role for mucosal antibodies, resulting in systemic vasculitis, and steroids seem not of benefit, or even counterproductive. Considerable progress has been made early on regarding the IVIg regimen used in these patients. Next to the proven dose dependency of beneficial effects [47], 2gr/kg BW given in 1 day proved superior (with no increase in adverse events), rather than spreading the IVIg course over multiple days. This also underscores the importance of the pharmacokinetics of IVIg: one massive boost in IgG is more efficacious here than multiple smaller peaks [48]. Further progress in this field discriminates between patients requiring either 1, 2 or even 4 gr/kg (for initial IVIg-resistant patients) of IVIg all given at a rate of 1 gr/kg BW an hour [49]. The 'luxury' that these IVIg-treated diseases have in common for comparative studies are relatively easy to measure and are reliable clinical and physiological markers for IVIg response, things sorely lacking in the field of GBS [50].

Nonetheless, speculation on a potentially increased efficacy for a shorter treatment duration was made immediately after proof of IVIg's effectiveness in GBS [51]. A small number of studies have been performed assessing different IVIg regimens. The only study in adults compared a 3-day regimen versus a 6-day regimen of 0.4 gr/kg BW in GBS patients unsuitable for plasma exchange. Whilst the authors mentioned several study limitations, they found a tendency for better efficacy with the 6-day regimen [52]. However, the pharmacokinetics of IVIg were not tested here, since the authors essentially compared 1.2 g/kg (3 days) versus 2.4 g/kg (6 days). Later on this hiatus was assessed in a study on childhood GBS. The authors found, contrary to what was expected and what was previously published in smaller studies, no difference between 2g/kg over 2 days versus the exact same amount over 5 days. While the 2-day course was safe, increased treatment-related fluctuations were reported, and the authors speculated on still ongoing disease, not sufficiently covered by the 2 days of treatment [53]. Yet, this hypothesis could not be proven since IgG serum levels were not available. Also, this study was performed in children, thought to have a relative mild disease course compared to adults [54]. Children, of course, also differ from adults in their body composition and subsequently in a key pharmacokinetic parameter: the volume of distribution [55]. Dosing of IVIg however, is still based on actual bodyweight for all patients (NB Imbach's study in 1981 were all children). Peculiar, since IVIg's pharmacokinetics (a relative small volume of distribution) imply that it hardly distributes into body fat [56]. Taking account for this fact is dosing based on adjusted or ideal bodyweight. However, this is largely motivated by cost-saving and to prevent overdosing in case of obesity [57], rather than by improving efficacy.

Future Perspectives

It seems that almost every aspect of GBS seems challenging to resolve, and this certainly applies to treatment with IVIg. Even in 2016, we at least know that IVIg works and that a prolonged increment in IgG levels after IVIg seems to positively affect outcome in GBS. Have we then made no further advancement these past 3 decades of IVIg treatment for GBS? That is definitely not the case. The coming years will allow us to clarify some mysteries concerning immunoglobulin therapy in GBS. Will a second dose of IVIg be beneficial in severe patients? Will subcutaneous IgG, providing a more favourable pharmacokinetic profile over time, offer new therapeutic options?

In addition, improvements and alternatives to polyvalent immunoglobulin therapy are well underway. This will allow us to finally decrease the enormous list of postulated IVIg working mechanisms. Some promising developments with IgG-based mAbs are eculizumab in GBS, an IgG2/4 hybrid blocking the

complement cascade; a mAb targeting the FcRn receptor, increasing the catabolism of auto-antibodies [58]; and mAbs directed against FcγRIIb, the inhibitory Fc-receptor with the potential to enhance existing therapies [59,60]. Alternatively to a mAb against FcγRIIb, a recombinant soluble variant of this receptor is currently being tested for its anti-inflammatory effects [61]. IVIg itself is also subject to potential improvement: a highly sialylated version has been developed, aiming to exploit the anti-inflammatory effects described when this carbohydrate is present on the IgG molecule [62]. The cationization of IVIg is also being explored, which should increase the bio-distribution, making it easier for IgG to enter organs or cross the blood-brain/nerve barrier [63]. A very appealing alternative is an IVIg biomimetic, which does not focus on one mechanism of action, but rather is capable of binding or blocking a number of receptors and complement factors [64]. It is hoped that one of these directions or other developments not mentioned here will take IVIg's place. For now, IVIg remains the cornerstone of GBS treatment and somehow tailoring the dose to the patient's needs may already yield benefit.

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Gangliosides and Guillain-Barré Syndrome: The Facts, 20 Years after the Withdrawal of the Ganglioside-Based Medications

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The Ganglioside-Based Medications: Cronassial and Sygen

Gangliosides, sialic acid-containing glycosphingolipids, are components of the external layer of all plasma membranes and are particularly abundant in neurons. Their oligosaccharide chains are protruding towards the extracellular aqueous environment, anchored to the membrane *via* the ceramide hydrophobic moiety, which is inserted in the lipid bilayer.

Ganglioside research developed at the beginning of the 20th century; however, it was necessary to wait until the 1960s to have their structures elucidated. Then, this family of glycoconjugates immediately attracted the interest of several scientists, whose work convincingly showed that gangliosides are important physiological regulators of a variety of neuronal processes, like neuritogenesis, synaptogenesis and cell-cell interactions such those involved in the migration of neuronal precursors and the wrapping of myelin around axons. In particular, the finding that the administration of ganglioside mixtures to animal models of peripheral sympathetic regeneration and reinnervation was able to improve the recovery processes of both cholinergic and adrenergic nerve fibres was extremely appealing, suggesting that gangliosides might play a relevant role in neural regeneration after injuries that could be pharmacologically exploited [1]. Subsequently, and up to today [2], a great number of basic, preclinical and clinical studies supported the notion that the administration of exogenous gangliosides could be effective in enhancing recovery from both peripheral and central nervous system injuries.

The most relevant clinical trials involving gangliosides suggested that the administration of exogenous gangliosides

- improved both electromyographic findings and clinical symptoms in patients with diabetic peripheral neuropathy;
- had some success in patients with uremic neuropathy;
- had some neuro-protective effectiveness for acute ischemic stroke and spinal cord injuries; and
- gave promising results in the prevention and treatment of degenerative diseases of the central nervous system, especially Parkinson's and Alzheimer's disease.

The research on the effects of gangliosides received a big impulse from the availability of great quantities of pure ganglioside molecules, GM1 and polysialylated gangliosides, following the introduction in the drug market of ganglioside-containing formulations.

In 1973, the drug Cronassial, containing the highly purified ganglioside mixture from calf brains, was launched in Italy and prescribed for peripheral neuropathies and back pain. From 1976, the drug was commercialized under several different names in 20 countries in Europe, South and Central America, Asia and Africa. Later, in 1985, the drug Sygen, containing highly purified GM1 and prescribed for neurodegenerative diseases and cerebral and spinal injuries, flanked Cronassial in Italy and later in Argentina, Brazil, Greece and Singapore. FIDIA S.p.A. prepared the gangliosides used for these formulations in its plants in the city of Abano Terme in Italy, and later in other countries.

For several years both Cronassial and Sygen were extensively prescribed for the therapy of nervous system diseases to the satisfaction of both doctors and patients.

Sygen was entered in new trials for the treatment of central nervous system diseases like cerebral ischemia and dementia, stroke and spinal cord injury; however, many of these trials could not be completed. In fact, at the beginning of the 1990s, some scientists claimed that the presence of gangliosides in the bloodstream was able to stimulate the production of anti-ganglioside antibodies. Anti-ganglioside antibodies raised by the administration of ganglioside-containing drugs were indicated as responsible for the occurrence of GBS, due to their interaction with the axonal surface at the node of Ranvier. This opened a fierce debate on the safety of gangliosides as drugs. Some scientists were against the therapy and others in favour. The discussion also involved the Italian drug committee that, after over 16 million prescriptions and a few cases of GBS claimed to be derived by the therapy, decided to withdraw gangliosides at the end of 1993, in Italy. The same occurred later in other countries. However, Brazil and China still prescribe Sygen and a generic 'monosialotetrahexosylganglioside sodium', respectively. In some countries, GM1 is still involved in clinical trials at different stages.

After over 20 years since the withdrawal of the ganglioside-containing drugs in many countries, the notion that the administration of gangliosides is not associated with the appearance of anti-ganglioside antibodies in serum, nor with the onset of GBS, seems to be sounder.

Immunogenicity of Gangliosides

Several lines of evidence show that oligosaccharide chains are haptens, and, in the majority of cases, they do not display immunogenic properties, as do not the soluble gangliosides. On the other hand, gangliosides become immunogenic when carried by an adjuvant, such as a microorganism.

The production of antisera against gangliosides in experimental animals invariably requires heavy manipulation of the material used for immunization. All procedures reported in the literature are based on the mixing or coupling of gangliosides with a variety of strongly immunogenic carrier substances, such as heterologous whole serum, methylated bovine serum albumin, heterologous glycoprotein, *Mycoplasma* membrane proteins and Freund's complete adjuvant. To the best of our knowledge, there is only a single paper claiming immunization against gangliosides in experimental animals without use of carrier proteins or immunologic adjuvants [3].

Information on the onset of an autoimmune neurological syndrome upon administration of gangliosides is very rare. In addition, this was obtained by injection of liposomes composed of gangliosides, egg lecithin and cholesterol, and methylated bovine serum albumin. In addition, liposomes were emulsified in complete Freund's adjuvant [4]. No reports are available on the onset of an autoimmune neurological syndrome following controlled ganglioside injections in a large cohort of humans that exceeds 15,000

units.

On the other hand, convincing proof that highly purified gangliosides are not immunogenic when injected in patients comes from the extensive use of gangliosides for 20 years, and by the clinical trials developed to understand some pharmacological properties of gangliosides on neurodegenerative diseases, as neuro-protective compounds and as promoters of spinal cord recovery following injury:

- Five Alzheimer's disease patients received up to 30 mg/day of GM1 ganglioside by continuous injection into the brain lateral ventricles for 12 months. None of these patients developed serum antibodies recognizing gangliosides in an ELISA test. None developed GBS or any other neurological autoimmune syndrome. Instead, these patients became more active, had improved reading comprehension and were able to perform activities such as writing reports and short letters on a computer [5].
- In 2 trials, about 100 Parkinson's disease patients received 100mg of GM1 daily, both intravenously and by subcutaneous injection, for up to 2 years. None developed GBS or any other neurological autoimmune syndrome. The treatment improved motor symptoms and lowered the disease symptoms' progression [6].

Both the above trials were carried out with the same GM1 used for the preparation of Sygen.

- Oxaliplatin is a very powerful drug against gastrointestinal tumours; however, it is characterized by severe peripheral neurotoxicity. Sixty patients with gastrointestinal tumours were injected daily with 100mg of GM1 for 3 days following chemotherapy treatment. The treatment showed some minor protection against the oxaliplatin neurotoxicity, but no case of GBS onset was reported [7].
- Several trials were carried out on over 700 patients to determine the properties of GM1 in recovering spinal cord injury, but no reports on the onset of peripheral neuropathies are available [8].

These are a few examples of the controlled administration of gangliosides, clearly proving that continuous administration of highly purified gangliosides to a very high number of patients do not lead to serum anti-ganglioside antibody production.

Ganglioside Mimicry

Guillain-Barré syndrome and related neuropathies, which were among the first to be associated with anti-oligosaccharide antibodies, are preceded in two-thirds of cases by *Campylobacter jejuni*, *Mycoplasma pneumoniae* or cytomegalovirus infections. Infection with *C. jejuni*, usually contracted by consumption of raw or undercooked poultry, unpasteurized milk or contaminated water, is the most common cause of bacterial gastroenteritis. The isolation rate of *C. jejuni* from stool culture of GBS patients ranges from 8% to 50%. Some of these patients developed serum antibodies recognizing gangliosides by ELISA or by immune-TLC staining.

The above-mentioned microorganisms display complex glycoconjugates with terminal carbohydrate sequences similar to those present in the cell membrane glycoconjugates of humans. Bacteria isolated from the stool culture of GBS patients were analysed for their membrane complex lipid composition, and found to contain terminal oligosaccharide structures overlapping to those of neuronal gangliosides. These microorganism-associated structures are believed to be responsible for an 'oligosaccharide mimicry' and for the immunogenic response [9].

Epidemiological Studies

The above-mentioned studies in our opinion convincingly demonstrate that there has never been any relationship among administration of gangliosides, anti-ganglioside antibodies and GBS. However, a further piece of evidence should dissipate any possible scepticism with this regard.

At the time of the first case reports, a retrospective cohort study based on administrative data found no cases of GBS among 13,373 subjects prescribed with gangliosides between 1988 and 1990 in an Italian district [10]. Spanish epidemiological data regarding 17 patients with GBS did not show any association with ganglioside treatment [11]. A subsequent study found 42 cases of GBS among 579,725 discharge codes referring to the year 1989 in 3 Italian districts. In another Italian district, 9 cases of GBS were reported in subjects receiving ganglioside treatment from 1981 to 1993; among those 9 patients, 7 received gangliosides *after* the diagnosis of GBS; the risk of GBS in subjects exposed to gangliosides was not significantly higher as compared with nonexposed subjects. A Spanish study showed that the incidence of GBS was not higher in geographical areas where gangliosides were frequently prescribed than in those in which they were not prescribed [12]. The local health district (LHD) of Ferrara, Italy reported an epidemiological investigation on the possible relationship between ganglioside therapy and GBS by a systematic comparison of the incidence of GBS in the period between 1988 and 1993 (when ganglioside-based drugs were widely prescribed) vs. the 1994–2001 period (following withdrawal of such drugs) [10]. The study found that the incidence of GBS increased from 1981 to 1993, especially in urban areas and among elderly subjects; however, only one of the GBS cases occurred during treatment with gangliosides; moreover, no decline in incidence was observed from 1992 to 1993, the year in which gangliosides were withdrawn from the Italian market. When extending case ascertainment up to 2001, only a small decline in the incidence of GBS was found that might be better explained by fluctuations in GBS incidence than by the withdrawal of ganglioside treatment.

In the LHD of Ferrara the resident mean population was 165,239, with 77,630 men and 87,609 women, in the years 1994–2001. The cases of GBS were 24.3 in the years 1981–1987, 20.1 in the years 1988–1993 and 25.4 in the years 1994–2001.

In the years 1981–1993 there was an incidence rate of GBS of 1.87 per 100,000 population, which became 2.30 for men and 1.48 for women. In the years 1994–2001, after ganglioside withdrawal, the investigation identified an incidence rate of 1.97 per 100,000 population, 2.25 for men and 1.71 for women. Recalling that the onset of GBS is age related, the age-adjusted rates became 1.65 and 1.66 per 100,000 in the years 1981–1993 and 1994–2001, respectively. Considering the calendar years, the highest rate was in 1997, 4.22 per 100,000, while the lowest one was in 1981, 0.54 per 100,000.

These studies clearly support no action of ganglioside treatment in onset of GBS.

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41

My Top 10 Images

Rhona McGonigal

Introduction

As the old adage goes, ‘A picture is worth a thousand words’, and I think this is particularly true in scientific research. We might not fully digest or remember the exact text in every article we read, but incredible images will convey novel information with immediate effect and spring to mind effortlessly over the years. Scientific images are at the heart of our belief in our data and findings as we can see the results with our own eyes.

To tackle this Top 10 I had to decide whether to pick the most visually pleasing images, or figures that changed the way we thought about aspects of GBS. Conveniently, often the images fit into both categories! Ten is really not enough; there are countless inspiring high quality images. Here I opted to follow quite a personal route, picking the images and figures that have been the most remarkable, influential and meaningful to my own line of research.

Guillain, Barré and Strohl

No book on GBS would be complete without a tribute to the three forefathers of Guillain-Barré syndrome, French army physicians Georges Guillain, Jean Alexandre Barré and Andre Strohl who reported the first cases of what we now know as GBS in 1916 ([Figure 41.1](#)) [1].

AIDP and AMAN Pathology

I could have chosen every single incredible image from both Griffin and colleagues [2] and Hafer-Macko and colleagues [3] but then thankfully the authors created these beautiful overview figures ([Figures 41.2](#) and [41.3](#)) in a subsequent review of human immune-mediated neuropathies [4] so I wasn't forced to choose. Animal models have of course been indispensable in furthering our understanding of GBS, but I think where possible it's beneficial that we use patient autopsy tissue as a reference point, and these studies have provided a wonderful foundation for our knowledge of disease processes. These figures clearly demonstrate the differing patterns of nerve fibre targeting and mode of injury in the demyelinating and axonal variants of GBS. Complement deposition occurs on the axolemma (primarily at the nodes of Ranvier) in AMAN and AMSAN patients or on the Schwann cell abaxonal membrane in AIDP, where it is likely involved in membrane disruption and vesiculation, respectively. Complement activation is followed by subsequent macrophage invasion at each respective membrane where they likely participate

in opening of the periaxonal space and degeneration of axons (AMAN), and removal of myelin debris (AIDP). Thorough ultrastructural and immunohistochemical analysis of nerve pathology in patients has highlighted the disease processes and aspects we must simulate when developing animal models.

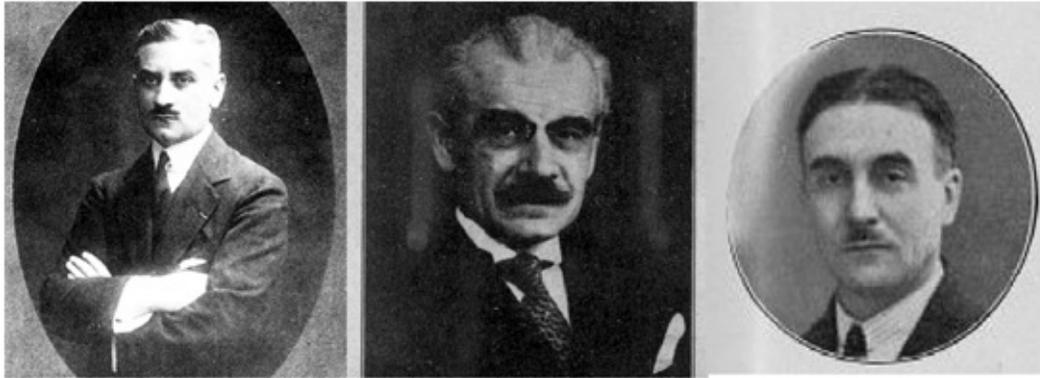


Figure 41.1 Georges Guillain, Jean Alexandre Barré and Andre Strohl. Public domain.

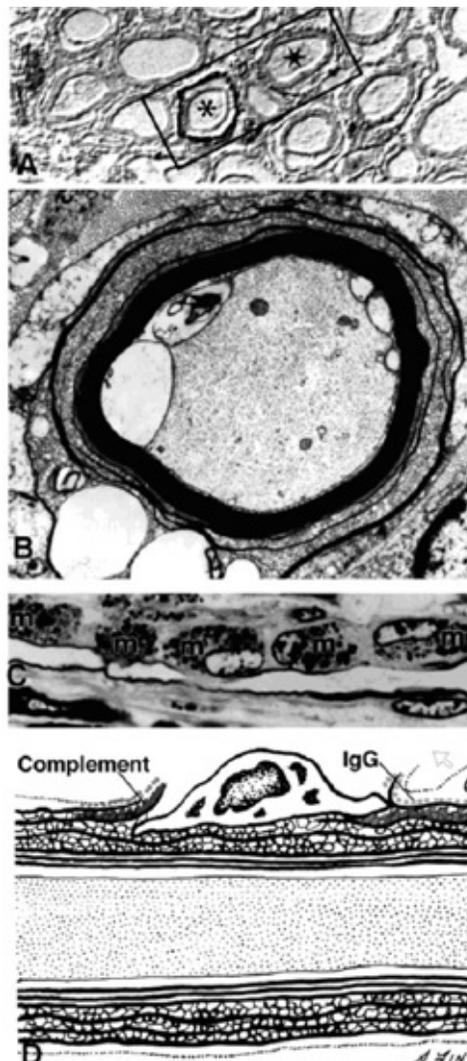


Figure 41.2 Immunopathology of AIDP. (A) Two fibres from an early AIDP case ringed by positive immunostaining for C3d are indicated by asterisks (*). (B) Activation of complement followed by calcium entry and vesicular demyelination are a hypothesized sequence of events in AIDP. (C) Macrophages attracted by complement activation remove myelin. (D) Schematic depiction of proposed pathogenesis in AIDP, with antibody binding to myelin followed by complement activation and macrophage-mediated demyelination. Images (A, B, C) adapted with permission from [19]. Image D reproduced with permission from [4].

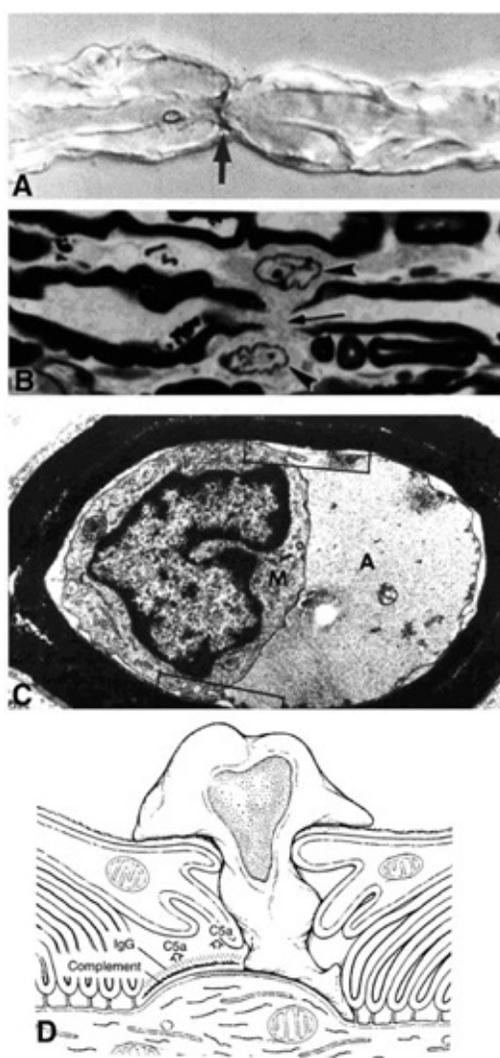


Figure 41.3 Immunopathology of AMAN. (A) C3d deposition in a node of Ranvier, demonstrated by immunostaining in the ventral root of a patient with AMAN. (B) Macrophages overlying a lengthened node of Ranvier in the ventral root of a patient with AMAN. (C) Macrophage within the periaxonal space and (boxed region) extending processes around the axon (D) Schematic depiction of proposed pathogenesis in AMAN, showing antibody binding to the axonal targets followed by complement activation and macrophage invasion through the node of Ranvier. Images A and D adapted with permission from [4]. Images B and C reproduced with permission from [2].

Complexities of Ganglioside Distribution and Membrane Interactions

The blots in [Figure 41.4](#) are wonderfully simple images, but the information that they convey both individually and together fundamentally changes the way ganglioside-antibody interactions are understood. Anti-ganglioside antibodies are found in approximately 60% of patient sera and are associated with pathogenesis through binding gangliosides on peripheral nerve. The following is a description of just two complexities that we are currently aware of.

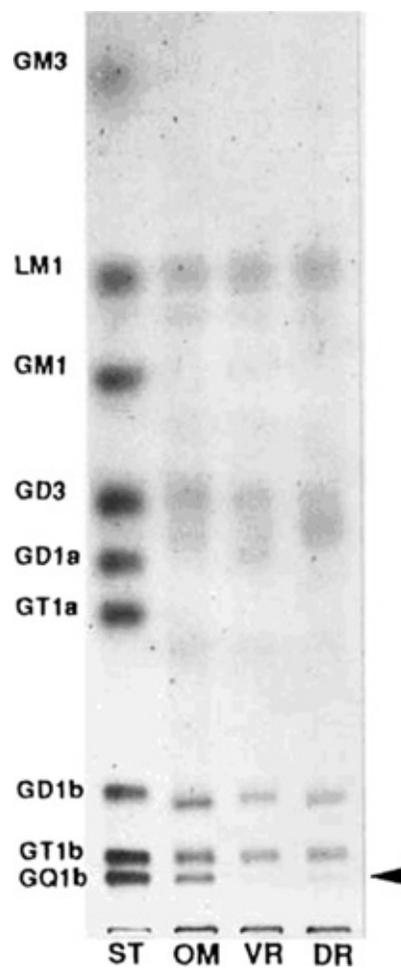


Figure 41.4a Ganglioside patterns. Ganglioside fractions corresponding to 4.0 mg wet weight of tissue were chromatographed on an HPTLC plate, then stained with resorcinol. The oculomotor nerve (OM) has more GQ than do the ventral root (VR) and dorsal root (DR) of the lumbar spinal cord for the same wet weight (arrowhead). ST = mixture of known standard gangliosides (500 ng each). Reproduced with permission from [5].

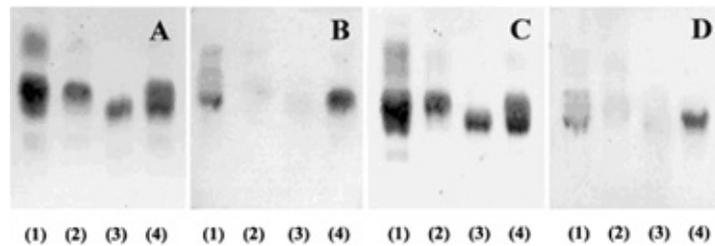


Figure 41.4b Thin-layer chromatogram (TLC) immunostaining of sera from Patients 1 and 3. TLC results in plates A and C are made visible by the orcinol reagent. Bovine brain gangliosides extracted by 0.1M ammonium acetate are in lane (1), GD1a (3 μ g) in lane (2), GD1b (3 μ g) in lane (3), and both GD1a and GD1b (3 μ g each) in lane (4). Plate B shows the TLC immunostaining of the serum from Patient 1, and plate D that of the serum from Patient 3. Compared with the slight immunostaining present in lanes (2) and (3), the overlapping portion of GD1a and GD1b in lane (4) is strongly immunostained. Reproduced with permission from [6].

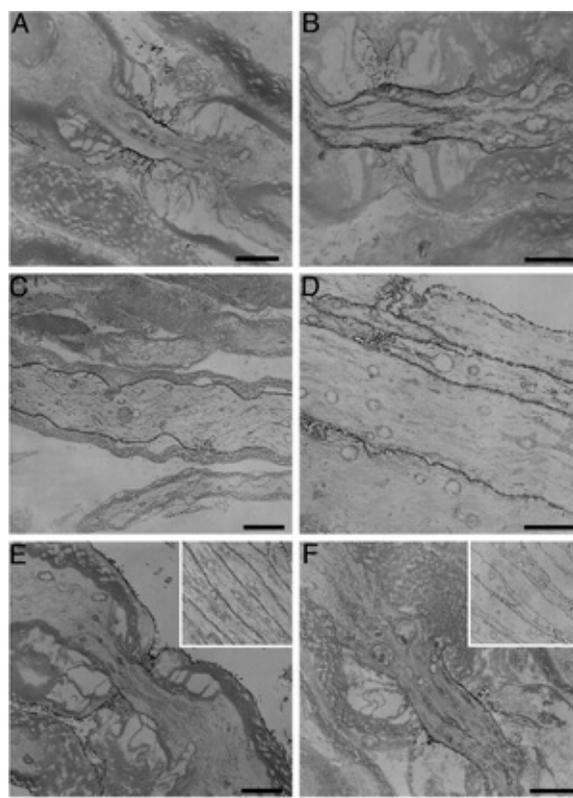


Figure 41.5 Immunoelectron microscopy of mouse spinal roots. (A) CT binding to nodal axolemma; (B) CT binding extending to paranodal axolemma; (C) CT binding to intermodal axolemma in an area where myelin is thinned and retracted; (D) CT binding to axolemma of amyelinated axons in a dystrophic spinal root; (E) PNA binding to nodal axolemma, the outside of Schwann cells, and microvilli of the terminal myelin loops; inset shows PNA binding to amyelinated axons in a dystrophic spinal root; (F) TTC binding to nodal axolemma; inset shows TTC binding to amyelinated axons in a dystrophic spinal root. Scale bar represents 5 μ m. Reproduced with permission from [7].

In a series of experiments, Chiba and colleagues demonstrated for the first time a relationship between anti-GQ1b antibody serum levels and patients with ophthalmoplegia in MFS and GBS and the underlying cause [5]. The landmark finding is illustrated in the blot where GQ1b content is significantly greater in the human oculomotor nerve compared to ventral and dorsal roots. This study demonstrates that ganglioside expression occurred at different densities in various nerve membranes throughout the body, and thereby could uniquely influence site-specific pathogenesis and clinical manifestation.

A decade later, Kaida and colleagues observed another new feature of patient anti-ganglioside antibodies [6]. It is possible to find antibodies in patient sera that bind to gangliosides in complex and their newly formed glycoepitopes, rather than single ganglioside epitopes. In the TLC blot from this article, lanes 2 (single GD1a) and 3 (single GD1b) show no or weak bands when patient serum is overlaid, while lane 4 (GD1a + GD1b) shows prominent binding. Gangliosides can form clusters in the plasma membrane, but until this study it had never been shown that antibodies could exclusively target these clusters instead of individual lipids. Antibody binding to complexes may correlate with a specific GBS phenotype just as GQ1b binding is associated with ophthalmoplegia. Additionally, currently not all GBS patients have identifiable serum antibodies; assessing patient sera for complexes as well as single gangliosides may account for this gap.

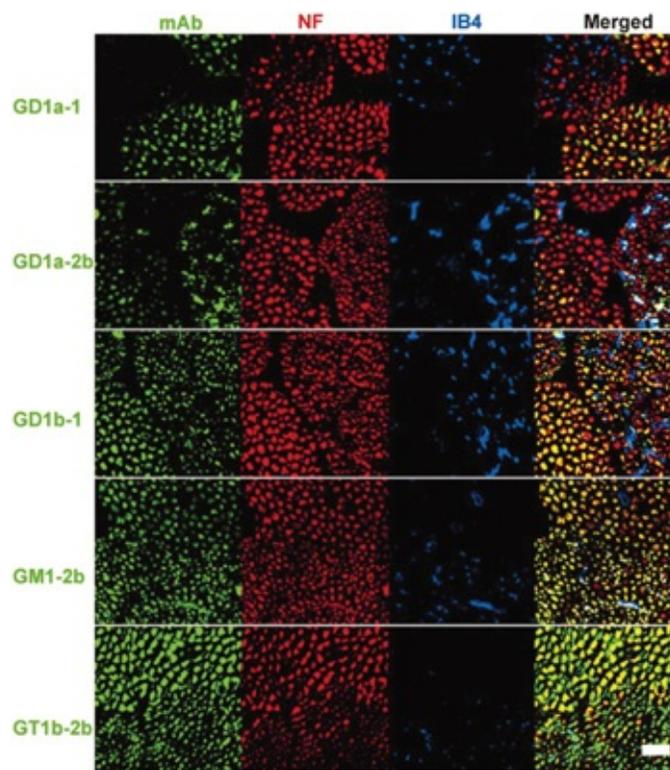


Figure 41.6 Fresh-frozen cross-sections of rat cauda equina triple-labelled with anti-ganglioside mAbs (green), neurofilament (red) and IB-4 (blue). Co-localization of three labels is also shown (merged). The mAbs included in this figure are shown on the left. Bar = 20 mm. Reproduced with permission from [8].

Localisation of Gangliosides in the PNS

Figures 41.5 and 41.6, from the work of Sheikh and colleagues [7] and Gong and colleagues [8], are as meaningful and interesting now as when they were first published. It is highly relevant to the above ideas that gangliosides are not homogeneously expressed and that not all characteristics of anti-ganglioside antibody binding can be revealed by array. Indeed, it is now known that antibodies binding similarly on array can bind differently in processed or living tissue [9].

It has been known for many years that anti-ganglioside antibodies are found at high titres in patient serum, and that some of these antibodies are associated with clinical symptoms implying specific nerves or sites are targeted (e.g. anti-GD1a Ab in AMAN). Why are some membranes (e.g. motor axons) preferentially targeted when gangliosides are comparably expressed in myelin and axon fractions? Does localisation of gangliosides differ, just as the density of GQ1b differs among nerves? Detailed and thorough ganglioside localisation is essential to answering these questions, and as such ganglioside localisation was first probed using indirect markers (e.g. cholera toxin is a known receptor for GM1) but high-affinity monoclonal antibodies now prevail as a more precise tool.

Figure 41.5 shows immunoelectron microscopy of cholera toxin binding in mouse spinal roots. In these striking ultrastructural images, cholera toxin binds specifically to the nodal axolemma and paranodes in normal nerve. However, binding is not so restricted in transverse cryosections where Schwann cell membrane and compact myelin are also positive. Tissue was processed differently for immunoelectron microscopy and cryosectioning and that likely underlies these slightly different results. Additional binding is found along the internode in areas of thinned myelin and all the way along unmyelinated fibres from amyelinated dystrophic mice indicating that GM1 is present on the entire axon but its accessibility is restricted under normal conditions. Taken together, these results raised awareness that there are many technical considerations to be made when interpreting binding and localisation data. And unfortunately a

beautiful image does not reflect the whole story!

In [Figure 41.6](#), the authors used high-affinity mouse monoclonal antibodies raised against the four major brain complex gangliosides to probe the peripheral nerve and produce these memorable images. The immunohistochemical results clearly demonstrate that antibodies can differentially bind motor and sensory fibres, and in this scenario axonal but not myelin membranes. This indicated that gangliosides can be inaccessible to circulating antibody binding (e.g. in compact myelin), displayed within the membrane in an unfavourable manner for binding, and that individual antibodies can exhibit fine specificity. This finding has further highlighted the causal relationship between certain anti-ganglioside antibodies, their preferential fibre system/cell type binding, and the ultimate clinical manifestation.

Complement-Mediated Nodal Injury in a Rabbit Model of GBS

The active immunisation rabbit GBS model has provided great insight into the pathomechanisms of axonal GBS [10,11]. It has also helped to establish the node of Ranvier as a primary site for immune attack associated with dysfunction. [Figure 41.7](#), from Susuki and colleagues [12], clearly indicates Na channel disruption at the node of Ranvier associated with complement deposition and nodal lengthening in the acute disease phase. Disruption to Na channel clustering critically impairs saltatory conduction and aligns with the motor nerve conduction block observed in rabbits and humans. This finding forms the foundation for the fascinating new concept of nodo-paranodopathy, whereby antibody binding and complement activation at the NoR can cause a continuum of dysfunction ranging from reversible conduction block and recovery to axonal degeneration and poor outcome [13].

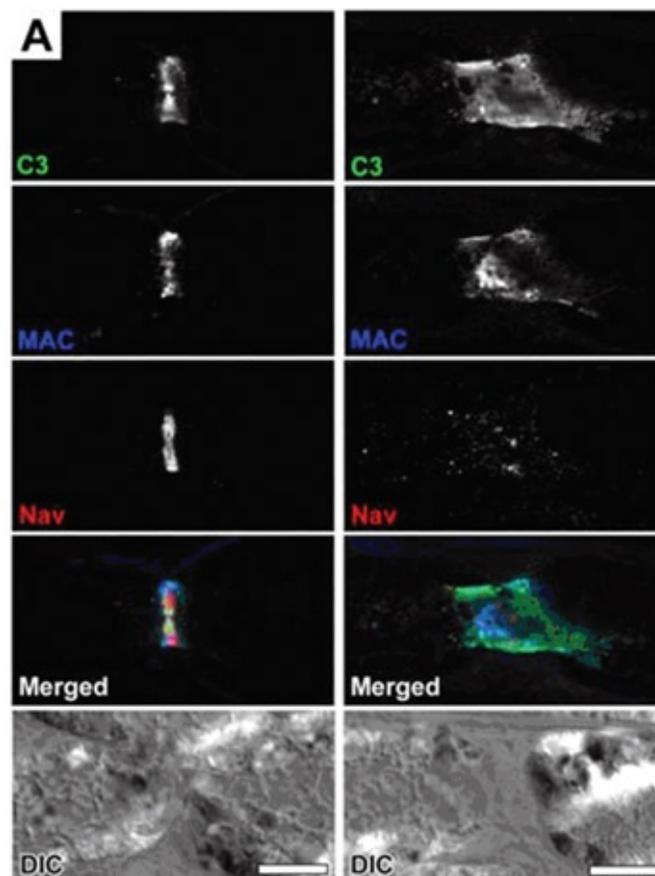


Figure 41.7 Lesions at the nodes of Ranvier with complement deposition and disrupted Na_v channel clusters. (A) Disruption of Na_v channel clusters at the acute progressive phase. Both C3 (green) and MAC (blue) staining is present at nodes. Clusters of

Na_v channels (red) are preserved at lesions restricted to the node (left column), whereas Na_v channels almost disappear at lengthened nodes with extended C3 and MAC staining (right column). Nerve fibres run horizontally in both columns. Scale bars, $10\mu\text{m}$. Reproduced with permission from [12].

Complement Inhibition as a Therapeutic for Attenuating Peripheral Nerve Degeneration in a Mouse Model of GBS

The powerful potential of using an antibody that binds and inhibits complement component C5 to attenuate pathology in a GBS disease scenario is outlined by [Figure 41.8](#). Mice that are transgenically engineered to endogenously express cyan fluorescent protein (CFP) in the cytoplasm of their axons are a useful tool for live-imaging experiments ... and also produce rather eye-catching images! Here, the authors used these mice to show the effect of blocking the formation of the complement cascade's terminal MAC pore on peripheral intramuscular nerve bundles subjected to anti-ganglioside antibody and a source of complement [14]. The fluorescent signal (white) is absent from the most terminal branches of the intramuscular nerve bundles and weak more proximally, indicating a severe disruption to the nerve membrane that coincided with loss of function. This is completely prevented by the blockade of MAC pore formation by anti-C5 antibody, eculizumab (Alexion Pharmaceuticals Ltd).

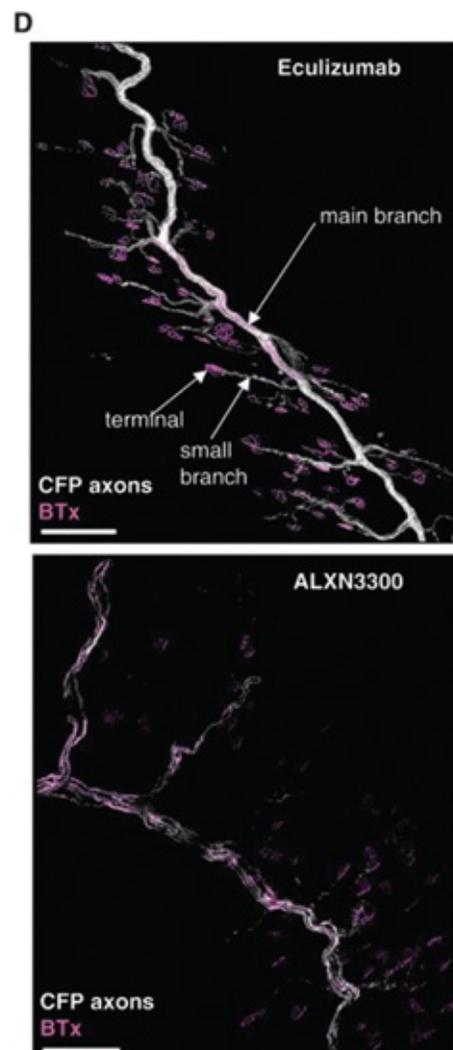


Figure 41.8 Complement inhibition with eculizumab attenuates nerve injury in an animal model of GBS. Antiganglioside antibodies and complement injure distal nerves resulting in axonal injury and loss of fluorescent signal (bottom panel; NMJs are labelled with bungarotoxin in red; fluorescent axonal signal in white). The addition of the complement inhibitor eculizumab preserves axonal integrity (top panel). Reproduced with permission from [14].

Serum IgG from Guillain-Barré Syndrome AMAN and AIDP Variants, and Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) Patients Bind the Nodes of Ranvier and Paranodes, Respectively, and This Binding Is Blocked by Soluble Antigens to Nodal Proteins

Figure 41.9 elegantly adds to the evidence corroborating the recent theory that antibodies targeting nodal proteins can be found in patients [15]. It is unclear whether these antibodies to nodal proteins exist either as part of the initial disease pathogenesis or are produced subsequently due to the unveiling of epitopes leading to disease augmentation. Evidence from animal models [16], points towards the targeting of peripheral myelin protein in the demyelinating phenotype. The number of patients who have anti-nodal protein antibodies is very small, but the summation of antibodies to each individual protein could account for many patients. The fact that the node is the general target regardless of specific epitope could underlie a similar pathogenesis among patients with differing preceding infections.

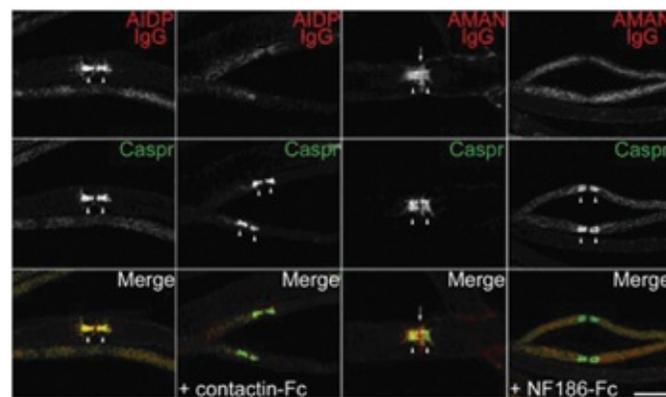


Figure 41.9 The binding of Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP) patients' IgG to nodes of Ranvier is blocked by soluble antigens. Mouse sciatic nerve fibres were incubated with sera (red) from (A, B) acute inflammatory demyelinating polyneuropathy (AIDP) or (C, D) acute motor axonal neuropathy (AMAN) patients and were stained for Caspr (green) to label paranodes. Pre-incubation of AIDP serum with soluble contactin-Fc (B) abolished the binding of IgG to contactin at paranodes (arrowheads). Also, pre-incubation of AMAN serum with soluble NF186-Fc (D) abolished the binding of IgG to neurofascin at nodes (arrows) and paranodes (arrowheads). Scale bar: 10µm. Reproduced with permission from [15].

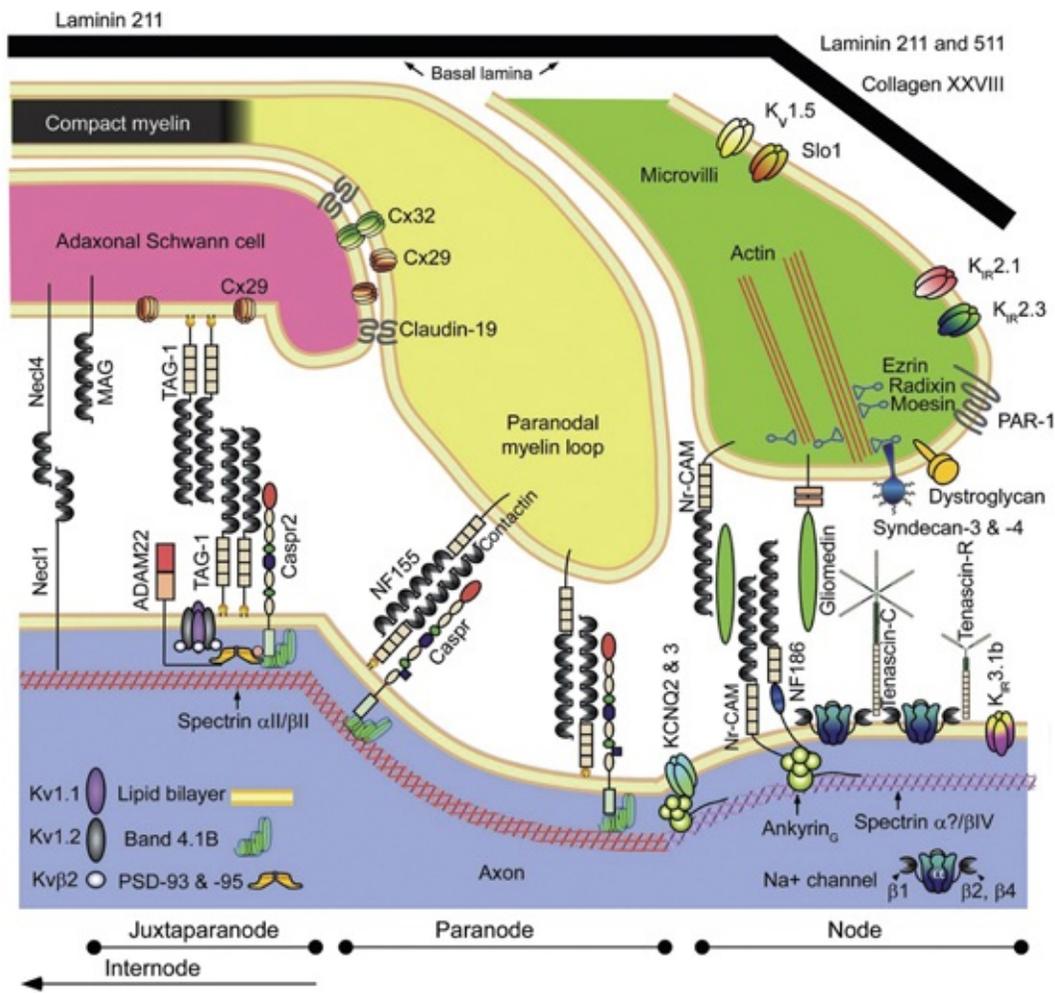


Figure 41.10a Molecular components at the node of Ranvier

Schematic drawing shows some of the molecular components of the peripheral nervous system nodal region, which can be divided into the node, paranode, and juxtapanode. At the node, gliomedin, Nr-CAM, and neurofascin (NF) 186 link the Schwann cell microvilli to the nodal axolemma. In the paranode, NF155 interacts with Caspr and contactin heterodimers to link the glial and axonal membranes. In the juxtapanode, TAG-1 homodimers link the adaxonal Schwann cell membrane to the axonal membrane. In the internodal region, Necl4 interacts with Necl1, and myelin-associated glycoprotein (MAG) has several potential axonal ligands. Reproduced with permission from [17].

Molecular Components of the Nodes of Ranvier

Schematics themselves have become more sophisticated over the years and form a really important reference point for our understanding. As we add more pieces to the puzzle through our research, the schematics can be revised and edited, but I think they form a neat starting point for new researchers and a helpful quick reminder for those already immersed in the field. I have chosen the particular schematic in [Figure 41.10a](#) [17] because at first glance it looks complex and daunting, but what I think it also reveals is that we need to look at the whole picture and not isolated elements. The node of Ranvier is a highly specialised structure with a critical role in nerve conduction. It is a known site of antibody binding, disruption and injury in GBS, but the specific mechanisms are yet to be revealed. With the recent emergence of antibodies that bind nodal proteins in GBS patient sera, the interest in this structure is even greater. Therefore, this figure can be used to guide us when we study antibody-nodal protein interactions and remind us that changes to one component will influence many other parts.

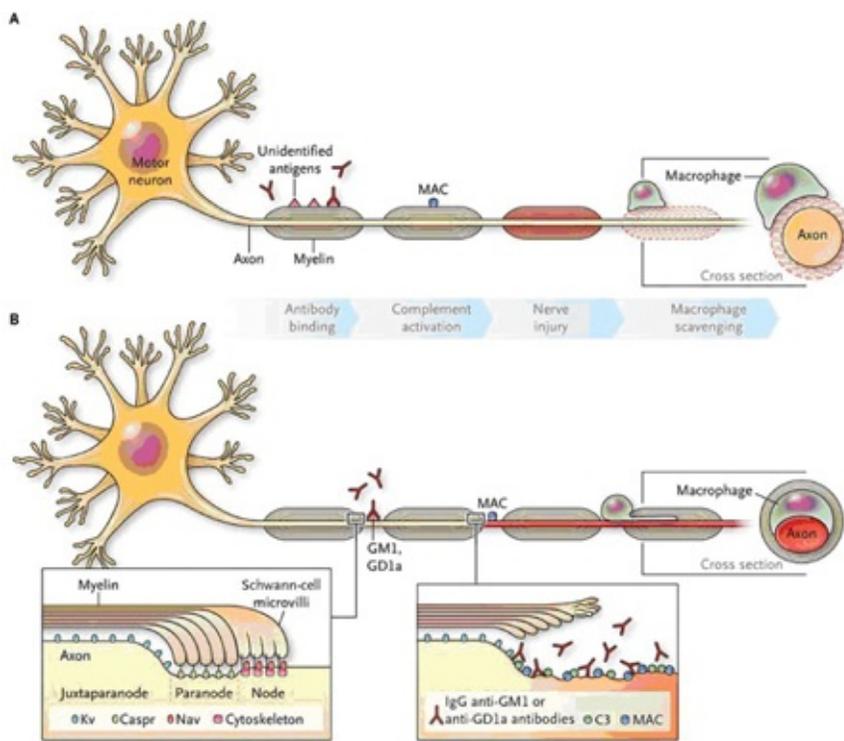


Figure 41.10b Possible immunopathogenesis of the Guillain-Barré syndrome

Panel A shows the immunopathogenesis of acute inflammatory demyelinating polyneuropathy. Although autoantigens have yet to be unequivocally identified, autoantibodies may bind to myelin antigens and activate complement. This is followed by the formation of membrane-attack complex (MAC) on the outer surface of Schwann cells and the initiation of vesicular degeneration. Macrophages subsequently invade myelin and act as scavengers to remove myelin debris. Panel B shows the immunopathogenesis of acute motor axonal neuropathy. Myelinated axons are divided into four functional regions: the nodes of Ranvier, paranodes, juxtapanodes, and internodes. Gangliosides GM1 and GD1a are strongly expressed at the nodes of Ranvier, where the voltage-gated sodium (Na_v) channels are localized. Contactin associated protein (Caspr) and voltage-gated potassium (K_v) channels are respectively present at the paranodes and juxtapanodes. IgG anti-GM1 or anti-GD1a autoantibodies bind to the nodal axolemma, leading to MAC formation. This results in the disappearance of Na_v clusters and the detachment of paranodal myelin, which can lead to nerve-conduction failure and muscle weakness. Axonal degeneration may follow at a later stage. Macrophages subsequently invade from the nodes into the periaxonal space, scavenging the injured axons. Reproduced with permission from [18].

Possible Pathogenesis of the Guillain-Barré Syndrome

Schematics themselves have become more and more sophisticated over the years, but the general principles hold true. I wanted to include [Figure 41.10b](#) [18] as I think the image is a neat point to work from and also to refer back to whenever new discoveries emerge.

Conclusion

These 10 images are just the tip of the iceberg of influential images in the GBS field. There has been such a diverse array of incredible images in just 100 years. With all of the advances in imaging and labelling technology, what will the next centenary of GBS research generate?

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ANIMAL MODELS

Animal Models: Anti-Ganglioside Antibodies as Causative Factor for GBS

Susumu Kusunoki

Introduction

Guillain-Barré syndrome (GBS) is an acute, self-limited peripheral neuropathy often preceded by an infection. The pathogenetic mechanism of GBS is considered to be autoimmunity. Antibodies against glycolipids, including gangliosides, have been reported to be present in around 60% of the sera from GBS patients in the acute phase. They are actually useful diagnostic markers of GBS. In contrast, it was an issue of debate whether anti-ganglioside antibodies were directly involved in the pathogenesis of GBS. Now, the pathogenetic roles are recognised, at least for some of the anti-ganglioside antibodies, because the neuropathic effects of those antibodies have been shown in in vivo animal models. In this chapter, 10 papers regarding the animal models of anti-ganglioside antibody-mediated neuropathy are selected and briefly introduced ([Table 42.1](#)).

Nagai Y, et al. Ganglioside syndrome, a new autoimmune neurologic disorder, experimentally induced with brain gangliosides. *Neuroscience Letters*, 1976

Nagai and colleagues investigated the modulation of the immunologic response to myelin basic protein (MBP) by the formation of a conjugate of MBP and acidic sphingoglycolipids [1]. During the course of their investigations, they noticed that rabbits intensively immunised with total brain gangliosides sometimes developed neurologic symptoms and signs. They then immunised rabbits with purified gangliosides. As a result, 4 of the 8 rabbits which had been injected with GD1a ganglioside developed neurological symptoms and signs. When they immunised rabbits with GM1 ganglioside, the rabbits were also affected with neurological disease, although the rate of induction of neurological problems was lower than it had been with GD1a. The rabbits injected with GM1 had high titres of antibody activities to GM1, but those injected with GD1a produced a small or negligible amount of antibody activities. This paper is the first to describe the animal model with robust neurological symptoms and signs with the sensitisation with ganglioside. However, descriptions of the neurological symptoms and signs, the serological examinations, and pathological investigations were not sufficient enough to evaluate in detail. They described that the rabbits injected with GD1a ganglioside developed a state of rigid or spastic paralysis whereas those injected with GM1 ganglioside developed flaccid paralysis. If so, the rabbits

injected with GD1a ganglioside might develop a central nervous system disease. But, in any case, it can be said that this work paved the way to the later research of autoimmune neuropathies with anti-ganglioside antibodies.

Table 42.1 Animal model of autoimmune neuropathy mediated by anti-glycolipid antibodies

| Target | Animal | Type ^a | Sensitisation | Clinical feature | Reference(s) |
|-------------------------|--------------|-------------------|------------------|---|---------------|
| GM1 or GD1a | rabbit | ? | active | limb paralysis | (1) |
| GalC | rabbit & rat | D | active & passive | motor & sensory neuropathy | (2), (3) |
| GD1b | rabbit | A | active | sensory ataxic neuropathy | (4), (5), (8) |
| BBG ^b or GM1 | rabbit | A | active | motor neuropathy | (6), (7) |
| GD1a | mouse | A | passive | ? | (9) |
| GQ1b | mouse | A | passive | motor neuropathy respiratory insufficiency | (10) |

^a D: demyelinating, A: axonal

^b BBG: bovine brain gangliosides

Saida T, et al. Experimental allergic neuritis induced by sensitization with galactocerebroside. *Science*, 1979

Saida and colleagues immunised rabbits with galactocerebroside (GalC), a major glycolipid in central and peripheral nervous systems [2]. They reported that 13 of 31 sensitised rabbits showed flaccid paresis and hypesthesia of 4 limbs. Some of the rabbits showed respiratory paresis. Electrophysiologically, multifocal conduction block and reduction of motor conduction velocities were revealed. Pathological findings showed multifocal demyelinating lesions with macrophage infiltration in the peripheral nervous system, primarily in spinal ganglia, roots, and cauda equina. This is the first established animal model of autoimmune neuropathy in which glycolipid is the target molecule.

Saida K, et al. In vivo demyelination induced by intraneural injection of anti-galactocerebroside serum: a morphologic study. *American Journal of Pathology*, 1979

Saida and colleagues performed intraneural injection of rats with rabbit anti-GalC serum and found that there were demyelinating changes followed by macrophage infiltration [3]. Demyelinating activity of the antiserum was removed by preincubation with GalC and was lost when the serums were heated at 56 degrees centigrade for 30 minutes, indicating that the pathogenetic mechanism was anti-GalC antibody-dependent and complement-mediated. Although the pathogenetic roles of anti-GalC antibodies were shown in this work, clinical relevance was not fully recognised at that time because a significant association between anti-GalC antibody and human diseases had not been reported. It was not until 1995 that the presence of anti-GalC antibodies in GBS subsequent to mycoplasma infection was first reported [4].

Kusunoki S, et al. Experimental sensory neuropathy induced by sensitization with ganglioside GD1b. *Annals of Neurology*, 1996

Even after the publication of the animal model of demyelinating neuropathy caused by anti-GalC antibody, the role of anti-ganglioside antibodies in the pathogenesis was still a matter of controversy because there was no established animal model induced by gangliosides. One reason for the difficulty of development of the animal model was the differences in the distribution of individual gangliosides from species to species. It was reported that antibody activities to gangliosides with disialosyl residue, including GD1b, were associated with sensory ataxic neuropathy. Kusunoki and colleagues found that GD1b was densely localised in the large neurons in human dorsal root ganglia. Because such localisation of GD1b ganglioside was also seen in rabbits, they hit upon an idea that the effective animal model would be developed by the sensitisation of the rabbits with GD1b ganglioside. They then immunised rabbits with GD1b ganglioside and succeeded in developing the animal model with robust neurological signs [5]. Muscle power in these rabbits was intact but the rabbits showed awkward movements. Pathologically, axonal degeneration was observed in the dorsal root and dorsal column of the spinal cord, whereas the ventral root was completely intact. Therefore, the rabbits were diagnosed with sensory ataxic neuropathy both clinically and pathologically. This rabbit model, GD1b-induced sensory ataxic neuropathy (GD1b-induced SAN), was the first established animal model of autoimmune neuropathy targeting ganglioside. The titres of the anti-GD1b antibodies were increased. In contrast, no lymphocytic infiltration was observed in the affected areas, indicating that the autoantibodies play the crucial roles in this animal model.

Kusunoki S, et al. Monospecific anti-GD1b IgG is required to induce rabbit ataxic neuropathy. *Annals of Neurology*, 1999

In Kusunoki and colleagues' 1999 study, the incidence of GD1b-induced SAN was about 50% [6]. One reason for that, they reasoned, might be differences in the fine specificity of the antibodies. Injection with GD1b ganglioside could cause the production of the antibodies to GM1 as well as GD1b because those 2 gangliosides share Gal-GalNAc residue. Using the GM1-affinity column, the antibodies specific to GD1b gangliosides could be separated from those binding to both GD1b and GM1. The authors then examined the titres of the antibodies specific to GD1b and found that the titres were significantly higher in the affected rabbits than in the unaffected ones. This shows that the antibodies specific to GD1b are required to induce sensory ataxic neuropathy in rabbits. It was reported in another paper that passive transfer of the antiserum caused neuropathological changes similar to the GD1b-induced SAN [7]. Taken together, the antibodies specific to GD1b ganglioside should be the main causative factor in this animal model. The association between the antibody activities highly specific to GD1b and the ataxia in GBS has later been shown by the investigation of the acute-phase sera from GBS patients [8].

Yuki N, et al. Animal model of axonal Guillain-Barré syndrome induced by sensitization with GM1 ganglioside. *Annals of Neurology*, 2001

Anti-GM1 IgG antibody is frequently detected in GBS subsequent to *Campylobacter jejuni* infection [9]. GBS subtypes of those cases are predominantly acute motor axonal neuropathy (AMAN) type. There was a controversy over whether or not immunisation of the animals with GM1 ganglioside would cause

neurological problems. Yuki and colleagues sensitized rabbits with bovine brain ganglioside mixture (BBG) containing GM1 or purified GM1 according to the procedure of GD1b-induced SAN model [10]. Results showed that all of the 13 rabbits inoculated with BBG and 9 of the 11 rabbits inoculated with purified GM1 developed acute motor neuropathy. The rabbits inoculated with BBG had anti-GM1 antibodies. Pathological findings showed axonal degeneration with neither lymphocytic infiltration nor demyelination. This study indicated that anti-GM1 antibody is a causative factor and this model should be a useful animal model for investigation of AMAN. The authors used functional grades (FG) from 0 (normal) to 5 (dead). In BBG-inoculated rabbits, 9 of 13 rabbits showed FG of 4 (showing severe weakness of the 4 limbs, which were spread out) at the nadir and one rabbit showed FG of 3 (showing moderate weakness of the 4 limbs and unable to walk). Therefore 10 of the 13 rabbits were unable to walk at the nadir. In contrast, in GM1-inoculated rabbits, only 3 of 11 rabbits were FG of 3 or more (2 were FG of 5). This suggests that BBG may be a more effective agent for the induction of this animal model than purified GM1.

Susuki K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *The Journal of Neuroscience*, 2007

In this study, Susuki and colleagues investigated the molecular organisation at the nodes of Ranvier immunohistochemically, using the BBG-induced motor axonal neuropathy model described above [11]. In the acute phase, voltage-gated sodium channel clusters were disrupted or disappeared at the nodes. The deposition of IgG and complement products were observed. Pathological changes were also detected in paranodal axo-glial junctions. This paper revealed the precise molecular pathogenetic mechanisms of BBG-induced motor axonal neuropathy and clearly showed that it was a complement-mediated disease. The results shown in this report should give us very important clues to elaborating novel therapeutic methods for AMAN.

Takada K, et al. Apoptosis of primary sensory neurons in GD1b-induced sensory ataxic neuropathy. *Experimental Neurology*, 2008

In a GD1b-induced SAN model, axonal degeneration with macrophage infiltration was observed in the dorsal root and in the dorsal column of the spinal cord. However, in spite of the looseness of the blood-nerve barrier in DRG, there were no significant pathological changes in DRG. It was possible that apoptosis of the large sensory neurons occur in this animal model. To find out, Takada and colleagues performed a TUNEL assay of the DRG from the affected rabbits and discovered that a subset of neurons were TUNEL positive [12]. Anti-caspase 3 antibody also immunostained some of the DRG neurons. TUNEL positivity was found in the neurons with large diameter. Therefore, apoptosis of the large primary sensory neurons subsequent to the binding of the GD1b-specific antibodies should be one important pathogenetic mechanism of GD1b-induced SAN. In addition to complement-mediated membrane attack, apoptotic mechanisms after the binding of the antibodies should be considered for the possible mechanism of the anti-ganglioside antibody-mediated neuropathies or neuronopathies.

Sheikh KA, et al. An anti-ganglioside antibody-secreting hybridoma

induces neuropathy in mice. *Annals of Neurology*, 2004

In order to develop passive transfer model of anti-ganglioside antibody-mediated neuropathies, Sheikh and colleagues performed intraperitoneal implantation of hybridoma-secreting anti-ganglioside antibodies, reacting with GD1a ganglioside, in mice [13]. As a result, approximately half the animals implanted with the hybridoma developed a patchy, predominantly axonal neuropathy in a small proportion of nerve fibres. Passive transfer with systemically administered antibodies did not cause such pathological changes. The authors discussed whether hybridoma implantation might make the blood-nerve barrier leaky. Even in the mice implanted with the hybridoma, neuropathological changes were mild. This study showed that, in addition to the antibodies, other factors such as antibody accessibility were important. It also indicated that mouse nerves might be resistant to anti-ganglioside antibody-mediated injury.

Halstead SK, et al. Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. *Brain*, 2008

Dr Hugh Willison's group reported that anti-GQ1b antibodies were able to bind and disrupt presynaptic motor nerve terminals at the neuromuscular junction (NMJ) using mouse hemi-diaphragm model [14]. Based on this finding, Halstead and colleagues injected balb/c mice intraperitoneally with anti-GQ1b antibody, followed by an intraperitoneal injection of normal human serum. Mice treated as described above showed breathing difficulties [15]. Anti-GQ1b antibodies are detected in about 90% of the sera from patients with Miller Fisher syndrome (MFS) in the acute phase. Breathing difficulty is not observed in MFS. However, when anti-GQ1b antibody is positive in patients with GBS with ophthalmoplegia, the patients more frequently need mechanical ventilation than anti-GQ1b-negative patients [16]. Therefore, this mouse model is a nice model of GBS with respiratory insufficiency. Halstead and colleagues reported that eculizumab, which blocks the formation of human C5a and C5b-9, protected mice from respiratory paralysis in this model by preventing complement-mediated damage at the NMJ. This work provides us with the rationale for the clinical trials of eculizumab for GBS. The clinical trials of eculizumab are currently underway at time of writing. If they show good results, we can obtain a novel therapeutic method for GBS, especially intractable cases.

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Experimental Autoimmune Neuritis and Spontaneous Autoimmune Polyneuropathy

Betty Soliven

Introduction

Animal models, in spite of their limitations, are essential research tools for deciphering disease mechanisms in autoimmune diseases. The use of animal models has facilitated the discovery of new susceptibility genes, detailed immunologic, and morphologic and functional studies as well as the development of novel therapeutic strategies. Within the context of Guillain-Barré syndrome and other autoimmune neuropathies, these models include experimental autoimmune neuritis (EAN), antibody (Ab)-mediated experimental autoimmune neuropathy and spontaneous autoimmune polyneuropathy (SAP). As the crucial role of antibodies (Abs) against glycolipids in GBS will be discussed elsewhere, we will focus on lessons learnt from EAN and SAP in this chapter.

Experimental Autoimmune Neuritis (EAN)

Waksman BH and Adams RD. Allergic neuritis: an experimental disease of rabbits induced by the injection of peripheral nervous tissue and adjuvants. *Journal of Experimental Medicine*, 1955; Aström KE, Webster HD and Arnason BG. The initial lesion in experimental allergic neuritis. A phase and electron microscopic study. *Journal of Experimental Medicine*, 1968; Asbury AK, Arnason BG and Adams RD. The inflammatory lesion in idiopathic polyneuritis. Its role in pathogenesis. *Medicine*, 1969

The Discovery of EAN and the Role of Cell-Mediated Immunity

EAN was first induced in rabbits by Waksman and Adams in 1955 by immunization with sciatic-nerve homogenates with Freund's adjuvants [1]. After a latent period of about 2 weeks, animals developed weakness and ataxia that reached their maximum in 3 days, with most of them recovering within the next 6 days. Aside from the typical albumino-cytologic dissociation in the CSF, these animals exhibited lesions in nerve roots, spinal ganglia and peripheral nerves that were characterized by perivascular infiltration with mononuclear cells, segmental demyelination and varying degrees of axonal degeneration. Subsequent successful transfer of disease with lymphocytes from immunized animals and reports on similar autopsy findings of 19 fatal cases of human GBS had led to conclusions that cellular immunity to myelin components is the underlying culprit in GBS [2–4]. Of note, Dr Waksman has also been recognized for his

pioneering work on circulating T lymphocytes as well as the role of thymus in immune response and tolerance.

Brostoff SW, Levit S and Powers JM. Induction of experimental allergic neuritis with a peptide from myelin P2 basic protein. *Nature*, 1977

Although myelin P0 is the most abundant myelin protein in the PNS, the first myelin peptide used to induce EAN was a 21 amino acid P2 peptide from the NH₂ terminal [5]. This was followed by studies on EAN induced by other myelin proteins such as myelin P0 and PMP22. In spite of a failure to translate to successful identification of antigenic targets of autoreactive T cells in GBS, EAN has provided valuable information such as the role of costimulatory signals, adhesion molecules and various cytokines, as well as mechanisms of injury. We have learnt that EAN is alleviated in CD28-deficient C57BL/6 mice immunized with P0 peptide 180–199 [6]. Recovery from EAN is associated with M2 milieu promoted by IL-10 and IL-4 [7]. The crucial role of macrophages in EAN is also demonstrated by altering the balance of M1/M2 macrophages in TNF- α knockout (KO) mice [8]. B cells contribute to the pathogenesis of EAN via CD40L-CD40 interactions, regulatory mechanisms and production of auto-Abs. B cells play a suppressive role during the induction of EAN, but enhance the severity of EAN during peak disease [9,10]. The latter may be due to auto-Abs to multiple myelin proteins and/or gangliosides in EAN induced by peripheral nerve myelin, which could contribute significantly to demyelination or conduction failure after disruption of the blood nerve barrier by myelin-reactive T cells.

Novakovic SD, et al. Disruption and reorganization of sodium channels in experimental allergic neuritis. *Muscle Nerve*, 1998; Lonigro A and Devaux JJ. Disruption of neurofascin and gliomedin at nodes of Ranvier precedes demyelination in experimental allergic neuritis. *Brain*, 2009

Mechanisms of Conduction Failure in EAN: Demyelination vs Nodal-Paranodal Dysfunction

Weakness in GBS and EAN arises from either axonal loss or conduction failure. Conduction failure in demyelinated fibres occurs as a consequence of capacitance impedance mismatch, leakage of currents and exposure of internodal K⁺ channels. However, conduction failure in EAN and GBS often precede the onset of demyelination, and is attributed to paranodal retraction and disruption of nodal Na⁺ channels [11,12].

Whether axonal or demyelinating features predominate in EAN depends on the disease severity and method of disease induction. Adoptive transfer of P2-reactive T cell lines in Lewis rats led to axonal dysfunction or degeneration with minimal demyelination, whereas adoptive transfer of myelin-sensitized lymph node cells (T cells and B cells) led to prominent demyelinating features [13]. Interestingly, disappearance of adhesion molecules neurofascin 186 and gliomedin, thought to be mediated by Abs against these proteins, was observed in EAN induced by peripheral myelin, but not in EAN induced by P2 [12]. These findings indicate that the antigenic targets of immune attack extend to nodal and paranodal proteins and is not restricted to myelin proteins. Indeed, immunization with gliomedin induces a progressive neuropathy that is characterized by nodal disruption and demyelination [14]. In addition, antibodies to neurofascin cause exacerbation and prolongation of adoptive transfer EAN [15]. Taken together, these results suggest that early conduction failure in EAN and GBS is due to nodal/paranodal dysfunction, which can be reversed by plasmapheresis or intravenous immunoglobulin (IVIg) before

demyelination and axonal degeneration develop.

EAN: Development of Therapies

Although T cells dominated the fields of EAN and GBS research for three decades, anti-myelin Abs in sera from EAN and GBS had been demonstrated, albeit not correlating well with clinical severity. A possible role of Abs or other serum factors in the pathogenesis of EAN is supported by the work of Antony and colleagues demonstrating the beneficial effect of plasma exchange in rabbits when given prior to the establishment of clinical disease [16]. The effectiveness of IVIg as a treatment of EAN was published in 1997, the same year that results of the Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial were published in *The Lancet* [17,18]. The study by Gabriel and colleagues demonstrated that the Ig treatment from disease onset was more beneficial than treatment from immunization and was associated with a decrease in rat anti-myelin Abs [17]. Miyagi and colleagues found that the intact IVIg, but not F(ab')₂ ameliorated the clinical course of EAN induced by P2 [18].

Because of the natural history of GBS and the favourable response to intravenous Ig and plasma exchange, many studies on experimental therapeutics in EAN were not translated to clinical trials or use for this disorder. I was involved in a study demonstrating the suppressive effect of a β 2 adrenergic agonist terbutaline on EAN in Lewis rats even when given after disease onset [19]. The immunomodulatory effect of cAMP-elevating agents in EAN was demonstrated by another agent, Rolipram, a phosphodiesterase-4 inhibitor, which induced downregulation of interferon- γ and pro-inflammatory chemokines, and upregulation of interleukin 4 [20]. But no clinical trials on cAMP-elevating agents ensued from these investigations. On the other hand, studies on the role of complement activation in EAN and related animal models have led to an ongoing clinical trial of eculizumab in GBS [21,22]. Fingolimod, an S1P receptor modulator studied in EAN and B7-2 knockout non-obese diabetic (NOD) mice, is currently being investigated in a clinical trial of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) [23, 24].

Spontaneous Autoimmune Polyneuropathy (SAP) in the NOD Mouse Model

Salomon B, et al. Development of spontaneous autoimmune peripheral polyneuropathy in B7-2-deficient NOD mice. *Journal of Experimental Medicine*, 2001; Kim HJ, et al. Targeting of myelin protein zero in a spontaneous autoimmune polyneuropathy. *Journal of Immunology*, 2008; Su MA, et al. Defective autoimmune regulator-dependent central tolerance to myelin protein zero is linked to autoimmune peripheral neuropathy. *Journal of Immunology*, 2012; Meyer zu Horste G, et al. Thymic epithelium determines a spontaneous chronic neuritis in Icam1(tm1Jcgr)NOD mice. *Journal of Immunology*, 2014

Most human autoimmune diseases occur spontaneously in genetically susceptible individuals, albeit with some exogenous triggers that are often unknown or not easily identified. In animals, a naturally occurring model of spontaneous, organ-specific autoimmunity is the NOD mouse, which exhibits increased susceptibility to multiple autoimmune diseases, including type 1 diabetes, thyroiditis, sialadenitis, gastritis, and inflammatory neuropathy.

Disease manifestations in NOD mice can vary, depending on the cytokine and costimulatory milieu. The B7-1/B7-2:CD28/CTLA4 costimulatory pathway is critical to the regulation of lymphocyte activation and homeostasis of regulatory T cells (Tregs) [25,26]. The B7-2 knockout NOD mouse was originally

generated in Dr Bluestone's laboratory to study the role of costimulatory molecules in type 1 diabetes. He was still at the University of Chicago at that time. One of his postdocs, Dr Salomon, observed that elimination of B7-2 (CD86) in NOD mice led to protection against type 1 diabetes but triggered the development of progressive weakness at 6 to 7 months of age. I was asked to help with the analysis of the clinical phenotype. Electrophysiological and histological studies revealed features of demyelination and axonal loss, and inflammatory infiltrates in sciatic nerves and dorsal root ganglia, as can be seen in progressive form of CIDP [24,27]. I was intrigued by the concept that elimination of one molecule can alter the manifestation of autoimmune disease in susceptible animals. Because my main research interest at that time was in oligodendrocyte and Schwann cell biology rather than neuroimmunology, I did not pursue further investigations until 6 years later. Using different approaches, both Bluestone's group, at University of California San Francisco, and my group, at the University of Chicago, found that myelin P0 is the antigenic target in SAP in NOD mice. SAP is mediated by IFN- γ -secreting Th1 cells that are reactive against myelin P0, and at least 2 pathogenic P0 epitopes are involved, P0 (1–25) and P0 (180–199) [28–30]. Myelin P0 is an Aire-regulated, tissue-specific self-Ag in the thymus. Maureen Su and colleagues found that Aire-deficient mice have increased autoreactivity against P0 and develop autoimmune neuropathy that is mediated by IFN- γ similar to that observed in B7-2 knockout NOD mice [31].

Aside from T cells, sera from SAP mice contain Abs to P0, which may contribute to peripheral nerve injury [29]. We found that depletion of B cells and plasmablasts with anti-CD19 Ab led to the attenuation of SAP [32]. SAP is also attenuated by S1P receptor modulators such as Fingolimod and SEW2871 [24]. More recently, we have demonstrated that B7-2-deficient dendritic cells (DCs) exhibit impaired capacity to induce tolerance to P0, which can be rescued by preconditioning with IL-10 [33]. Another way to overcome impaired DC function is by transduction with lentiviral vectors expressing vasoactive intestinal peptide (LV-VIP-BCD11b+ DCs), which delays the onset of disease and attenuates clinical severity in SAP [34].

Of note, P0 is expressed not only in the PNS myelin but also by peri-islet Schwann cells, suggesting a potential mechanism linking the islet and PNS autoimmunity [29]. The shift from islet to PNS autoimmunity is also observed in intercellular adhesion molecule1 (ICAM-1) deficient NOD mice, and is due to altered thymic selection rather than loss of adhesive activity of ICAM-1. In contrast to B7-2 KO NOD mice, ICAM-1 deficient NOD mice exhibit Th17 bias instead of Th1 bias [35].

Overall, these studies demonstrate that a progressive autoimmune neuropathy mimicking CIDP can be triggered by changes in costimulatory molecules/pathways. It is tempting to speculate that similar mechanisms occur in humans in that GBS is monophasic in most patients but can switch to relapsing and remitting CIDP or progressive CIDP when regulatory mechanisms are impaired due to altered costimulatory or cytokine milieu.

Conclusions

Based on time course, EAN would be considered more relevant to GBS while SAP would be more pertinent to CIDP. The popularity of EAN induced by PNS myelin or myelin protein has been challenged to some extent by the discovery of anti-glycolipid Abs in GBS variants, and by successful establishment of animal models of AMAN and ataxic neuropathy in rabbits and mice. Nonetheless, EAN and SAP have facilitated investigations on the pathogenicity of PNS-reactive T cells and auto-Abs against PNS antigens, as well as regulatory mechanisms pertinent to disease development. In addition, these models have been used for the development of novel therapeutic strategies.

There are many investigators who have contributed significantly to the advancement of our understanding of pathogenesis of GBS. Within the context of EAN and SAP, my top 10 publications were chosen based on their impact or implications on the field of neuroimmunology of PNS. The three early publications by investigators at Harvard (Waksman, Adams, Arnason, Asbury and Webster) led to the decades of emphasis on the role of PNS-reactive T cells and other effector mechanisms in GBS. Brostoff and colleagues were the first to show that EAN can be induced by a peripheral myelin peptide. The pathology of EAN consists of inflammatory infiltrates, demyelination and varying degrees of axonal degeneration. The realization that nodal and paranodal dysfunction plays an important role in the pathophysiology of EAN is highlighted by the work of Shrager's group, and Lonigro and Devaux. Last but not the least, studies on SAP by different groups of investigators (Bluestone's, Soliven's, Su's, Keiseier's) have illustrated the complexity of regulatory mechanisms (thymic selection, Tregs, DCs), and how elimination of a single molecule could shift the manifestations from one disease phenotype to another in susceptible animals or individuals. No doubt, we have come a long way in our understanding of GBS, CIDP and other inflammatory neuropathies, though some mysteries remain to be unravelled in the future.

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Spontaneous Models of Guillain-Barré Syndrome in Animals

Angie Rupp

Introduction

Spontaneous conditions similar to those of Guillain-Barré syndrome (GBS), leading to acute ascending paralysis and associated with polyradiculoneuropathies of unknown origin, have been reported in a number of different animal species. Unfortunately, in many cases, comparison and interpretation of these reports is challenging, since depending on when these examinations were conducted and whether the animals survived, the extent and modality of investigations carried out often varies.

The majority of descriptions focus on dogs, cats and chickens. Individual case reports include the description of a chimpanzee with acute onset of ascending, symmetrical, monophasic flaccid paralysis and high protein in the CSF, possibly associated with a preceding rabies vaccination [1], and polyradiculoneuritis with mononuclear cell infiltrates, demyelination and Schwann cell proliferation in a 6-week-old goat [2].

Dogs: The Early Investigations into Coonhound Paralysis

In 1954 a disease resembling GBS in dogs was described by Kingma and Catcott and termed ‘coonhound paralysis’, since for induction of the disease the bite (or even only a scratch) of a racoon was considered to be necessary. However, it was not until the late 1960s to 1980s, that coonhound paralysis underwent extensive further examinations on both the clinical and experimental level and was discussed as a potential canine equivalent to GBS [3].

Initially this condition was predominantly seen as an occupational hazard in ‘coonhounds’—dogs that hunt racoons, such as Redbone hounds, Walker hounds and Black and Tan hounds and other large-breed dogs—which had experienced a close encounter with a racoon resulting in a bite (Figure 44.1). Within 7–14 days of being bitten by a racoon, these dogs exhibited an acute, symmetrical, ascending paralysis, occasionally also involving the tail, neck and trunk. Hyperaesthesia, involvement of cranial nerves, death (presumed to be caused by respiratory failure) and multiple episodes after repeated bites were also observed. It does, however, need to be noted, that only a few dogs bitten by racoons actually develop coonhound paralysis.

The detailed histological investigations conducted in dogs succumbing to the disease revealed the most striking changes to be in the ventral roots (Figure 44.2) with lumbosacral areas more involved than thoracic or cervical areas, and the myelin sheaths affected more than the axons. The extent of changes

varied in peripheral nerves; however, both in nerves and nerve roots predominantly large-diameter nerve fibres were affected. The myelin damage exhibited a segmental pattern with the myelin appearing either swollen or pale, or fragmented into globules. Complete loss of myelin resulted in empty Schwann cell sheaths. Associated axons ranged from appearing quite normal to undergoing degeneration. In other cases, the axons were more frequently involved when compared to the myelin sheaths [4]. The intraneural inflammatory infiltrates varied both in intensity and quality between the different dogs assessed and consisted of macrophages, plasma cells, lymphocytes and variable numbers of neutrophils, with severity of the infiltrates reflecting the extent of the damage. Prominent infiltrates invariably were associated with myelin and axonal damage; however, swelling and breakdown of myelin also could be observed in areas of sparse or absent inflammation. Similar to reports in GBS, macrophages were present within the periaxonal spaces.



Figure 44.1 Redbone coonhound in the recovery phase of coonhound paralysis

Note the marked muscle atrophy and decubital ulcers acquired during the paralytic phase of disease. Reproduced with permission from [21].

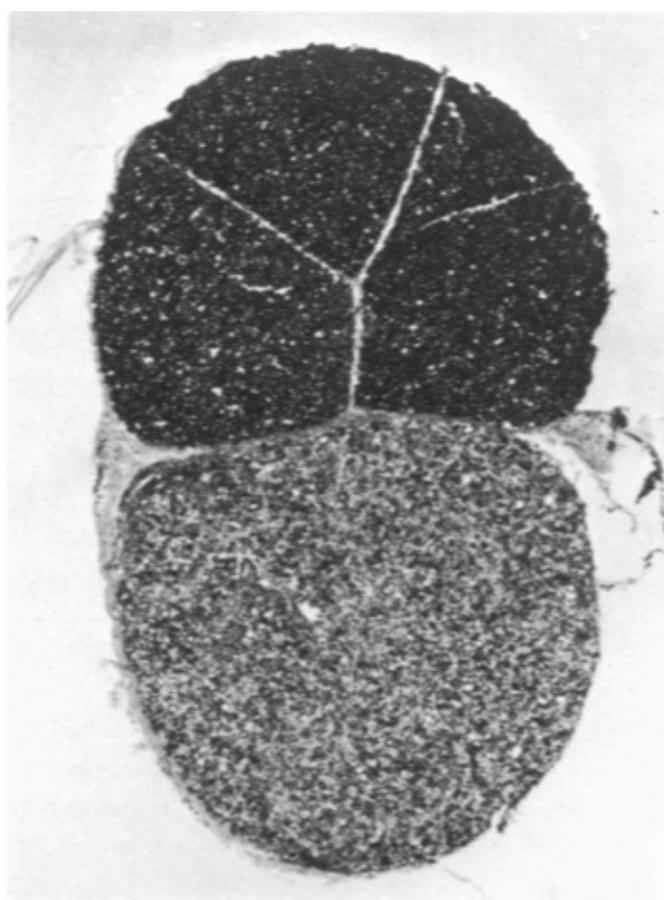


Figure 44.2 Transverse section of a lumbar spinal nerve from a dog with coonhound paralysis stained with Luxol fast blue (myelin stain).

The ventral root (lower half) exhibits marked demyelination, whilst the upper half (dorsal root) is considered within normal limits. Reproduced with permission from [21].

The cerebrospinal fluid exhibited variable protein levels, with elevations more consistently observed following lumbar puncture when compared to cisternal puncture [4].

Roughly the same time as these very detailed examinations into coonhound paralysis were carried out, further investigations by other groups examining dogs with similar clinical presentation in which, however, a preceding raccoon bite was not reported or could be excluded, indicated that a raccoon encounter did not appear to be necessary to induce a disease of great similarity with coonhound paralysis [5].

Dogs: Current Perspectives and Clinical Implications

Currently, acute canine polyradiculoneuritis (ACP) is subclassified into coonhound paralysis, idiopathic polyradiculoneuritis and post-vaccination polyradiculoneuritis, the latter of which is very rare. Most dog breeds, ranging from toy to large breeds and including cross-breeds, may be affected. However, despite representing the most common acute canine polyneuropathy, ACP is only observed sporadically.

Clinically, ACP dogs show a short-strided and stiff gait, which within 2 to 4 days progresses to lower motor neuron tetraparesis or flaccid paralysis [6]. Symptoms usually start in the hind limbs and then progress to the forelimbs; the progressive phase tends to last for roughly 5 days, occasionally longer. Some dogs continue to exhibit voluntary movement of all 4 limbs throughout the disease, whilst others suffer complete paralysis of limbs and neck, and may experience respiratory compromise, requiring mechanical ventilation. Muscle atrophy develops within 7 to 10 days and hypo- or areflexia, a decrease

or loss of muscle tone and in many cases hyperesthesia of the limbs and trunk are noted. Owners often recognize a change or loss in bark and some dogs develop bilateral facial paralysis and tongue weakness. Generally, however, dogs remain alert and responsive throughout the disease, continue to eat and drink normally and in the vast majority of cases remain in control of their urination and defecation and even continue to wag their tail.

In electrophysiological investigations, ACP dogs exhibit signs both for demyelinating and axonal neuropathies, combined with evidence of muscle denervation (Figure 44.3). Some investigations have revealed demyelinating and axonal changes in the ventral roots, with a prominent axonopathy overshadowing the demyelination in the peripheral nerves [7]. Prolonged F-wave latencies or unrecordable F waves are seen as early as 4 days from disease onset.

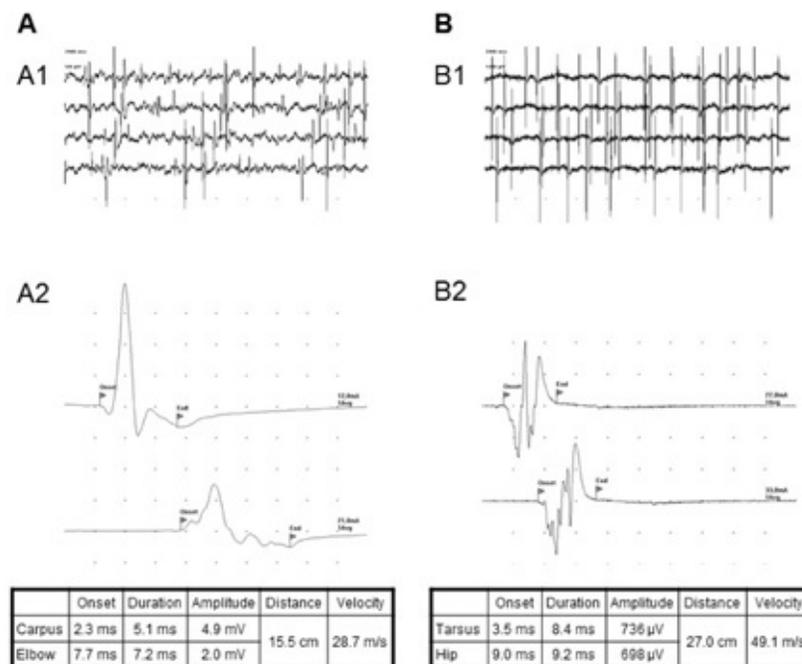


Figure 44.3 Electrophysiological examinations of two ACP dogs, both of which exhibited anti-GM2 Abs

A1/B1 spontaneous activity in electromyographic assessments of the tibial cranial muscle; A2/B2 motor nerve conduction studies of ulnar (A2) and sciatic/tibial (B2) nerve. Note the reduction of CMAP amplitude and CMAP dispersion in the 2nd trace of dog A. This dog also exhibited a reduced MNCV (28.7 m/s). Dog B exhibited a vast reduction of its CMAP amplitude in both traces (< 1 mV) with a MNCV (49.1 m/s) at the lower end of the physiological reference range.

Divisions on the abscissa are 2 ms (A2) and 5 ms (B2). Divisions on the ordinate are 1 mV (A2) and 200 μ V (B2). Reproduced with permission from [9].

Nerve biopsies exhibit no changes or mildly reduced myelinated fibre density, occasional myelin ovoids and mild inflammatory infiltrates, whilst muscles exhibit changes consistent with denervation atrophy [5,8].

Routinely, ACP patients receive no treatment and all efforts are put into supportive care and rehabilitation. Dogs need to be turned every few hours to prevent pressure sores, and ventilated and hand fed if necessary. Intensive physiotherapy in the form of passive range-of-motion exercises, massages, encouraging the dog to ambulate with support (in a sling or cart), hydrotherapy (swimming) and walking on a treadmill aim to minimize muscle atrophy, help rebuild muscle and keep the joints mobile. Most dogs make a complete recovery, which can take between 3 weeks and a half a year [6]. However, depending on the disease severity and the degree of muscle atrophy, some dogs may retain residual deficits. Death due to respiratory paralysis or concurring pneumonia occurs in roughly 10% of ACP patients [8,9]. Similar to

GBS patients and the early reports of coonhound paralysis, multiple episodes of ACP are possible [3,5,8].

Considering the clinical, electrophysiological and pathological findings, the latter obtained in the early investigations into coonhound paralysis, ACP indeed in many respects needs to be considered strikingly comparable to GBS.

Dogs: Possible Pathophysiology and Aetiological Link to GBS

Potential antecedent events in ACP include a recent upper respiratory or gastrointestinal tract infection, vaccination or the bite of a raccoon. However, host factors also are considered to contribute to disease susceptibility since in a number of cases related animals have been affected and early investigations into coonhound paralysis revealed that the induction of lesions and early clinical signs following inoculation of dogs with canine sciatic nerve emulsified in Freud's complete adjuvant (experimental allergic neuritis) could only be induced in offspring from coonhound-type dogs who had recovered from coonhound paralysis [10]. Similarly, the reproduction of coonhound paralysis with raccoon saliva had only been possible in dogs who had previously experienced naturally occurring coonhound paralysis [11].

More recent investigations were able to provide a potential aetiological and pathophysiological link to GBS by examining the incidence of serum anti-ganglioside antibodies (Abs) in a small Italian cohort of ACP dogs, local control dogs and dogs with idiopathic epilepsy. Here, 15 out of 25 ACP dogs, 1 of 19 control dogs and 0 of 15 dogs with idiopathic epilepsy exhibited such Abs, the vast majority of which were reactive with ganglioside GM2 [9]. These findings were confirmed in a larger, ongoing, multicentre extension of the pilot study, at this stage incorporating 10 institutions from 4 European countries and including dogs with other cranial and peripheral neuropathies of unknown origin, myopathies and neuromuscular disorders (OMN group), which altogether are examining 162 canine sera. Here, 32 of 73 ACP dogs exhibited anti-GM2 Abs, 4 of 73 exhibited anti-GA1 Abs and 13 of 73 exhibited both (overall 67%), whilst only 3 of 40 (7.5%) OMN dogs and 5 of 49 (10%) with non-neurological control sera exhibited such Abs ([Figure 44.4](#)).

The concurrent staining investigations carried out with anti-GM2 Ab-containing canine sera on murine sciatic nerves and murine monoclonal anti-GM2 Abs on canine sciatic nerves indicated that GM2 was localized to the abaxonal Schwann cell surface and multifocally in the axonal areas. Experimental support for potential demyelinating properties of ACP serum is lent by the observation that intraneural injection of canine ACP serum into rat sciatic nerves in conjunction with a source of complement induces demyelination at a higher frequency when compared to control serum [12]. Also the observation that the application of IVIg in ACP dogs has shown a clear trend towards treated dogs recovering faster than nontreated dogs [8] provides a further potential pathophysiological link between ACP and GBS.

Chickens: Marek's Disease

Roughly around the same time as the extensive investigations into coonhound paralysis began, Marek's disease in chickens was reported to represent a model for GBS [13], since its histological features in the early course of the disease were considered to be indistinguishable from those of the demyelinating form of GBS, AIDP.

The infection of chickens with Marek's disease virus, a cell-associated lymphotropic herpes virus, results—in its classic form—in a paralytic, demyelinating peripheral neuropathy, with affected chickens

exhibiting asymmetrical, partial paresis of the wings and legs, progressing to complete paralysis of one or more extremities, torticollis, paralysis or dilation of the crop and respiratory distress. Grossly, one or more peripheral nerves are enlarged, usually involving the brachial and sciatic plexus and nerve trunks, the coeliac plexus, abdominal vagus and intercostal nerves. Histologically, a spectrum of proliferative (neoplastic), inflammatory (lymphoplasmacytic infiltrates with small numbers of macrophages) or minor inflammatory changes (termed A-, B- and C-type lesions, respectively) are observed, some of which are combined with demyelination (A and B types) or Schwann cell proliferation (B types). In experimental disease, the proliferative A type precedes the inflammatory infiltrates of the B type [14].

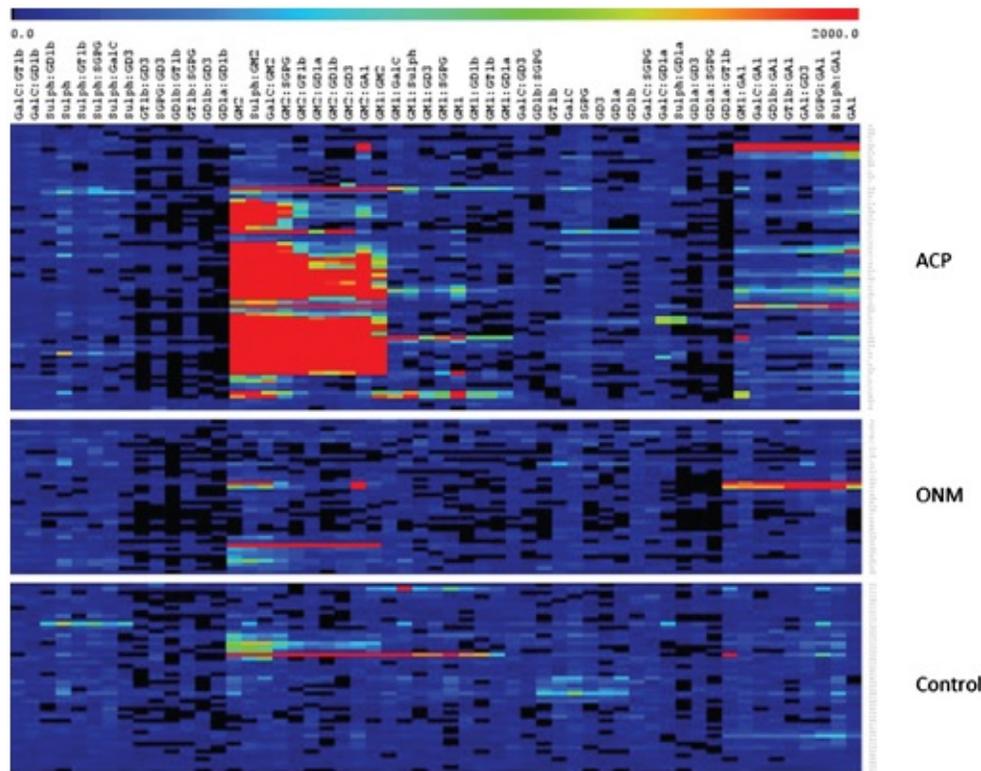


Figure 44.4 Heat map depicting the examination of 162 canine sera for anti-ganglioside Abs.

Overall 49/73 ACP sera contained either anti-GM2, anti-GA1 Abs or both, whilst only 3/40 ONM dogs and 5/49 of non-neurological control sera exhibited such Abs. Black corresponds to Ab-negative signals. The intensity of Ab reactivity increases of blue to green and red, with red indicating the strongest signals.

Closer research into the pathophysiological events associated with the severe polyneuritis and associated demyelination in Marek's disease revealed an underlying latent viral infection of the satellite cells, nonmyelinating Schwann cells and lymphocytes. Whilst thymectomy apparently resulted in reducing the incidence of disease and the predominant population of cells associated with the nerve lesions were T cells, IgG antibodies against peripheral nerve myelin were seen, the quantity of which, however, did not appear to correlate with the intensity of the nerve lesions. These anti-myelin antibodies were considered a possible consequence of a latent viral infection resulting in expression of a viral-induced antigen on the cell surface, which in turn was detected by reactive lymphocytes and resulted in neural injury including disruption of myelin sheaths via 'bystander demyelination' [13].

Chickens: Acute Paretic Syndrome in Juvenile White Leghorn Chickens

More recently, very thorough investigations [15] have described a sporadic, acute, parietic syndrome in juvenile White Leghorn chickens apparently not associated with Marek's disease virus (MDV) and characterised by cell-mediated, inflammatory demyelination present in cranial nerves, spinal nerves and nerve roots. Clinically, these animals exhibit progressive asymmetric paresis with intermittent relative extensor hypertonicity. Grossly, cranial nerves and spinal nerve roots are thickened, whilst histological examinations reveal multifocal lymphohistiocytic infiltrates within the proximal aspects of some of the cranial nerves as well as the dorsal and ventral roots, which colocalise with demyelinated and hypomyelinated axons. Similar to findings in GBS, electron microscopical investigations reveal macrophages invading the myelin sheaths (Figure 44.5). Additionally, a genetic susceptibility factor confined to the avian major histocompatibility complex was found. Whether this syndrome corresponds to a previously described syndrome of spastic paralysis in young White Leghorn pullets, which is not associated with MDV and appears to have a genetic disposition [16] is not clear; however, the findings in these chickens combined with the fact that between 1% and 4% of chickens are reported to spontaneously be affected, indicate that this disorder may present a valuable future model for AIDP.

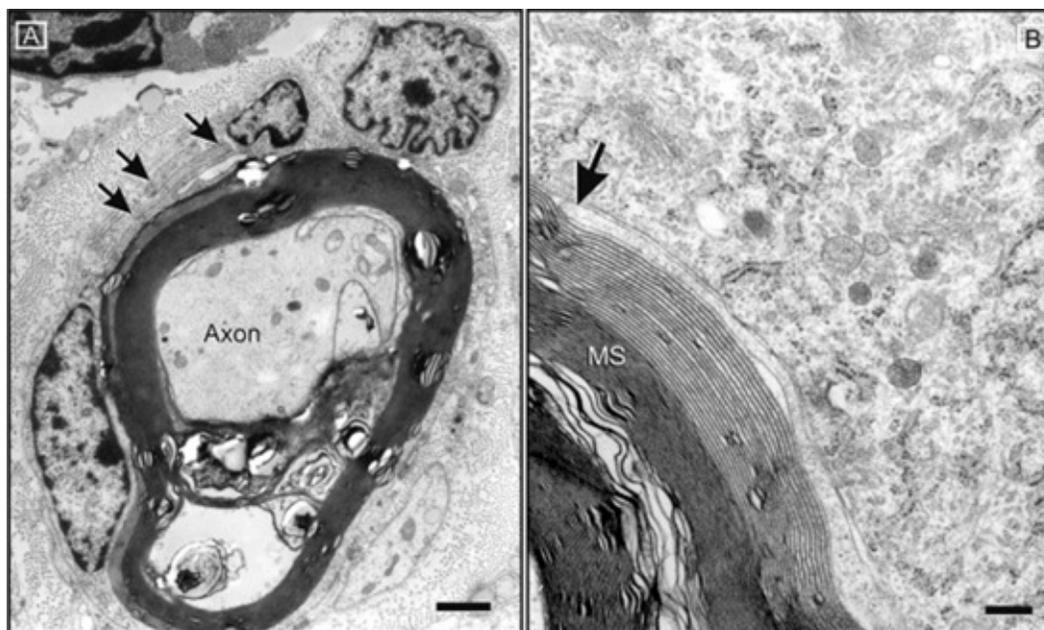


Figure 44.5 Macrophage-mediated myelin stripping in Acute Parietic syndrome in juvenile White Leghorn chickens

Macrophage processes (arrows) are seen splitting the outer mesaxon (A) and invading the myelin along the intraperiod line (arrow in B). MS = myelin sheath. Scale bar = 2 μ m. Reproduced with permission from [15].

Cats

Cats are only rarely affected by acute idiopathic poly(radiculo)neuropathies and in this species the comparison to GBS is hindered most by the inconsistency of the examinations carried out in each report. The largest case series describes 9 young (4-month-old to 4-year-old) cats with acute ascending tetraparesis or tetraplegia, a loss of spinal reflexes, and variable dyspnoea and involvement of the cranial nerves [17]. The nadir of clinical signs was reached 3 days after onset and 7 out of 9 cats made a complete recovery with supportive treatment over the following 4–6 weeks, whilst the remaining 2 cats were euthanized following progressive respiratory distress. Histologically, the ventral roots and multisegmentally the peripheral nerves, including the phrenic and intercostal nerves, exhibited moderate to severe axonal degeneration, demyelination and histiocytic infiltrates. Considering that the cause of the

clinical and pathological changes could not be elucidated, these cases were classified as idiopathic with an immune-mediated aetiology discussed.

Similar conclusions were reached in a report describing a young adult Bengal cat [18], who presented with rapidly progressive, symmetrical and flaccid tetraparesis and upon electrophysiological examination of muscles and nerves revealed changes consistent with generalised axonal neuropathy predominantly affecting the ventral nerve roots, and in an adult domestic shorthair cat who presented with acute pelvic limb weakness progressing to nonambulatory tetraparesis over 3 days, proprioceptive deficits, cranial nerve involvement, absent spinal reflexes and absent superficial pain perception [19]. Whilst the latter of these cats—euthanized following progressive respiratory compromise—exhibited an acute motor and sensory polyradiculoneuritis combined with demyelination and axonal degeneration in histological examinations, the young Bengal survived and—similar to reports of GBS patients—was reported to have experienced multiple episodes of varying intensity. The cat recovered from each of these with supportive care.

Horses

Originally, equine cauda equina neuritis also had been considered a possible equine equivalent to GBS due to the lymphoplasmacytic and histiocytic infiltrates combined with demyelination present in the sacral and caudal spinal nerve roots and occasionally other (including sciatic and facial) nerves [20]. However, the chronic course of this disease, the aetiology of which remains unknown, is considered to possibly represent an immunogenic response to a persisting herpes virus infection, an infection of other aetiology or antigens released by trauma or infection. This contrasts with GBS, making this disease a less compatible model.

Conclusion

Spontaneous animal models of GBS are present in numerous animal species, with the most promising models at this stage represented by chickens and dogs, the former of which (chickens) recommend themselves due to the numerical availability of subjects for examination, whilst the latter of which (dogs) are more amenable to more detailed and advanced investigations, including neurological, electrophysiological and CSF examinations, which combined with the heterogeneity of the canine strains involved is likely to mimic the situation in human GBS patients better.

Following a relatively long period of paucity of reports on the subject of acute polyradiculoneuropathies in animals, this fascinating field currently is experiencing a revival and the results of these investigations are likely to be of interest for human and animal patients alike.

Acknowledgment

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ANTIBODIES

Anti-Ganglioside Antibodies in Guillain-Barré Syndrome and Variants

Eduardo Nobile-Orazio, Marinella Carpo and Claudia Giannotta

Introduction

Since 1988 antibodies to glycolipids and in particular to gangliosides have become a major actor in the diagnosis and pathogenesis of Guillain-Barré syndrome and of its variants. A large number of studies progressively showed an increased prevalence of these antibodies in GBS and its variants, their association with specific clinical forms, the antecedent infective agents leading to their production, their effect in vitro and the animal models of GBS induced by immunization with these gangliosides. Even if the results of this data point to a direct role of these antibodies in the pathogenesis of GBS, there are still some aspects that need to be clarified to definitely confirm this hypothesis. I will here review the most brilliant studies that in my opinion opened the way to a better comprehension of the immune pathogenesis of GBS.

Before the Beginning: Are there anti-ganglioside antibodies? Ilyas et al. 1984,

The story of the association of anti-ganglioside antibodies with neuropathy started with the description of a reactivity to a ganglioside that ultimately proved not to be a ganglioside. Ilyas and colleagues first reported in 1984 [1] that sera from 3 patients with neuropathy and IgM monoclonal gammopathy reacted with the nerve antigen myelin-associated glycoprotein (MAG) bound to an antigen in the carbohydrate moiety of this molecule, which was also present in a glycolipid of peripheral nerve that was deemed to be a ganglioside. This cross-reacting glycolipid was later found not to be a ganglioside but 2 glycosphingolipids named sulfoglucuronylparagloboside (SGPG) and sulfoglucuronylactosaminylparagloboside (SGLPG) [2]. The same or a closely related reactive epitope was reported the previous year to react with the mouse IgM monoclonal antibodies H-NK1 directed against a surface epitope expressed by human natural killer cell and by several neural cell adhesion molecules [3]. This study opened the way to the search for antibodies to gangliosides in patients with different forms of neuropathy, leading in the following years to the association of IgG antibodies to different gangliosides in patients with Guillain-Barré syndrome and its variants and of IgM antibodies to GM1 and other gangliosides in patients with multifocal motor neuropathy [4,5].

A New Entry: Anti-GM1 Antibodies in Guillain-Barré Syndrome: Yuki et al. 1990

The real start of the association of anti-gangliosides antibodies and Guillain-Barré syndrome was the fundamental 1990 study by Yuki and colleagues [6] that reported on 2 patients who developed an acute axonal polyneuropathy after *Campylobacter jejuni* enteritis. Both patients had high titres of serum IgG antibodies to the gangliosides GM1 that progressively decreased during recovery. Despite its relevance, this manuscript was accepted at *Neurology* only as a brief communication, and there were many discussions about this study. I remember that in those days there was some scepticism among experts about the existence of an axonal form of GBS that was considered to be only a demyelinating neuropathy, and indeed the term ‘GBS’ did not appear in the title. Even though a previous study by Feasby and colleagues in 1986 [7] had already described 5 patients with an acute axonal form of GBS, I remember an expert at a meeting saying that “there is no such thing as an axonal Guillain-Barré”. This may also explain why the concomitant report of an epidemic among people living in the country in northern China of a disease that is now considered to be axonal GBS was originally postulated to be a different disease, termed ‘acute motor axonal neuropathy’ (AMAN) [8,9] which was only later included under GBS [10,11].

To make things more complicated, during those years an influential Italian pharmaceutical company was producing therapeutic agents for neuropathy containing a mixture of gangliosides (GM1, GD1a, GD1b and GT1b; Cronassial) or GM1 alone (Sygen) so that the association of antibodies to gangliosides and GBS was quite disturbing, considering that some reports of GBS after treatment with this therapy had started to appear [12]. It is now accepted by the neurological community that the paper by Yuki and colleagues included most of the current knowledge that a predominantly axonal form of GBS may be induced by an antecedent infection by *Campylobacter jejuni* that by a mechanism of molecular mimicry may induce the production of IgG antibodies to GM1 in some patients. Subsequent studies showed that anti-GM1 IgG antibodies occur in 30–40% of patients with GBS, are more common in patients with a predominantly motor and axonal form of GBS, may be also found in those with demyelinating GBS and are mostly associated with an antecedent *Campylobacter jejuni* enteritis. Studies also showed that either this infection or the associated antibodies or both are associated with a less favourable prognosis. Not all studies were concordant on each of these aspects [13–17].

A Second New Entry: Anti-GQ1b Antibodies in Miller Fisher Syndrome. Chiba et al. 1992

In 1992 Chiba and colleagues [18] made another fundamental discovery, reporting the presence of high titres of anti-GQ1b IgG antibodies in 6 patients with the Miller Fisher syndrome (MFS), a variant of GBS characterized by the concomitant presence of ophthalmoplegia, ataxia and areflexia. Since then this reactivity has been reported in approximately 90% of patients with MFS [19,20], and in patients with Bickerstaff’s brain-stem encephalitis [21] which differs from MFS in the presence of concomitant signs of central nervous system (CNS) involvement, including impairment of consciousness or pyramidal signs, in some patients with GBS and ophthalmoplegia [22] or with acute ophthalmoparesis (reviewed in [23]). This has also led to the proposal of the term ‘anti-GQ1b syndrome’ for patients with these antibodies and a number of acute clinical syndromes characterized by the presence of oculomotor impairment with or without ataxia. I have to say that the close association of these antibodies with this syndrome has led to an amazing increase in the diagnosis of MFS, at least in Italy, where a number of patients with

ophthalmoparesis and these antibodies were diagnosed to have MFS even when tetraplegic and with assisted respiratory ventilation. The selective impairment of the oculomotor nerve was related to the abundant expression of GQ1b in these nerves [22]. Also, in these cases the presence of these antibodies was associated with an antecedent *Campylobacter jejuni* infection [24], even if the pathogenic bacteria were later reported to differ from those associated with anti-GM1 antibodies (see below).

Other Anti-Ganglioside Antibodies in Axonal GBS. Mostly a Japanese story.

Several antibodies to different gangliosides appeared in the following years and were variably associated with different clinical and electrophysiological presentations of GBS, different antecedent events and possibly pathogenetic mechanisms, and to the definition of a number of new functional or topographic variants of GBS. In 1992 Yuki and colleagues reported 2 patients with an explosive, motor GBS rapidly evolving into respiratory insufficiency and mainly axonal impairment who had high titres of anti-GD1a IgG antibodies [25]. Subsequent studies showed that these antibodies are present in approximately 5–20% of patients with GBS, most of whom have a severe axonal form [26–28]. Studies also reported an association of this reactivity with an antecedent infection by *Campylobacter jejuni* [29]. A closely related reactivity against N-acetylgalactosaminyl GD1a (GalNAcGD1a) [30] and often with the cross-reacting GM1b ganglioside [31] was subsequently reported in approximately 20% of patients with GBS and was initially associated with an axonal motor form of GBS and antecedent *Campylobacter jejuni* infection [32] and occasionally with *Mycoplasma pneumoniae* infection [33]. More heterogeneous clinical presentations were, however, later associated with these reactivities, indicating that these antibody reactivities are not always strictly associated with a definite clinical presentation.

Anti-Ganglioside Antibodies in Other Forms of GBS. Willison et al, 1994; Irie et al, 1996

New antibody reactivities were also associated with demyelinating forms of GBS. In 1996 Irie et al. reported 3 patients with GBS and an acute cytomegalovirus (CMV) infection revealed by increased anti-CMV IgM antibodies who had high titres of anti-GM2 IgG and IgM antibodies [34]. Subsequent series reported this reactivity in up to 10% of patients. These antibodies were later found to cross-react with CMV-infected fibroblasts, supporting the hypothesis of a possible mechanism of molecular mimicry between the pathogen and the ganglioside [35]. These studies opened the way to the possible understanding of the pathogenesis of demyelinating GBS since most patients with GBS after CMV infection appeared to have a demyelinating GBS with more prominent sensory impairment than patients with an antecedent *Campylobacter jejuni* infection or without a known infection [36]. The relation between anti-GM2 antibodies and CMV-associated GBS was not, however, always confirmed in subsequent studies [37,38].

Another infrequent reactivity with ganglioside was described in 1994 by Willison and colleagues, who reported a patient with an acute sensory neuropathy without ophthalmoplegia with IgG antibodies to GD1b and GD3 but not to GQ1b, distinguishing him from MFS [39]. A few other patients with acute ataxic neuropathy and a selective reactivity to GD1b were later reported [40,41]. A more extensive study [42] reported that 9 out of 445 patients (2%) with GBS had a selective reactivity with GD1b. All these patients had sensory motor GBS, with electrophysiological signs of demyelination in the majority, while

none had signs of primary axonal degeneration. These findings led to the very interesting animal model of GBS induced immunization with GD1b (see below).

Another peculiar association is that of a selective IgG reactivity with GT1a with or without concomitant reactivity with GQ1b in a form of GBS with prominent or exclusive cervico-brachial-oropharyngeal impairment. This was initially reported in a patient with a pharyngeal-cervical brachial variant of GBS with a separate, concomitant lower reactivity with GD1a [43]. A similar selective reactivity with GT1a was subsequently reported in a few other patients with oropharyngeal palsy, neck weakness or polyneuritis cranialis [44,45], while in the majority of positive patients the correlation was difficult to ascertain considering the frequent concomitant reactivity to GQ1b. The difficulty in defining the clinical correlate of this reactivity also derives from another study confirming the frequent concomitant reactivity with GQ1b [46]. Other reactivities were subsequently reported in a small number of patients against the gangliosides GM1 α , GM1 (NeuGC), GD1 α , GalNAcGM1b, GD3, GT3, GT1b, GQ1 β , 9-O-acetyl GD1b and others. I have to say that some of these studies are complicated by the fact that a number of patients have concomitant reactivities to other gangliosides making it difficult to interpret the clinical correlation reported for each of these reactivities.

Anti-Ganglioside Antibodies and *Campylobacter Jejuni* Serotypes. Mostly Yuki

As mentioned before, anti-ganglioside antibodies in both GBS and MFS were frequently associated with an antecedent *Campylobacter jejuni* infection but it was unclear how the same pathogen could be associated with different clinical conditions. Kuroki and colleagues were the first to relate the presence anti-GM1 IgG antibodies in GBS with an antecedent infection by *Campylobacter jejuni* of the Penner 19 serogroup [47,48]. Yuki and colleagues subsequently reported that the *Campylobacter jejuni* isolates from stool cultures of most patients with GBS (51%) was of the serotype Penner 19 while the majority of the isolates from patients with MFS (71%) was of Penner 2 serotype [49]. In addition, most patients with Penner 19 isolates had anti-GM1 IgG antibodies while the majority of those with Penner 2 serotype had anti-GQ1b antibodies.

This study supported the hypothesis that molecular mimicry between the epitopes expressed on the surface of the infectious agent and certain a ganglioside was responsible for inducing an immune response that also affected the nerve. A further support to this theory derives from a study by the same group that showed that the polymorphism of the genes of *Campylobacter jejuni* affecting the patients resulted in the expression on the surface of the bacteria of the lipooligosaccharide (LOS) bearing a GQ1b or GM1 and GD1a epitope [50]. In addition, most patients infected by GQ1b-*Campylobacter jejuni* developed anti-GQ1b antibodies and MFS, while those infected by a GM1-GD1a-*Campylobacter jejuni* most frequently developed anti-GM1 and anti-GD1 antibodies and had limb weakness. This might also explain while only a minority of patients with *Campylobacter jejuni* infection develop GBS (1.17/1000, 77 times greater risk than in the general population) and that the probability of developing GBS in the 2 months following *Campylobacter jejuni* is less than 2/10,000 [51]. The hypothesis that the immunologic predisposition of the host may also contribute to this susceptibility has also been investigated with inconclusive results [52,53].

The In Vitro Effect of Anti-GM1 Antibodies. Buchwald et al. 1998

The possible mechanism by which anti-GM1 antibodies may affect peripheral nerve function was examined in an in vitro model by Buchwald and colleagues, who adopted the same macro-patch-clamp system used to study neuromuscular transmission in myasthenia gravis [54]. Using this model the authors found that serum and purified IgG from patients with GBS containing anti-GM1 antibodies consistently reduced the evoked quantal release at the neuromuscular junction in the mouse hemidiaphragm. A similar effect was, however, obtained with sera from GBS patients not containing anti-GM1 antibodies, leaving it unclear whether this effect was caused by undetectable levels of these antibodies or other factors in the sera of the patients. The same authors later reported that this blocking effect was reduced in a dose-dependent way by the addition of the intravenous immunoglobulin used for therapy [55].

The In Vitro Effect of Anti-GQ1b Antibodies: α -Latrotoxin Comes to Neurology. Willison et al, 1994

The mechanism by which anti GQ1b IgG antibodies can affect peripheral nerves were mainly examined by Willison and colleagues. They first examined the in vitro effect on a mouse phrenic nerve/diaphragm preparation of the sera from 3 patients with MFS and high anti-GQ1b antibodies [56]. Contrary to what one would theoretically expect, soon after addition of the sera, there was a markedly increased frequency of miniature endplate potentials followed by a complete block of neuromuscular transmission. In a subsequent study [57] they showed that anti-GQ1b antibodies bind at the neuromuscular junctions, inducing a massive quantal release of acetylcholine from nerve terminals followed by a block of neuromuscular transmission. This effect resembled that of the neurotoxin α -latrotoxin, a component of black widow spider venom, raising the possibility that the antibodies bound to the same receptor as bound by this toxin. The effect of anti-GQ1b antibodies was strictly dependent on the activation of complement components but neither the classical pathway activation nor the formation of membrane attack complexes was required, supporting the possibility of the activation of the alternative pathway.

In a subsequent study the authors found that these antibodies mediated complement-dependent destruction of the motor nerve terminal [58]. Somehow, contrasting results were reported at the same time by Buchwald and colleagues, who examined the in vitro effect on the neuromuscular transmission of anti-GQ1b containing IgG from patients with MFS using the same model used to study the effect of anti-GM1 antibodies (see above) [59,60]. They found that IgG from patients with MFS had a combined pre- and postsynaptic action blocking the neuromuscular transmission sera. In this case, however, a similar effect was obtained with sera from patients in whom anti-GQ1b antibodies were not found. In general both groups showed that sera from patients with MFS impaired neuromuscular transmission even if in both studies it remains unclear how these impairments can be related to a disease where weakness is mainly of the oculomotor nerve while limb weakness is by definition mild or absent.

Animal Models of Anti-Ganglioside Neuropathy. Kusunoki et al, 1996

The first animal model of anti-ganglioside neuropathy was reported in 1996 by Kusunoki and colleagues, who induced an ataxic sensory axonal neuropathy in rabbits by immunization with ganglioside GD1b [61]. Three of the 6 immunized rabbits developed a sensory neuropathy without motor involvement that was associated with axonal loss in the dorsal roots and sciatic nerve and with loss of nerve cell bodies in dorsal root ganglia. All animals developed antibodies to GD1b. This presentation was similar to what had been observed in some patients with anti-GD1b antibodies and an acute sensory ataxic neuropathy

(see above). A similar model of anti-ganglioside neuropathy was later induced in rabbits by immunization with a mixture of ganglioside containing GM1 or with GM1 alone [62]. In this case the animals developed high titres of anti-GM1 antibodies associated with the acute onset of flaccid paresis with a monophasic illness course. Pathological studies revealed axonal degeneration with deposits of IgG on the axons of anterior roots. This animal model strictly resembled human axonal GBS. In addition, similar to what had been observed in human GBS, treatment of the immunized rabbits with intravenous immunoglobulin induced a faster recovery of GBS compared to untreated animals [63]. A similar axonal neuropathy was induced in mice by intraperitoneal implantation of hybridoma-secreting antibodies to the gangliosides GD1a/GT1b-2b [64]. This experimental model may explain the already mentioned neuropathy induced in humans by treatment with gangliosides; subsequent studies revealed the ability to induce an acute axonal GBS by sensitization with the lipooligosaccharide of *Campylobacter jejuni* isolated from patients with GBS [65,66]. Immunized animals developed high titres of serum anti-GM1 antibodies, followed by an acute flaccid paresis. Pathological studies revealed signs of Wallerian degeneration in some animals with deposits of IgG in the nerve. In addition, the same pathological features observed in some patients with acute GBS of macrophage infiltrating between the myelin and axon were found in some animals [65]. I think that these experiments clearly showed how exposure of the animals to *Campylobacter jejuni* may induce the development of anti-GM1 antibodies and an acute axonal GBS.

A Personal View

I think that there is considerable evidence from the above-mentioned studies that anti-ganglioside antibodies play an important role in the pathogenesis of GBS and MFS. As neurologists, however, we are a little bit spoiled by what we observed in myasthenia gravis, where 80–90% of patients have antibodies to an acetylcholine receptor, while a consistent proportion of the negative patients have antibodies to other components at the neuromuscular junction, including MUSK and LRP4. This is what we observe in MFS, where approximately the same proportion of patients have anti-GQ1b antibodies. In patients with GBS, however, no more than 50% have antibodies to one or more gangliosides, suggesting that what we observe is probably only part of the story. It is true that more recent studies have shown that some patients with GBS or MFS have antibodies to a complex of gangliosides [67,68] even if these patients only represent a minority. It remains unclear why a difference in the prevalence of antecedent infection from country to country does not result in consistent differences in the incidence of GBS [69] nor is it clear why a similar proportion of patients in different countries eventually develop the same disease from different triggering events. Even if we now have fantastic evidence on how the mechanism of molecular mimicry between external pathogens and our molecules may explain the disease, in almost 50% of the patients the triggering events and the mechanism by which they can induce GBS remain to be clarified.

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Antibodies to Ganglioside Complexes

Kenichi Kaida

Introduction

In Guillain-Barré syndrome (GBS) and its variants, antibodies to glycolipids, especially *N*-acetylneuraminic acid- (sialic-acid) bearing glycosphingolipids, which are referred to as gangliosides, have been tested in acute phase sera as diagnostic markers. Generally, to detect pathogenic antibodies, purified single substances have been used as test antigens. Thorough purification of natural substances and avoidance of antigen contamination provide accurate and reliable results. It has been the same in measurement of anti-ganglioside antibodies. Over 10 years ago, we detected IgG antibodies specifically reacting with a mixture of 2 different gangliosides in sera from patients with GBS, and named such mixture as ganglioside complex (GSC). In thin-layer chromatogram (TLC) immunostaining assay, the anti-GSC antibodies do not bind to each constituent ganglioside, but bind to overlapping portions of 2 gangliosides. Currently, antibodies to GSCs as well as single glycolipid antigens have been routinely tested in serum samples from patients with GBS or other immune-mediated neuropathy.

Kaida K, et al. Ganglioside complexes as new target antigens in Guillain-Barré syndrome. *Annals of Neurology*, 2004

In the late 1990s, Dr Susumu Kusunoki found that serum IgG from a patient with severe GBS distinctly reacted with unknown antigens just below a level of GD1a in TLC immunostaining assay using crude bovine brain ganglioside fraction which was obtained through DEAE Sephadex A-25 column by extraction with 0.1M ammonium acetate. Conventional ELISA screening for serum anti-ganglioside antibodies showed negative results for GD1a, GalNAc-GD1a, and GD1b. Identification of the invisible targets for the immunoreaction on a TLC plate was one of tasks the visionary supervisor Dr Kusunoki assigned to a graduate student. In spite of considerable laboratory work with use of various purification methods, we could not purify and confirm the unknown target glycolipids. Antibody activities to ganglioside mix were negative. Three years passed before we confirmed that the immunostaining revealed a specific reaction against a GSC, GD1a/GD1b complex ([Figure 46.1](#)). A study of a mixture of GM1 and a phospholipid [1] gave us a clue. In 2004, the mission 'invisible' was accomplished with publication in *Annals of Neurology* [2].

Considering that glycosphingolipids form a cluster with other glycolipids in lipid rafts, glycosphingolipid-enriched membranes, it is no wonder that clustered glycol-epitopes in the rafts are targeted by anti-ganglioside antibodies. Ligands of adhesion molecules such as selectins and siglecs

(sialic acid-recognizing immunoglobulin-superfamily lectins) are assumed to comprise complex glycoconjugates, which are packed closely in the cell membrane to form rigid, rod-like configurations with multiple valency and strict binding specificity [3]. Anti-GSC antibodies are real examples of such a concept.

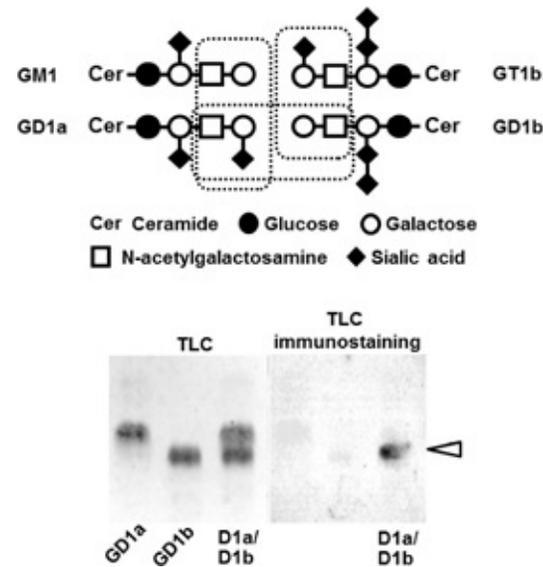


Figure 46.1 Carbohydrate structures of ganglioside complexes (GSCs) and thin-layer chromatogram (TLC) immunostaining of the serum of patient with GBS.

Pattern diagrams of GSCs are shown: GM1/GD1a, GD1a/GD1b, GM1/GT1b, and GD1b/GT1b. Squares with dotted lines illustrate putative antigenic epitopes for anti-GSC antibodies. In TLC immunostaining, the developing solvent consisted of chloroform, methanol and 0.2% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (50:45:10, v/v). Lower left panel: TLC results visualised by orcinol reagent; lower right panel: TLC immunostaining. Positive immunostaining on the right panel indicates an antibody reaction to the GD1a-GD1b complex (GD1a/GD1b) (reproduced with permission from [2]).

Kaida K, et al. Anti-ganglioside complex antibodies in Miller Fisher syndrome. *Journal of Neurology Neurosurgery and Psychiatry*, 2006

We conducted a survey of antibodies to GSC containing GQ1b or GT1a in Miller Fisher syndrome (MFS) because they are key molecules in MFS, and we found IgG antibodies to GSC containing GQ1b or GT1a in half of MFS patients [4]. Binding reactivity of the MFS-associated anti-GSC antibodies is classified into 2 patterns: reactivity against a combination of $[\text{Gal}\beta 1-3\text{GalNAc}]$ and $[\text{NeuAc}\alpha 2-8 \text{NeuAc} \alpha 2-3\text{Gal}\beta 1-3\text{GalNAc}]$ in terminal residues of ganglio-N-tetraose structures, or reactivity against a combination of $[\text{NeuAc}\alpha 2-3\text{Gal}\beta 1-3\text{GalNAc}]$ and $[\text{NeuAc}\alpha 2-8 \text{NeuAc}\alpha 2-3\text{Gal}\beta 1-3\text{GalNAc}]$ in terminal residues (Figure 46.2). Such antibodies to GSC containing GQ1b or GT1a are also found in half of GBS patients with ophthalmoplegia [5]. Regarding anti-GSC antibodies, MFS is different from GBS in that there are no IgG antibodies to GSCs consisting of 2 of GM1, GD1a, GD1b and GT1b in MFS. Colocalization of GQ1b, GM1 and GD1a remains to be proved in nerve membranes of oculomotor nerves and dorsal root ganglion neurons.

Glycolipid complexes, GA1/GQ1b or GA1/GT1a, are targeted in some patients with MFS or GBS with ophthalmoplegia. Seventy percent of antibodies to GA1/GQ1b or GA1/GT1a, however, did not bind to GM1/GQ1b or GD1b/GQ1b, which are similar to GA1/GQ1b regarding terminal residues in the GSC [6]. Factors such as conformational epitopes or electric charges of sialic acids in GSC may influence the immune response to GSC in the nerve membrane.

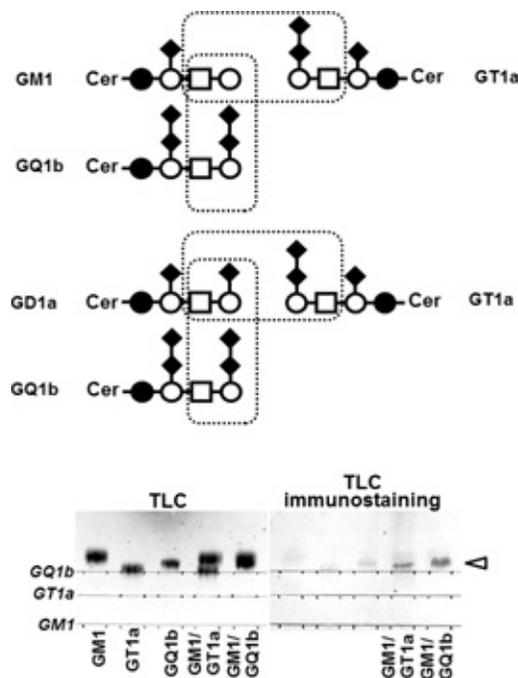


Figure 46.2 Carbohydrate structures of ganglioside complexes containing GQ1b or GT1a and TLC immunostaining of the serum of patient with Fisher syndrome.

Pattern diagrams of GSCs are shown: GM1/GQ1b, GM1/GT1a, GD1a/GQ1b, and GD1a/GT1a. Squares with dotted lines illustrate putative antigenic epitopes for anti-GSC antibodies. Lower left panel: TLC results visualised by orcinol reagent; lower right panel: TLC immunostaining. Positive immunostaining on the right panel indicates antibody reactions to GT1a/GM1 and GQ1b/GM1. Antibody reactions to GT1a, GD1b, GM1, GT1a and GQ1b were negative (reproduced with permission from [4]).

Kaida K, et al. Anti-ganglioside complex antibodies associated with severe disability in GBS. *Journal of Neuroimmunology*, 2007

At first we conducted a survey of IgG antibodies to GSCs consisting of 2 of 4 major gangliosides, GM1, GD1a, GD1b and GT1b, in consecutive Japanese patients with GBS, revealing that the frequency of anti-GSC antibodies was 17% in GBS [7]. In target GSC, the combination of gangliosides appears to be governed by a principle that a target molecule is an epitope formed by a combination of [Gal β 1-3GalNAc] and [NeuAc α 2-3Gal β 1-3GalNAc] in terminal moieties of ganglio-*N*-tetraose structures (Figure 46.1). GBS patients with such anti-GSC antibodies were characterized by having had an antecedent gastrointestinal infection, lower cranial nerve deficits and severe disability. The association of anti-GSC antibodies with severe disability should be confirmed in future prospective studies. The severity may be explained by tighter interactions between anti-GSC antibodies and GSC with multivalent glycol epitopes. Whether the tighter interactions induce stronger antibody-mediated immunoreactions remains to be confirmed.

Todeschini AR, et al. Ganglioside GM2/GM3 complex affixed on silica nanospheres strongly inhibits cell motility through CD82/cMet-mediated pathway. *Proceedings of the National Academy of Sciences of the United States of America*, 2008

Cell adhesion processes in the plasma membrane are regulated by carbohydrate-binding proteins such as selectins and Siglecs (sialic-acid-recognizing immunoglobulin-superfamily lectins), based on a cis- or trans-carbohydrate-to-carbohydrate interaction [8,9]. Ganglioside complexes providing clustered

carbohydrate epitopes are, therefore, likely to be influential on the cell adhesion process. Research by Todeschini and colleagues exhibited that a GM2/GM3 complex more efficiently inhibits cell motility through blocking of c-Met activation than GM2 or GM3 alone [10]. This was the first report showing that GSCs play a functional role in the cell function. One future task is to amass convincing evidence proving the hypothesis that GSCs or multivalent glycol epitopes in the cell membrane have a functional role in cell-to-cell recognition rather than isolated gangliosides or monovalent glycol epitopes.

Kaida K, et al. GM1/GalNAc-GD1a complex: a target for pure motor Guillain-Barré syndrome. *Neurology*, 2008

An antibody to a complex consisting of GM1 and GalNAc-GD1a (GM1/GalNAc-GD1a) is found in 5% of GBS patients. Anti-GM1/GalNAc-GD1a-positive patients usually develop a pure motor variant of GBS with preserved cranial and sensory nerves and, contrary to expectations, are characterized by experiencing a preceding respiratory infection and early conduction block (CB) in motor nerves. The CB arises at intermediate nerve segments of motor nerves, but not at common compression sites such as the wrists and elbows. In view of the rapid recovery of CB and no evidence of remyelination and axonal degeneration in subsequent electrophysiological studies, the CB is likely to result from reversible conduction failure caused by temporary blockade of voltage-gated sodium channels (Na_V) at the nodes of Ranvier. GM1 and GalNAc-GD1a may assemble and form a GM1/GalNAc-GD1a complex in the vicinity of Na_V clusters at nodal membranes in motor nerves, where antibody-antigen interactions may disturb regulatory functions of Na_V [11].

Kaida K, et al. GD1b-specific antibody induces ataxia in Guillain-Barré syndrome. *Neurology*, 2008

In the routine measurement of anti-GSC antibodies in sera from GBS patients, some IgG anti-GD1b antibodies revealed prominent decrease of anti-GD1b activities by the addition of gangliosides with 2 or more sialic acids to GD1b. In a larger number of GBS patients, we confirmed that such GD1b-specific antibodies were significantly associated with development of ataxia, whereas GBS patients without ataxia had anti-GD1b antibodies which equally reacted against a mixture of GD1b and other gangliosides, and often had antibodies to GSCs containing GD1b [12]. These findings suggest that colocalization of another ganglioside with GD1b in the cell membrane forms novel glycol epitopes, different from GD1b, and influences the accessibility of the anti-GD1b antibodies. That is, *cis*-interaction of the sugar chain of gangliosides in lipid rafts may modify the steric structure of the glycol epitopes in the cell membrane.

Greenshields KN, et al. The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice. *The Journal of Clinical Investigation*, 2009

In a sophisticated *ex vivo* study using GalNAc transferase-deficient and GD3 synthase-deficient mice, Greenshields and colleagues clearly showed that the glycolipid environment governs the accessibility and the avidity of anti-ganglioside antibodies [13]. A monoclonal anti-GM1 antibody (DG2) with activity not attenuated by GSC was found to bind to motor nerve terminals. In contrast, a monoclonal anti-GM1

antibody (DG1) with activity that was attenuated by GSC could not bind to motor nerve terminals. Conversion of GD1a to GM1 by neuraminidase treatment made it possible for DG1 to access to GM1 in the motor terminal axons. These study results clearly indicate that the glycolipid environment and antibody specificity are influential factors in antibody-antigen interactions. Based on the binding specificity, finally, the anti-ganglioside antibodies are classified into 3 types: complex-attenuated (DG1), complex-independent (DG2), and complex-enhanced. Neighbour glycolipids as well as anti-ganglioside antibody specificity play a crucial role in antibody binding and subsequent immunoreaction such as complement activation.

Zitman FM, et al. Neuropathophysiological potential of Guillain-Barré syndrome anti-ganglioside complex antibodies at mouse motor nerve terminals. *Clinical and Experimental Neuroimmunology*, 2011

Complement activation is considered to play a key role in anti-ganglioside antibody-mediated nerve injury in GBS and its variants, as shown in vitro and ex vivo. Zitman and colleagues showed, in the nerve-muscle co-culture ex vivo model, that anti-GSC antibodies from patients with GBS and its variants had a neurophysiological blocking effect at motor nerve terminals and induce complement-mediated nerve damage through an antigen-antibody interaction [14]. In addition, the authors showed that GSC such as GM1/GD1a actually exists as an available antigen in living neuronal membranes. Their study provides direct experimental evidence for the neuropathogenicity of anti-GSC antibodies in GBS.

Rinaldi S, et al. Combinatorial glycoarray. *Methods in Molecular Biology*, 2012

ELISA is an easy and universal method for screening antibodies. However, 96 wells in a conventional ELISA plate are not enough to test antibody reactivity against a multitude of glycolipid complexes. Rinaldi, Brennan and Willison (2012) developed a new screening method—combinatorial glycoarray—which is appropriate for testing antibody activities to many heteromeric complexes consisting of various lipids as well as glycolipid complexes [15]. Their method can test antibodies to many glycolipid complexes simultaneously, discover new anti-glycolipid antibodies and save scarce reagents.

As reported elsewhere [16], in multifocal motor neuropathy, IgM anti-GM1 antibodies revealed a stronger binding ability to GM1 ganglioside-containing lipid mixture than to GM1 alone. A recent study using the combinatorial glycoarrays with polyvinylidene difluoride (PVDF) membrane has pointed out that glycol epitopes newly formed in a mixture of glycolipid and lipid can be novel targets [17]. We should note that the sensitivity and the specificity of antibodies to such heteromeric complexes may depend upon the weight proportions of their constituents [17,18]. Mixture in equal amounts of the constituents does not necessarily provide the optimal sensitivity of the antibodies to the lipid complexes.

Mauri L, et al. Anti-GM1/GD1a complex antibodies in GBS sera specifically recognize the hybrid dimer GM1-GD1a. *Glycobiology*, 2012

There is little evidence to elucidate the details of a real glycolipid environment in the nerve membrane. Reconstructing biological membrane models faithfully is an urgent issue. It is, however, difficult at present to artificially reproduce the same lipid environment as lipid rafts of the plasma membrane. It is

unclear how GSCs are formed, are distributed and function in the nervous system. In 2012, Mauri and colleagues provided a dimeric GM1-GD1a hybrid ganglioside derivative, which contains 2 structurally different oligosaccharide chains [19]. Such synthetic dimeric hybrids mimic GSCs and are useful for studies on anti-GSC antibody-mediated immunoreaction to carbohydrates. The combinatory glycoarray systems are important tools for extensively investigating anti-ganglioside antibody-mediated glycoconjugate recognition, as well as for detecting novel anti-GSC antibodies. It is, however, nearly impossible to adjust the condition of the glycoarray systems regarding their glycolipid density and the clustering of sugar ligands. Antibody-mediated carbohydrate recognition on the cell surface is regulated by the chemical property of the constituents of glycoconjugates in the cell membrane. Additionally, the nature of the glycoconjugates is governed by parameters consisting mainly of multivalency of sugar epitopes, their orientation and conformational flexibility of their presentation, and ligand density and spacing of interaction partners [20] (figure 46.3). Synthetic clustered glycolipids of different valencies vary in carbohydrate density on a PVDF or a polystyrene surface and are useful to adjust some of the above parameters and to make efficient use of the present glycoarray systems.

Perspectives

In addition to the GM1-GD1a hybrid, a dimeric GM1 hybrid (GM1-GM1 dimer) and a dimeric GD1a hybrid (GD1a-GD1a dimer) are also important tools for analysing anti-ganglioside antibody-carbohydrate interaction on the cell surface [21]. We should equip a series of synthetic mono-, di-, and trivalent gangliosides to improve our understanding of anti-ganglioside antibody-mediated carbohydrate recognition.

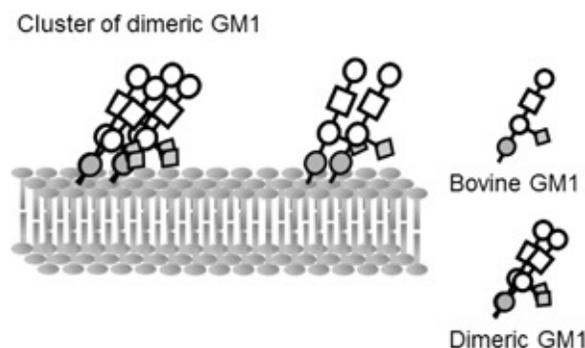


Figure 46.3 Structures of GM1 dimer and natural GM1 in the cell membrane.

Clusters of synthetic GM1 dimers are assumed to be more multivalent and denser than those of natural (bovine) GM1 at nerve membranes.

A recent study has shown that GM1-like and GD1a-like lipooligosaccharides may form a GM1b epitope, inducing the development of anti-GM1b antibodies [22]. This result indicates that the complex of 2 different structures may form a new molecular mimicry, although it is unclear whether the anti-GM1/GD1a antibodies and the anti-GM1b antibodies bind to the same carbohydrates in living nerve membrane. We should carefully interpret the configuration of clustered glycol epitopes in GSCs.

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Antibody and B Cell Immunology in GBS: From Evolution to Current Concepts

Ruth Huizinga

Introduction

Serum anti-glycolipid antibodies are a hallmark of the Guillain-Barré syndrome (GBS) and have been demonstrated to induce complement-mediated nerve damage [1]. The pathogenic antibodies are produced by plasma cells, which differentiate from B cells. Here I discuss my 10 favourite papers that have shed important light on the immunological mechanisms of the induction of anti-glycolipid antibodies in GBS.

Cook SD, et al. The Guillain-Barré syndrome. Relationship of circulating immunocytes to disease activity. *Archives of Neurology*,1970

One of the earliest studies that I could find on PubMed, suggesting that a *systemic* immune response plays a role in the pathogenesis of GBS, is from Cook and colleagues [2]. The paper reports the presence of atypical basophilic mononuclear cells in the blood of patients with GBS, so-called atypical leukocytes or circulating immunocytes. They found many of these cells proliferating, as indicated by the uptake of tritiated thymidine, demonstrating DNA synthesis. This was analysed cell-by-cell (imagine all the counting that it required!). Sixty-four percent of the GBS patients, mostly in the acute stage of the disease, had increased numbers of these DNA-synthesizing cells. The numbers subsequently declined to basal levels. Importantly, there was a correlation between the number of DNA-synthesizing cells and the onset of clinical recovery. The responses in peripheral blood were reminiscent of the responses seen during immune reactions in man and animals immunised with peripheral nervous system tissue in complete Freund's adjuvant (CFA) [3]. Building further onto the landmark histopathology study [4], showing that mononuclear infiltrates are present in demyelinating areas of peripheral nerves of GBS patients, the authors proposed the concept that “antigenic stimulation occurs in patients with GBS, resulting in the presence of circulating DNA synthesizing cells which may play an important role in the development of peripheral nerve injury” [2]. The sequence of events turned out to be correct as we now consider GBS to be a post-infectious disease.

Willison HJ, et al. Immunoglobulin subclass distribution and binding characteristics of anti-GQ1b antibodies in Miller Fisher syndrome.

Journal of Neuroimmunology, 1994

After the discovery that antibodies in GBS patients were recognising glycolipids [5], an important question arose as to what subclasses were produced. Since antibodies to lipopolysaccharides (LPS) and bacterial capsular polysaccharides are predominantly of the IgG2 subclass [6,7], Hugh Willison and co-workers must have been surprised to find that the antibodies were actually of the IgG1 and IgG3 subclasses [8]. Interestingly, some patients with MFS, but not GBS, did have low levels of IgG2 anti-GQ1b, suggesting that a typical carbohydrate antibody response to glycolipids can occur in some instances. The 'atypical immune response', as mentioned in the discussion of the paper [8], has been confirmed by many other groups and was found to be related to preceding infections, where gastrointestinal infections were linked to IgG1 and respiratory infections to both IgG1 and IgG3 [9]. Immunisation of humans with GM2 combined with *Bacillus Calmette-Guérin* also resulted in the induction of IgG1 and IgG3 antibodies to GM2 [10], indicating that the IgG 1/3 antibody response to glycolipids is not a unique feature of patients with GBS. The finding of IgG1 and IgG3 preference has led to the assumption that T cells are required for the development of anti-glycolipid antibodies. However, the exact phenotype of the cells that induce class switch recombination in GBS remains unknown.

Heidenreich F, et al. T cell-dependent activity of ganglioside GM1-specific B cells in Guillain-Barré syndrome and multifocal motor neuropathy in vitro. *Journal of Neuroimmunology, 1994*

The paper by Heidenreich and colleagues is of particular interest because it provides evidence that GM1-reactive B cells are present in the peripheral blood of patients with GBS [11]. Although the study mainly focussed on the in vitro production of anti-GM1 IgM, and this was also found in some disease control patients, in 2 GBS cases evidence was found for the production of anti-GM1 IgG. In addition, in 2 patients in vitro IgM anti-GM1 production was found early in the disease course and in unstimulated cultures, suggesting that plasmablasts were present in the cell preparation. The mitogen used for stimulating the cells was derived from pokeweed. Recently, these preparations were further examined and were found to contain several Toll-like receptor (TLR) ligands [12]. These ligands may cause production of cytokines that cause activation and differentiation of B cells. In conclusion, the data suggests that GBS patients have circulating B cells or plasmablasts that can secrete anti-glycolipid antibodies in vitro. This implies that the cloning and detailed characterisation of these B cells should in principle be possible.

Freimer ML, et al. Gangliosides elicit a T-cell independent antibody response. *Journal of Autoimmunity, 1993*

So what are the real requirements for the activation of anti-glycolipid reactive B cells? An elegant animal study performed by Freimer and colleagues demonstrates that, at least in mice, B cells can be activated to produce anti-GM1 IgM using GM1-containing liposomes, with lipid A as an adjuvant [13]. The response was also present in nude mice, lacking a thymus. Upon each booster, there was an increase in antibody levels, suggesting a step-wise expansion of GM1-specific B cells. Hence the data nicely indicates that activation of B cells is possible in a thymus-independent manner, without the help of T cells and NK cells, which also develop in the thymus. In a control experiment using the protein antigen albumin, no IgM or IgG responses could be induced, as expected. In the study, absolutely no anti-GM1 IgG response could

be measured, not even IgG3, which is the mouse equivalent of the thymus-independent antibody response. This finding may be explained by later studies using ganglioside-deficient mice (see below). In principle, this study shows that a thymus-independent response to glycolipids can occur and, possibly, that this is the reason for the presence of low levels of IgM antibodies against glycolipids in healthy humans [14], which are most likely induced because of cross-reactivity with bacterial antigens.

Wirguin I, et al. Induction of anti-GM1 ganglioside antibodies by *Campylobacter jejuni* lipopolysaccharides. *Journal of Neuroimmunology*, 1997

The ‘antigenic stimulation’ in GBS patients, as observed by Cook and colleagues, is now known to be caused by various pathogens, most frequently *Campylobacter jejuni* [15]. Therefore the Norman Latov’s group wondered whether immunisation with lipooligosaccharides (LOS) derived from *C. jejuni* would give rise to anti-glycolipids [16]. They were able to induce high titres of anti-GM1 IgM antibodies in rats that were immunised with keyhole limpet hemocyanin (KLH) prior to injection with *C. jejuni* LOS. The specificity of the antibodies indicated that stimulation through the B-cell receptor is required. KLH is glycosylated and immunisation induces antibodies to the Gal-GalNAc epitope [17]. The findings suggest that the subsequent challenge with *C. jejuni* LOS is further stimulating the Gal-GalNAc-specific B cells induced by KLH to produce antibodies that are also cross-reactive with GM1. As many individuals have antibodies to asialo-GM1, which also contains the Gal-GalNAc epitope, it will be interesting to compare the sequences of asialo-GM1 and GM1-reactive B cells.

Bowes T, et al. Tolerance to self gangliosides is the major factor restricting the antibody response to lipopolysaccharide core oligosaccharides in *Campylobacter jejuni* strains associated with Guillain-Barré syndrome. *Infection and Immunity*, 2002

Major progress was made in determining some of the mechanisms of anti-glycolipid antibody induction using mice that lack glycosyltransferases and are hence deficient in certain types of gangliosides. Using these mice, anti-glycolipid IgG could be induced not only in response to ganglioside liposomes, but also following immunisation with *C. jejuni* LOS [18]. In this model, ‘intermolecular’ T-cell help was provided by the addition of ovalbumin to the ganglioside liposomes, or by the use of CFA. This resulted in class switching to IgG1 and IgG2a/b. In the same paper it was shown that in ganglioside-sufficient, but TLR4 hyporesponsive mice, no anti-glycolipid antibodies could be induced. This experiment clearly demonstrates the important role of TLR4 activation. Mice immunised with LOS devoid of a ganglioside mimic did not produce cross-reactive antibodies, indicating that the exact mimic must be present. The mouse model was later used to demonstrate that the induction of anti-glycolipid antibodies can occur independent of CD1d [19].

Lee et al. Induction of human IgM and IgG anti-GM1 antibodies in transgenic mice in response to lipopolysaccharides from *Campylobacter jejuni*. *Journal of Neuroimmunology*, 2004

In the quest for a mouse model for anti-GM1 antibody-mediated neuropathy, the laboratory of Norman Latov generated a transgenic mouse with a population of B cells expressing an anti-GM1 B-cell receptor [20]. The sequence of the antibody was derived from a patient with multifocal motor neuropathy [21]. One of the most striking observations to me is that the B cells with anti-GM1 reactivity were not deleted due to negative selection during development, which is observed in other models of anti-carbohydrate antibodies, such as in transgenic anti- α Gal mice [22]. This was elegantly demonstrated using an idiotype antibody that recognises the anti-GM1 antibody. Apparently, the presence of GM1 epitopes is sufficiently low to allow normal development of these B cells. During steady state, the transgenic mice produced low levels of anti-GM1 IgM, as can be observed in some humans. Injection with only *C. jejuni* LOS, without proteins or other adjuvants, resulted in high titres of anti-GM1 IgM, and after 18 days, IgG responses also occurred (mainly IgG3 and IgG2b). Immunisation with GM1 alone or *E. coli* LPS did not induce anti-GM1 IgG antibodies, indicating that both the carbohydrate mimic and an innate trigger is required to overcome peripheral tolerance mechanisms. Although the antibodies were able to bind to mouse nerves and activate complement, the mice did not develop neuropathy and hence a complete animal model for GBS was still lacking.

Borsellino G, et al. Phenotypic and functional properties of gamma delta T cells from patients with Guillain-Barré syndrome. *Journal of Neuroimmunology*, 2000

The studies in rodents, discussed above, in which glycolipids predominantly induce antibodies with thymus-independent characteristics, suggest that T cells are required for switching to the isotypes associated with GBS. So what is known about T-cell responses in GBS? Evidence for involvement of $\gamma\delta$ T cells in GBS is provided by the 2000 study by Borsellino and colleagues [23]. Most importantly, the percentage of V δ 1 cells was significantly increased in the peripheral blood of GBS patients, and approximately half of the cells also expressed the NK cell marker NKRP1A. T-cell clones derived from these cells secreted high levels of IL-4. This study is in line with the observation that $\gamma\delta$ T cells were present in the peripheral nerves of a GBS patient with a preceding *C.jejuni* infection [24]. Together the data may suggest that a population of NK T cells may be increased in acute GBS. However, whether these cells are able to induce isotype switching of human glycolipid-reactive B cells remains to be determined.

Yuki N, et al. Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, 2004

The final ‘missing link’ experiment, to prove that cross-reactive antibodies are generated through a process of molecular mimicry and cause neuropathy, was reported by Yuki and colleagues in 2004 [25]. Rabbits were immunised at 3-week intervals with *C. jejuni* LOS dissolved in KLH and emulsified in CFA. In the high-dose group, all animals developed antibodies to GM1 and flaccid paralysis occurred at various time points. The rabbit model pathologically resembles human acute motor axonal neuropathy. Of note, the anti-GM1 titres gradually increased following booster immunisations, and the majority of the animals developed paralysis within 3 weeks after the anti-GM1 titre peak was reached. One animal started to recover 16 days after the onset of symptoms, hence mimicking the disease in humans.

Importantly, the study demonstrates that the induction of GM1 antibodies is not the result of a polyclonal B cell response due to TLR triggering, since immunisation of rabbits with LPS from *E. coli* or *S. minnesota* did not lead to the generation of cross-reactive antibodies. This proves again that the exact carbohydrate structure, mimicking gangliosides, needs to be present for the development of cross-reactive antibodies. What then makes the rabbit so successful? Although differences in B-cell development and mechanisms of affinity maturation may account for the ability of rabbits to produce high affinity antibodies to a diverse set of molecules [26], it is also intriguing that rabbits have high numbers of $\gamma\delta$ T cells and that the T cell receptor gamma loci have more sequence identity to humans than mice [27]. Finally, rabbits have more CD1 molecules compared to mice, which only express CD1d.

Kuijf MI, et al. LR4-mediated sensing of *Campylobacter jejuni* by dendritic cells is determined by sialylation. *Journal of Immunology*, 2010

I would like to end this monograph by briefly discussing the project that took me into the GBS field when I joined the laboratory of Bart Jacobs. The project underscores the long-standing fruitful collaboration between microbiologists, immunologists and neurologists at Erasmus MC. By using mutant *C. jejuni*, unable to sialylate LOS [28], the first author of the paper, Mark Kuijf, found that sialic acid on *C. jejuni* LOS, leading to mimicry with gangliosides, also resulted in a potent activation of human dendritic cells [29]. Thus the addition of only one sialic acid carbohydrate made LOS more potent by a factor of 100. So unlike the general notion that only lipid A is important for TLR4 signalling, this study clearly indicates that the carbohydrate composition also plays an important role. How can this strong innate response be linked to the development of anti-glycolipid antibodies? The activation of dendritic cells resulted in the production of cytokines that enhanced the proliferation of B cells. Hence the cytokine milieu produced by innate immune cells is facilitating the initiation of subsequent adaptive immune responses. Recent data further indicates that dendritic cells of almost all GBS patients showed a strong response to *C. jejuni* LOS by producing type I interferon, in contrast to control subjects [30]. This indicates that a strong innate immune response to *C. jejuni* may be a critical host condition for the development of GBS.

Conclusions and Future Perspectives

A hundred years of GBS research has resulted in the general elucidation of the pathogenesis of GBS. It is clear now that GBS is a post-infectious disease, mediated by B cells and antibodies that are cross-reactive with glycolipids in the peripheral nerve. Although B cells can be activated to produce anti-glycolipid antibodies in a thymus-independent manner in rodents, and most likely also in humans, for class switching to IgG1 and IgG3 additional B-cell help seems to be required. The exact cellular mechanism, however, remains to be determined. Both the presence of a carbohydrate mimic, which activates the B-cell receptor, and signalling through innate antigen receptors appears to be required for the development of cross-reactive antibodies. A great challenge for the future will be to reveal the factors that determine the magnitude and persistence of the anti-glycolipid antibody response in GBS. This may open up new ways for interfering with these responses early during disease.

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Myelin Antigens in Guillain-Barré Syndrome: EAN, the Pied Piper of Myelin

Emily K. Mathey and John Pollard

Myelin Becomes the Prime Suspect

The possibility that myelin antigens may play a pathogenic role in GBS was not seriously considered until the description of experimental autoimmune neuritis (EAN) by Waksman and Adams in 1955 [1]. Indeed, it was that discovery which provided the first indication that myelin might be selectively destroyed in GBS. Prior to that date, pathological studies, even the landmark report of Haymaker and Kernohan in 1949, which examined 50 fatal cases, did not mention demyelination, although the extent of nerve devastation was thoroughly documented [2]. The first major study to recognise specific myelin destruction in GBS was the postmortem analysis of 19 fatal cases by Asbury, Arnason and Adams from Massachusetts General Hospital in 1969 [3]. That study noted the association between demyelination and infiltrating inflammatory cells, thus suggesting some homology with EAN.

In the same year (1969) a number of publications appeared which examined the mechanism by which myelin was targeted in these disorders. Wisniewski, Terry, Whitaker, Cook and Dowling published an autopsy study which showed that myelin dissolution occurred in immediate proximity to invading macrophages and that lymphocytes were not present within the basal lamina of affected fibres [4]. Similar macrophage-mediated demyelination in EAN was reported by Lampert in 1969 [5]. In 1972 Prineas described changes in GBS of stripping and phagocytosis of compact myelin by macrophages following displacement of normal appearing Schwann cell cytoplasm away from the sheath [6]. (See [Figure 48.1.](#))

These dramatic illustrations suggested that if GBS and EAN were homologous autoimmune disorders then the target of immune attack did indeed appear to reside within compact myelin. Moreover the pathological changes in GBS and EAN were so similar that investigations into the pathogenesis of GBS were for some years dominated by studies in EAN. Studies of immune targets in myelin in the EAN model may, in retrospect, have retarded progress in understanding the pathogenesis of the human disease.

Wherefore Art Thou P0?

Although EAN has informed many areas of GBS pathogenesis with respect to identifying target antigens, the model has been akin to a reverse Pied Piper where the rats have lead us all on a merry dance towards the major myelin proteins. In 1979 Kadlubowski and Hughes identified P2 as the neuritogen responsible for induction of EAN after immunisation with whole peripheral nerve and was also shown to induce EAN by active immunisation in its purified form [7]. P2 is expressed in the compact myelin and is thought to

play a role in the stacking and stabilisation of apposing Schwann cell membranes as they come together to form myelin. Kadlubowski and Hughes finished their paper by stating, “Now that we have clearly identified the neuritogen involved in the animal model of Guillain-Barré Syndrome, we intend to extend our study to look for evidence of sensitisation to P2 in the human disease”. And so began the merry dance.

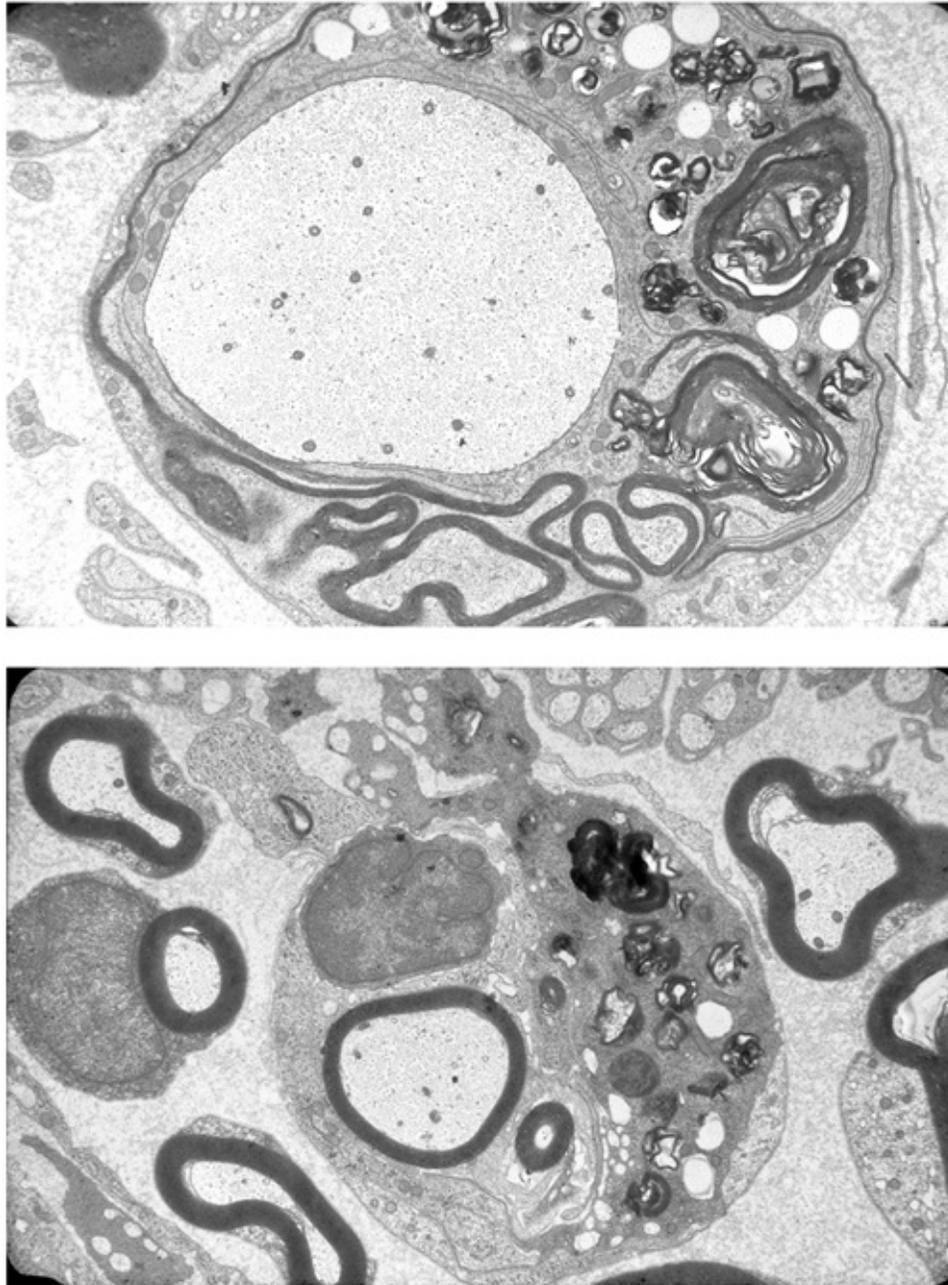


Figure 48.1 Myelin stripping and phagocytosis of compact myelin. Reproduced with permission from [6].

Over the next few decades researchers screened patient sera for antibodies to the major myelin proteins—particularly for those that could induce EAN, including P2, P0 and PMP-22. There have been numerous studies screening sera for antibody reactivity to these major myelin proteins, but thus far they have not proven to be key targets and there is no consensus as to their pathogenic relevance in those patients in whom antibodies have been detected.

P0 Protein

P0 is the most abundant protein in PNS myelin. It forms tetramers that interact in cis and transform the molecular glue that holds together the extracellular space of compact myelin. Immunisation with bovine P0 produces EAN in the Lewis rat and P0-specific T cells transfer EAN to naïve rats. In 1992 Khalili-Shirazi and colleagues reported T-cell responsiveness to purified P0 protein in 6 of 19 GBS cases, and IgG and IgM antibodies to P0 in about one-third of cases [8]. However, there is no convincing evidence from passive transfer studies for pathogenicity of these autoimmune responses to P0 in GBS.

PMP-22 Protein

Duplications or deletions in the gene for PMP-22 protein account for the majority of cases of hereditary neuropathy. PMP-22 is also neuritogenic in the Lewis rat, and antibodies to synthetic peptides representing either the first or second extracellular domains of PMP-22 have been reported in 58% of 19 GBS patients and 4% of 51 normal subjects [9]. Other groups have failed to detect such a clear relationship between GBS or CIDP and antibodies to this protein. Interestingly, antibodies to PMP-22 appear in 70% of patients with CMT1a and 60% of those with CMT2 and in 23% of normal people [10]. It has been suggested that such antibodies may play a role in patients with CMT1a who experience sudden deterioration.

Galactocerebroside

In 1978 and over the next few years the husband-and-wife team of Takahiko and Kyoko Saida, working in Philadelphia, published reports in *Nature* and *Science* which aroused considerable international interest [12,13]. These reports described a powerful demyelinating effect of rabbit EAN serum when injected into rat sciatic nerve. This effect was shown to be due to antibodies to galactocerebroside (GalC), and this glycolipid was shown to be neuritogenic in the rabbit. The Philadelphia group subsequently reported that sera from patients with GBS caused demyelination when injected into rat nerve [14], a finding confirmed by some but not by others, but none of the positive findings showed the potency of rabbit EAN serum and the technique of intraneural injection was felt to be unphysiological. Although GalC is highly enriched in compact myelin, it is also present in Schwann cells and the Saidas' group described extensive Schwann cell damage in their original description of anti-GalC-induced EAN. Samukawa and colleagues have reported antibodies to GalC in patients with GBS, some following CMV infection [15]. Passive transfer studies of these antibodies would be of great interest.

Molecular Mimicry in AIDP?

Thus far, the major advances in pathogenesis and defining pathogenic antigens, at least in the AMAN subtype, occurred in the 1990s in studies of human serum particularly by Nobuhiro Yuki in Japan. Due to the success of identifying the relationship between antecedent *Campylobacter jejuni* infection in AMAN and the generation of pathogenic anti-ganglioside antibodies, attempts have been made to identify similar mechanisms of molecular mimicry in AIDP. Recently Sawai and colleagues identified antibodies to the protein moesin in 5 out of 6 AIDP patients who had antecedent CMV infections [16]. Moesin was identified as a target in this case by using a proteomic approach to screen sera with proteins extracted from schwannoma cells and identify proteins that bound IgG using mass spectrometry. Moesin is part of the ERM (ezrin, radixin, moesin) family expressed on Schwann cell microvilli at the nodes of Ranvier and is among a growing group of proteins that may be relevant to demyelinating neuropathies but are not

located in the compact myelin. However, nothing is ever simple in the complicated world of GBS and another group were unable to detect an autoantibody response to any of the ERM proteins in AIDP with antecedent CMV nor in CIDP cases [11].

Searching for a Needle in a Haystack

Studies such as that by Sawai and colleagues [16] have come full circle compared with the approach of finding a protein that induces EAN and then searching for its relevance in the human disease. In the 1970s and 1980s the diversity of myelin proteins was severely underestimated due to the rudimentary methods available. At the time, separation of myelin proteins by gel electrophoresis and Coomassie staining could identify the 3 major bands of P0, P1 and P2 and little else. Novel proteins were gradually added to the cache as new methods of detection became available, and by 2010 there were about 40 known PNS myelin proteins.

In 2011 the first proteomic analysis of PNS myelin identified 545 proteins in mouse sciatic nerve, including 36 previously identified myelin proteins [17]. This study confirmed that PNS myelin is much more complex than previously thought and that in terms of identifying auto-antibodies to myelin antigens in GBS we have really only considered the tip of the iceberg. With this increase in the number of myelin proteins it is not in the least way feasible to test candidate antigens for their ability to induce EAN and then correlate this immune response with clinical serological analyses. Further advances will come from unbiased screening of patient sera and identification of novel targets in patients first, a strategy that has been recently successful in identifying novel autoantibody responses to the nodal regions in CIDP.

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Molecular Mimicry

Nobuhiro Yuki

From the time autoimmune diseases were first discovered, molecular mimicry between human tissue and micro-organisms had been proposed to be a pathogenic mechanism. However, until recently, no studies had convincingly demonstrated this [1] because 4 criteria had to be satisfied to conclude that a disease is triggered by molecular mimicry [2]: (A) establishment of an epidemiological association between an infectious disease and immune-mediated diseases; (B) identification of T cells or antibodies directed against human target antigens; (C) identification of microbial mimics of the target antigen; and (D) reproduction of the disease in an animal model.

Guillain–Barré syndrome (GBS) is the first disease to fulfil all the 4 criteria: (A) an epidemiological association between GBS and *Campylobacter jejuni* infection [3] has been established; (B) auto-antibodies against GM1 or GD1a in patients with GBS subsequent to *C. jejuni* enteritis [4,5] have been identified; (C) Molecular mimicry between GM1 or GD1a and lipooligosaccharides (LOSs) of *C. jejuni* isolated from GBS [6,7] has been demonstrated; and (D) a disease replica has been produced by sensitizing rabbits with GM1 or GM1-like LOS of *C. jejuni* and passive transfer of anti-GM1 or anti-GD1a antibodies in mice [8–11]. Here I describe how we and other investigators have shown that molecular mimicry is a cause of GBS.

Identification of Autoantibodies

Yuki N, et al. Acute axonal polyneuropathy associated with anti-GM1 antibodies following *Campylobacter* enteritis. *Neurology*, 1990; Chiba A, et al. Serum IgG antibody to ganglioside GQ1b is a possible marker of Miller Fisher syndrome. *Annals of Neurology*, 1992

My first encounter with a GBS patient was in 1989 when the individual was admitted to our hospital due to bilateral leg weakness followed by arm weakness developing over several days. He had had watery diarrhoea 1 week before the onset. He presented with tetraplegia and areflexia without any sensory signs, in contrast to the typical patient with GBS who usually describes a glove-and-stocking pattern of sensory impairment. Feasby and colleagues had reported “an acute axonal form of Guillain-Barré polyneuropathy” in 1986 [12], but at that time most clinicians believed GBS to be a demyelinating peripheral nerve disease. Repeated nerve conduction study results in our patient supported a diagnosis of axonal degeneration, and not demyelination. Although our patient complained of subjective distal paresthesia, his clinical and electrophysiological features were similar to amyotrophic lateral sclerosis (ALS). I recalled a paper I had read by Latov’s group published in 1986 in which his group reported a

patient with ALS-like disorder who had IgM M-protein against GM1 and who improved after immunotherapy [13], suggesting what we know now as multifocal motor neuropathy. Their report prompted me to consider the possibility that our patient might also have anti-GM1 antibodies. Our investigations revealed IgG, not IgM, antibodies reacting with GM1 [4]. The titres decreased with the course of the illness. A second patient with GBS who carried anti-GM1 IgG antibodies was later identified. This patient also had antecedent diarrhoea and pure motor weakness. Nerve conduction studies suggested axonal degeneration in motor nerves, but no demyelination.

The presence of watery diarrhoea prior to the illness in both patients provided the clue that a microbial agent might be the trigger for the development of GBS. There had been a few reports of association of GBS with diarrhoea or *C. jejuni* enteritis [14,15]. Although *C. jejuni* had not been widely recognized as an antecedent infectious agent of GBS at that time, both our patients were serologically confirmed as having had an antecedent *C. jejuni* infection [4]. We reported the 2 patients with axonal GBS following *C. jejuni* enteritis and positive anti-GM1 antibodies. In 1999 the Hopkins group extended the concept by showing anti-GD1a antibodies and axonal GBS associated with *C. jejuni* infection [16].

The Miller Fisher syndrome (MFS) is characterized by ophthalmoplegia, ataxia and areflexia [17]. Its link to GBS is strengthened by the observation that some MFS patients develop profound limb weakness typical of GBS during the clinical course of their illness [18]. Chiba and colleagues identified anti-GQ1b IgG antibodies in patients with MFS [19], thereby linking not only its clinical features to GBS but also the proposed immune mechanism.

Molecular Mimicry

Yuki N, et al. A bacterium lipopolysaccharide that elicits Guillain-Barré syndrome has a GM1 ganglioside-like structure. *The Journal of Experimental Medicine*, 1993; Yuki N, et al. Molecular mimicry between GQ1b ganglioside and lipopolysaccharides of *Campylobacter jejuni* isolated from patients with Fisher's syndrome. *Annals of Neurology*, (1994)

Administration of bovine brain gangliosides (BBG) was widely used in Western Europe and South America in the early 1990s. Since our case report of an ALS-like disorder after ganglioside therapy [20,21], there have been other reports of patients who developed GBS after ganglioside administration in Italy and Spain [22,23]. A causal association between ganglioside injection and the precipitation of GBS was inferred, and gangliosides were eventually withdrawn in Italy in 1993.

The occurrence of GBS in association with ganglioside administration suggested that *C. jejuni* might carry ganglioside-like structures. LOS constitutes the outer membrane of *C. jejuni*. I cultured a *C. jejuni* strain isolated from a patient with GBS who was positive for anti-GM1 IgG antibodies. I extracted LOS using the hot phenol-water technique and discovered that rabbit anti-GM1 antibodies and the cholera toxin B-subunit, a specific ligand for GM1-oligosaccharide, reacted with the LOS, as well as GM1, on thin-layer chromatogram plates, which consisted of silica beads [24]. This suggested that the LOS carried a GM1 epitope, and that silica bead column chromatography might be helpful in the purification of LOS with a GM1 epitope. The LOS was separated by column chromatography, and fractions were obtained that showed reactivity to rabbit anti-GM1 antibodies and cholera toxin B-subunit. By gas-liquid chromatography mass spectroscopy, I found that the purified LOS contained D-galactose, D-glucose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine and *N*-acetyl neuraminic acid, which are sugar components of GM1 ganglioside. ¹H nuclear magnetic resonance showed that the terminal tetrasaccharide

of the purified LOS was identical to that of GM1 (Figure 49.1) [7]. The bacterial strain also carried a GD1a-like LOS [25].

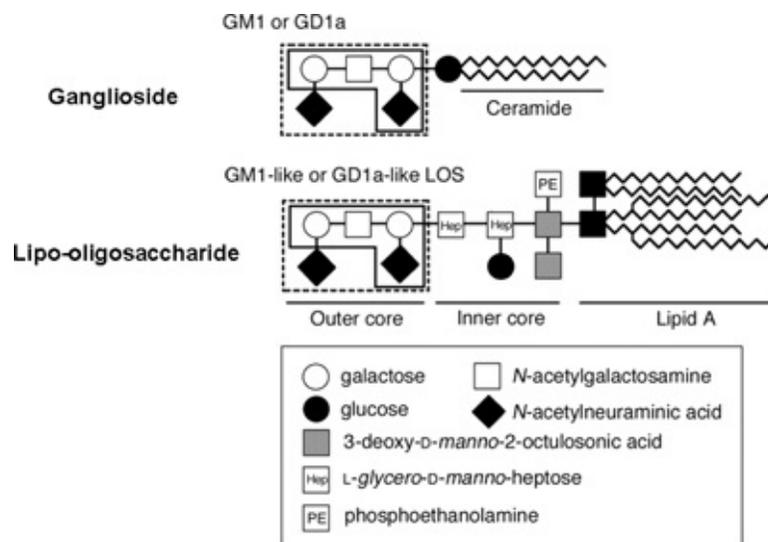
As there were reported cases of MFS subsequent to *C. jejuni* enteritis [26], I postulated that there were some strains of *C. jejuni* that had the GQ1b epitope. In 1993, when I investigated the presence of the GQ1b epitope in *C. jejuni* strains isolated from patients with enteritis, clinicians requested me to test anti-GQ1b antibodies in 2 patients with MFS from whom *C. jejuni* was isolated [27]. I therefore performed thin-layer chromatography immunostaining to show the presence of GQ1b-like LOS of *C. jejuni* from the patients using monoclonal anti-GQ1b antibody. In 1994, we reported the results, suggesting the existence of molecular mimicry between GQ1b and the *C. jejuni* LOS [28]. In 1997, Aspinall's group demonstrated that LOS of *C. jejuni* isolated from a patient with MFS carried GD1c-oligosaccharride (Figure 49.2) [29]. In collaboration with Gilbert, we were also able to demonstrate that *C. jejuni* isolated from patients with MFS bore GD1c-like or GT1a-like LOS mimicking GQ1b [6,30].

Epidemiological Association

Rees JH, et al. *Campylobacter jejuni* infection and Guillain-Barré syndrome. *The New England Journal of Medicine*, 1993

Hughes' group established an epidemiological association between *C. jejuni* infection and GBS through their prospective case-control study of 96 patients with GBS [3]. Patients and controls were systematically examined for evidence of *C. jejuni* infection and a recent *C. jejuni* infection was noted in 26% of the patients with GBS, compared with 2% of the household controls (a member of the patient's household) and 1% of the age-matched hospital controls.

Serological evidence of *C. jejuni* infection was found in 31% of 201 patients with GBS and 18% of 65 patients with MFS [31]. Between 2000 and 2003, we received 367 serum samples from patients with MFS, of which 73 samples with paired hospital controls were available for further analysis. During the same period, we received 1,814 serum samples from patients with GBS, and 73 samples were randomly selected as disease controls. From these samples, we demonstrated that the serologic evidence of recent *C. jejuni* (21%) infection was more common in patients with MFS than in the hospital controls [6].



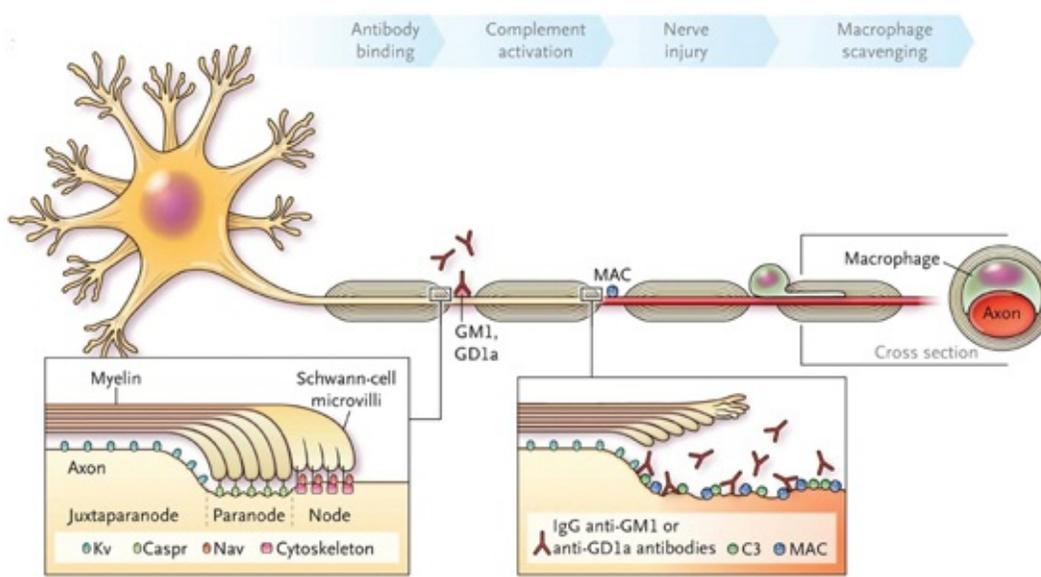


Figure 49.1 Molecular mimicry as a cause of Guillain-Barré syndrome

(A) Molecular mimicry exists between gangliosides (GM1 and GD1a) and *Campylobacter jejuni* lipooligosaccharides (LOSs). Infection by *C. jejuni* bearing GM1-like or GD1a-like LOSs may induce the production of anti-GM1 or anti-GD1a IgG antibodies in certain patients. Modified from 44 with permission.

(B) Myelinated axons are divided into 4 functional regions: the nodes of Ranvier, paranodes, juxtaparanodes and internodes. Gangliosides GM1 and GD1a are strongly expressed at the nodes of Ranvier, where the voltage-gated sodium (Na_V) channels are localized. Contactin-associated protein (Caspr) and voltage-gated potassium (K_V) channels are present at the paranodes and juxtaparanodes, respectively. Anti-GM1 or anti-GD1a IgG antibodies bind to the nodal axolemma, leading to membrane attack complex (MAC) formation. This results in the disappearance of Na_V clusters at the nodes, mislocalization of K_V clusters at the paranodes and the detachment of paranodal myelin, which can lead to nerve-conduction failure and muscle weakness. Axonal degeneration may follow at a later stage. Macrophages subsequently invade from the nodes into the periaxonal space, scavenging the injured axons. Modified from 43 with permission.

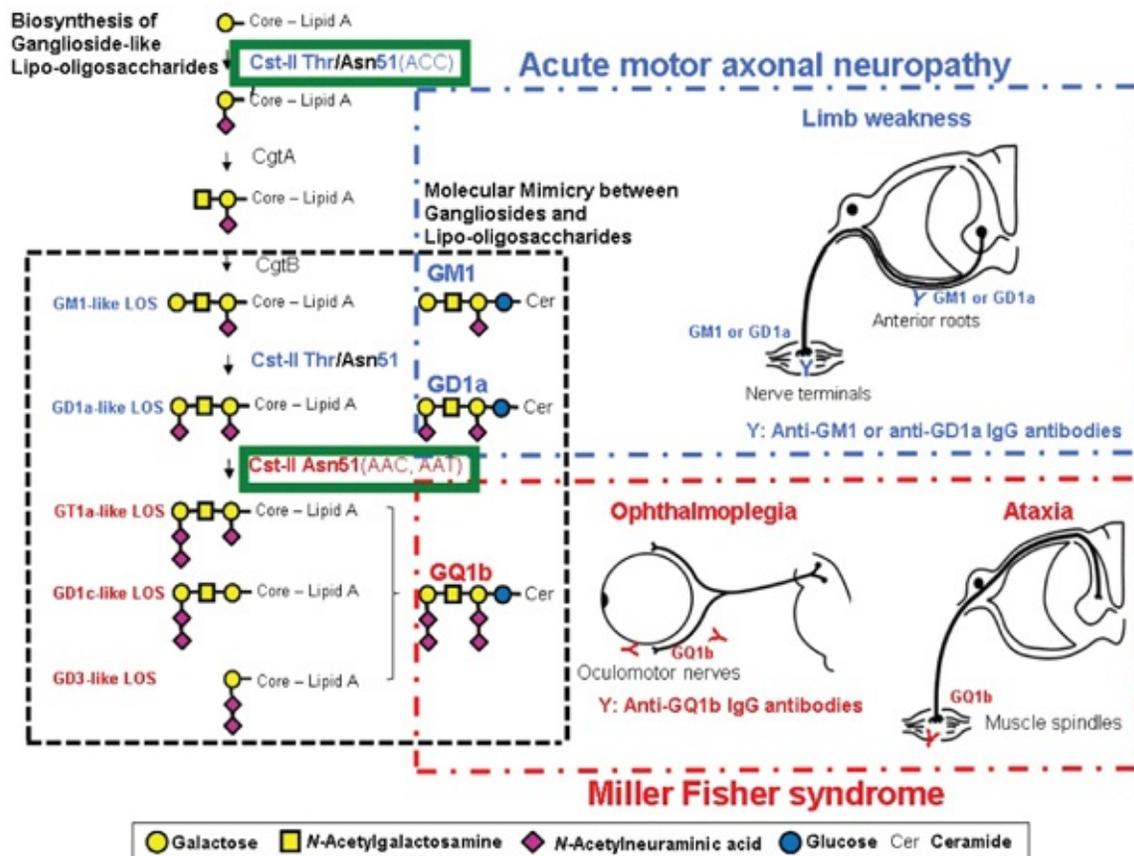


Figure 49.2 Bacterial gene polymorphism to determine clinical features of autoimmune diseases

Campylobacter sialyltransferase cst-II, N-acetylgalactosaminyltransferase cgtA and galactosyltransferase cgtB are instrumental in the biosynthesis of LOS. The 51st amino acid of cst-II determines its enzymatic activity. cst-II (Thr51) produces GM1-like and GD1a-like LOSs, whereas cst-II (Asn51) synthesizes GT1a-like, GD1c-like or GD3-like LOS, which mimics GQ1b. The *C. jejuni* carrying cst-II (Thr51) can express GM1-like or GD1a-like LOS. Infection by such a strain may induce anti-GM1 or anti-GD1a IgG antibodies. The auto-antibodies bind to GM1 or GD1a expressed on the motor nerves of the 4 limbs, causing acute motor axonal neuropathy. In contrast, *C. jejuni* that carries cst-II (Asn51) expresses GQ1b-mimicking LOS. Infection by these *C. jejuni* strains may induce anti-GQ1b IgG antibody production. The anti-GQ1b antibodies bind to GQ1b expressed on oculomotor nerves and muscle spindles in the limbs, leading to Miller Fisher syndrome. Modified from 43 with permission.

Animal Models

Yuki N, et al. Animal model of axonal Guillain-Barré syndrome induced by sensitization with GM1 ganglioside. *Annals of Neurology*, 2001; Yuki N, et al. Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, 2004; Goodfellow JA, et al. Overexpression of GD1a ganglioside sensitizes motor nerve terminals to anti-GD1a antibody-mediated injury in a model of acute motor axonal neuropathy. *The Journal of Neuroscience*, 2005; Susuki K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *The Journal of Neuroscience*, 2007

Kusunoki and colleagues induced acute sensory ataxic neuropathy by repeated sensitization of Japanese white rabbits with 0.5 mg of GD1b together with keyhole limpet hemocyanin and complete Freund's adjuvant [32]. I postulated that when we inoculated Japanese white rabbits with 2.5 mg of a BBG mixture (GM1 21%, GD1a 40%, GD1b 16%, GT1b 19%; Cronassial[®]) according to their protocol [32], at least some rabbits might develop flaccid paralysis or ataxia associated with anti-GD1a or anti-GD1b antibodies because the mixture contained 0.5 mg of GM1, 1 mg of GD1a or 0.4 mg of GD1b.

When we started our animal experiments in 1998, I was very sceptical as to whether the rabbits would develop muscle weakness. Surprisingly, all 13 rabbits inoculated with the ganglioside mixture (Cronassial[®]) developed flaccid paralysis [9]. Limb weakness progressed for 4 to 13 days (median, 5 days) after onset, indicating acute onset. Some of the rabbits began to recover spontaneously, suggesting a monophasic course of the illness as shown in patients with GBS. Unexpectedly, all the diseased rabbits developed high titres of anti-GM1 IgG antibodies, but not anti-GD1a antibodies. The antibody titres did not differ before and after the disease onset, but high affinity antibodies were detected only at the disease onset [33]. This suggested that a high affinity of anti-GM1 antibodies was essential for the development of the disease. We started inoculating rabbits with Sygen[®] (isolated GM1) when a few rabbits developed anti-GM1 IgG antibodies and acute flaccid paralysis [9]. Nine of 11 rabbits developed anti-GM1 IgG antibodies and acute flaccid paralysis. Pathological findings in the rabbit peripheral nerves were predominantly Wallerian-like degeneration with neither lymphocytic infiltration nor demyelination. IgG was deposited at the nodal or intranodal axolemma in the spinal anterior roots. Anterior spinal nerves showed macrophage infiltration in the periaxonal space, but the surrounding myelin sheaths remained almost intact [34]. In addition to these pathological findings, neurophysiological findings also corresponded well with those in human acute motor axonal neuropathy (AMAN), an axonal form of GBS.

We repeatedly injected Japanese white rabbits with *C. jejuni* LOS isolated from a patient with AMAN, which consisted of GM1-like and GD1a-like LOSs [6,8]. We started this experiment in 1999 when a few rabbits developed the disease by sensitization with the BBG mixture. First, we used 2.5 mg of *C. jejuni* LOS, as well as the BBG mixture experiment. Only 4 of 10 rabbits developed flaccid paralysis,

suggesting that sensitization had to be more frequent. Next, we sensitized 10 rabbits with 10 mg of *C. jejuni* LOS, and all the rabbits developed flaccid paralysis. The diseased rabbits had anti-GM1 IgG antibodies, but not anti-GD1a antibodies. The pathological findings, compatible with the features of human AMAN, were evidence that rabbits inoculated with *C. jejuni* LOS constitute a valid AMAN model.

I postulated that anti-GM1 IgG antibodies block voltage-gated sodium (Na_V) channels at the nodes of Ranvier [35]. Susuki and I began these series of experiments in 2004. We showed that in the spinal anterior roots of AMAN rabbits, IgG antibodies bound to nodes of Ranvier, where GM1 was highly expressed, and activated complement, resulting in the formation of membrane attack complex at the nodal axolemma [36]. Na_V channel clusters disappeared at lengthened nodes with complement deposition. There was paranodal detachment along with nodal lengthening, which was seen at the early phase in patients with AMAN [37]. These pathological changes are able to produce muscle weakness. Complement deposition was prominent at the acute progressive phase, but decreased with the clinical course of the disease.

Willison's group was successful in producing murine models of axonal GBS by the passive transfer of human or mice anti-GM1 or anti-GD1a antibodies in the presence of human complement [10,11]. Their excellent studies provided conclusive evidence of the pathogenic roles of anti-ganglioside antibodies and complement in the development of axonal GBS.

The possible pathogenesis of AMAN subsequent to *C. jejuni* enteritis are shown in [Figure 49.1](#): (A) infection by *C. jejuni* bearing GM1-like or GD1a-like LOS induces the production of anti-GM1 or anti-GD1a IgG antibodies [6,8]; (B) these autoantibodies bind to GM1 or GD1a at the nodes of Ranvier in peripheral motor nerves [36]; (C) bound anti-GM1 or anti-GD1a IgG antibodies induce local complement activation, resulting in the formation of membrane attack complex; (D) the autoimmune attack disrupts Na_V channel clusters, producing muscle weakness at the early phase of illness. Axonal degeneration subsequently occurs.

Bacterial Gene Polymorphism to Determine Clinical Features of Autoimmune Diseases

Koga M, et al. *Campylobacter* gene polymorphism as a determinant of clinical features of Guillain-Barré syndrome. *Neurology*, 2005

Ganglioside-like LOSs are synthesized by sialyltransferase *cst-II*, *N*-acetylgalactosaminyltransferase *cgtA* and galactosyltransferase *cgtB* [38]. LOS biosynthesis loci have been divided into several classes based on gene organization, and classes A, B and C carry *cgtA*, *cgtB*, and *cst-II*, and the strains belonging to these classes could express ganglioside-like LOSs [39]. In 2003, Gilbert and I began our collaborative work. Most isolates from GBS and MFS patients belonged to classes A, B or C, and the frequency was significantly higher than isolates from patients with uncomplicated enteritis [25]. In other words, *cgtA*, *cgtB* and *cst-II* are the genes responsible for the development of peripheral neuropathies. In contrast, two-thirds of enteritis strains belonged to the classes, and they did not always induce the development of GBS. This suggested that host factors are also important for the development of neuropathies after the bacterial infection.

In 2002, Gilbert and colleagues reported that *cst-II* sialyltransferase consists of 291 amino acids, with the 51st determining its enzymatic activity [38]. *Cst-II* (Thr51) has only α -2,3-sialyltransferase activity

(mono-functional) and produces GM1-like and GD1a-like LOSs (Figure 49.2). In contrast, cst-II (Asn51) has both α -2,3- and α -2,8-sialyltransferase activities (bi-functional), and synthesizes GT1a-like or GD1c-like LOSs, mimicking GQ1b. When I read their paper, I postulated that *C. jejuni* isolates from GBS had cst-II (Thr51) and that the isolates from MFS had cst-II (Asn51). Koga and I began experiments using the relevant isolates. We found that neuropathic strains were more frequently found to have cst-II, in particular cst-II (Thr51), than did enteritic ones (82% versus 52%) [40], whereas strains with cst-II (Thr51) had the GM1 and GD1a epitopes, strains with cst-II (Asn51) regularly expressed the GQ1b epitope. The presence of these bacterial epitopes in neuropathic patients corresponded to autoantibody reactivity. Patients infected with *C. jejuni* (cst-II Asn51) more often were positive for anti-GQ1b IgG antibodies and had ophthalmoparesis and ataxia. In contrast, patients who had *C. jejuni* (cst-II Thr51) were more frequently positive for anti-GM1 and anti-GD1a IgG antibodies and had limb weakness.

Why a microbial infection leads to the development of different autoimmune diseases has yet to be clarified. For example, the mechanism of how group A streptococcal infection induces acute rheumatic fever in some patients and acute glomerulonephritis in others is unknown. The mechanism of how *C. jejuni* induces GBS in some patients and MFS in others, however, was made clear by our findings.

The molecular pathogenesis of GBS or MFS subsequent to *C. jejuni* enteritis is as follows (see Figure 49.2): *C. jejuni* strains that carry cst-II (Thr51) express GM1-like or GD1a-like LOS on its cell surface and may induce anti-GM1 or anti-GD1a IgG antibodies in some infected patients. The autoantibodies bind to GM1 or GD1a expressed on the motor nerves of the limbs, producing AMAN. In contrast, *C. jejuni* strains that carry cst-II (Asn51) expresses GT1a-like or GD1c-like LOS on its cell surface, and infection by such a strain may induce anti-GQ1b antibodies in some patients. The autoantibodies bind to GQ1b expressed in the oculomotor nerves and muscle spindles [41,42], resulting in MFS.

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OUTCOME

50

Predicting Outcome in GBS

Christa Walgaard

Introduction

A neuromuscular professor in Rotterdam has a Mark Twain quote pinned to the wall of his room, which states “It is difficult to make predictions, especially about the future.” The relevance of this statement became apparent when the professor and I were trying to make predictions about the future of individual patients with the Guillain-Barré syndrome (GBS). And we are not the first to do so; in the last 100 years much hard work was done to make the best possible prognostication to guide patients and their families. And nowadays also their doctors to give the best suitable therapies, ultimately accomplishing personalized medicine.

Top 10

Guillain G, Barré JA, Strohl A. Sur un syndrome de radiculonévrite avec hyperalbuminose du liquide céphalo-rachidien sans réaction cellulaire. Remarques sur les caractères cliniques et graphiques des réflexes tendineux. *Bulletins et Mémoires de la Société médicale des Hôpitaux de Paris*, 1916.

In 1916 the 3 founders and name-givers of the Guillain-Barré syndrome described a flaccid paralysis in 2 formerly healthy soldiers [1]. This disease entity was different from the then much-more-prevalent cause of flaccid paralysis, polio myelitis, in 2 ways: the ‘dissociation albumin-cytologique’ and the much better prognosis. The authors stated, ‘The prognosis does not appear to be extremely serious, if we may judge from the course of the disease in our two patients; the first had almost recovered and the second was improving when they were discharged from the army’. In 1936 Guillain described 10 more cases, all with a favourable outcome [2]. The medical journals in those days must not have had as strict regulations on maximum word counts as we enjoy nowadays, as Guillain meticulously describes those 10 cases. In one of those cases he makes probably the first of many predictions of outcome in a patient with GBS: ‘Although physicians who had examined the patient had given an unfavourable prognosis, I predicted a favourable outcome.’ In those first decades after the first description of the disease the outcome looked good just because the diagnosis was GBS instead of polio myelitis.

Pleasure DE, et al. The prognosis of acute polyradiculoneuritis. *Neurology*, 1968.

At that time they were in such a way convinced that the prognosis of GBS was good, that patients with a

poor prognosis were labelled as having 'atypical polyneuritis'. Osler and Sidell suggested in 1960 that the eponym Guillain-Barré syndrome should be applied only to patients without atypical signs such as incomplete recovery [3]. However, in 1968, Pleasure, Lovelace and Duvoisin described a cohort of 49 patients and found no differences in the clinical picture between patients with GBS and 'atypical polyneuritis' as suggested by Osler and Sidell [4]. They concluded that patients with GBS can indeed have an unfavourable clinical course and outcome, probably due to severe demyelination and Wallerian degeneration.

Eberle E, et al. Early predictors of incomplete recovery in children with Guillain-Barré polyneuritis. *Journal of Pediatrics*, 1975.

Surprisingly, one of the first large cohorts analysed to find predictors of outcome included only children. This was a retrospective study of 47 children admitted to a rehabilitation centre between 1959 and 1972 [5]. The children were followed until full recovery or for at least 3 years without full recovery. Full recovery was defined as good and normal strength of all muscle groups (grading scale: zero, trace, poor, fair, good and normal) in the first 3 years after GBS. Thirty-six children (77%) made a full recovery, but all 47 children were ambulant after 3 years, even in the incomplete recovery group. Eberle and colleagues found muscle weakness of the upper and lower extremities, absence of deep tendon reflexes in lower extremities, low protein level in cerebrospinal fluid, long hospitalization time and longer time from maximal weakness until beginning of the improvement as significant predictors of incomplete recovery. This last predictor was seen as the most useful clinical predictor and plotted in a graph ([Figure 50.1](#)) as a tool for clinicians. They concluded that prospective studies would be needed to further investigate their findings. In hindsight it seems likely that the long period between maximal weakness and the beginning of improvement was caused by axonal damage.

Raman PT, Taori GM. Prognostic significance of electrodiagnostic studies in the Guillain-Barré syndrome. *Journal of Neurology Neurosurgery and Psychiatry*, 1976.

Only one year later Raman and Taori initiated a study of the prognostic significance of electrodiagnostic studies in GBS, the first to suggest that axonal damage is a poor prognostic sign [6]. They found striking differences in prognosis between patients who had no fibrillation potentials (81% good prognosis), indicating pure demyelinating disease, and patients with fibrillation potentials, with or without conduction velocity abnormalities (32% good prognosis), indicating (secondary) axonal damage. Debate about the optimal timing of nerve conduction studies and the phenomenon of switching between categories is still ongoing. However, in this study serial nerve conduction studies were performed biweekly and none of the 50 patients showed any significant alterations from the category originally noted.

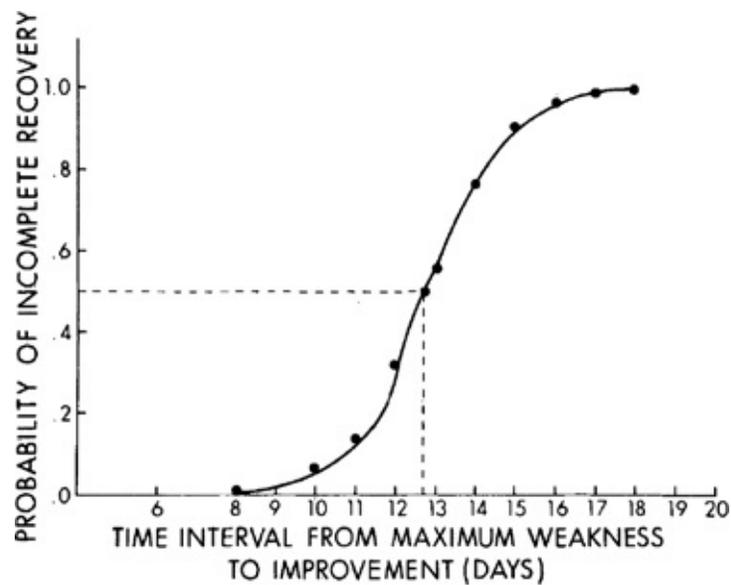


Figure 50.1 Probability of incomplete recovery versus time interval from maximum weakness to improvement (days). Reproduced with permission from [5]

Winer JB, et al. Prognosis in Guillain-Barré syndrome. *The Lancet*, 1985.

Winer and colleagues' short communication of only 2 pages in the *Lancet* in 1985 [7] is important, as it was the first paper that analysed prospectively collected trial data (on prednisolone [8] and plasma exchange [9]) of 71 patients to discover early predictors of poor prognosis. Mechanical ventilation, time to improvement of more than 1 month and a plateau time of more than 3 weeks were identified as significant predictors of poor outcome (restricted in manual activities at 12 months; see [Table 50.1](#)). Also, patients with a poor outcome had a higher mean peak deficit on a functional scale and a longer average time to onset of improvement.

Winer JB, et al. A prospective study of acute idiopathic neuropathy. I. Clinical features and their prognostic value. *Journal of Neurology Neurosurgery and Psychiatry*, 1988.

After their analysis of trial data, Winer and colleagues set up a prospective cohort study to answer all kinds of questions about GBS, including identifying predictors of outcome [10]. This study was published as a triptych in May 1988. One hundred patients with GBS in southeast England were included and followed for 12 months. As outcome measures Winer and colleagues used being bed-bound at 3 months and the inability to undertake manual work after one year. The 4 most discriminating variables are shown in [Table 50.2](#). This was the first study to find age as a significant predictor of poor outcome, which can be explained by different cut-offs used in former studies. After this study many more studies were published, confirming the following factors as predictors for poor outcome: age, clinical severity, diarrhoea and axonal features in nerve conduction studies.

Table 50.1 Significance of possible predictive factors. Reproduced with permission from [7]

| | Good outcome | | Poor outcome | | χ^2 (1df) |
|------------------------------|--------------|-----|--------------|-----|----------------|
| | No. | % | No. | % | |
| Requiring ventilation | 13 | 25 | 16 | 80 | 15.5** |
| Age 18 or more | 43 | 84 | 20 | 100 | 2.1 |
| Time to peak deficit <4 days | 7 | 14 | 7 | 35 | 2.9 |
| Time to improvement > 1 mo | 20 | 39 | 16 | 80 | 8.0* |
| Plateau time > 3 wk | 9 | 18 | 12 | 60 | 11.5** |
| CSF cell count > 10/ μ l | 0 | 0 | 2 | 10 | 2.2 |
| Presence of bulbar signs | 28 | 55 | 12 | 60 | 0 |
| Total | 51 | 100 | 20 | 100 | |

*p<0.01

**p<0.001

Table 50.2 Relative risks of poor outcome at 12 months attributable to the 4 most discriminating variables. Reproduced with permission from [7]

| | Relative risk | 95% confidence intervals |
|-------------------------------------|---------------|--------------------------|
| Age \geq 40 years | 4.4 | 1.2–16.4 |
| Disability grade 4 in \leq 4 days | 3.1 | 1.0–9.6 |
| Requiring ventilation | 2.6 | 0.9–7.6 |
| APB MAP \leq 1 mV | 3.8 | 1.0–14.0 |

van Koningsveld R, et al. A clinical prognostic scoring system for Guillain-Barré syndrome. *The Lancet Neurology*, 2007

In this landmark study, van Koningsveld and colleagues developed a simple clinical prognostic scoring system for use in clinical practice, which combined different statistical significant predictors for poor outcome after 6 months using prospectively collected trial data (Table 50.3 and Figure 50.2) [11].

Kuitwaard K, et al. Pharmacokinetics of intravenous immunoglobulin and outcome in Guillain-Barré syndrome. *Annals of Neurology*, 2009.

Immune therapy with IVIg and plasmapheresis undoubtedly improved the prognosis of GBS patients; however, not all patients do well after therapy. Kuitwaard and colleagues searched for an explanation and found that there is a large variability in IgG rise after IVIg treatment [12]. A multivariate analysis, correcting for known prognostic factors, indicated that a low rise in serum IgG after IVIg therapy was an independent predictor for poor outcome. This may suggest that some patients need a higher dosage of IVIg. Confirmation of this finding would be a first step towards personalized medicine in GBS; IVIg dosage can be tailored individually according to serum IgG rise. In the ongoing randomized placebo-controlled trial to the effect of a second IVIg dosage in GBS patients with a poor prognosis (SID-GBS trial) this will be tested.

Table 50.3 The Erasmus GBS outcome score. Reproduced with permission from [11]

| | Categories | Score |
|---|------------|-------|
| Age at onset | > 60 | 1 |
| | 41–60 | 0.5 |
| | ≤ 40 | 0 |
| Diarrhoea (≤ 4 weeks) | Absence | 0 |
| | Presence | 1 |
| GBS disability score (at 2 weeks after entry) | 0 or 1 | 1 |
| | 2 | 2 |
| | 3 | 3 |
| | 4 | 4 |
| | 5 | 5 |
| Erasmus GBS outcome score | | 1–7 |

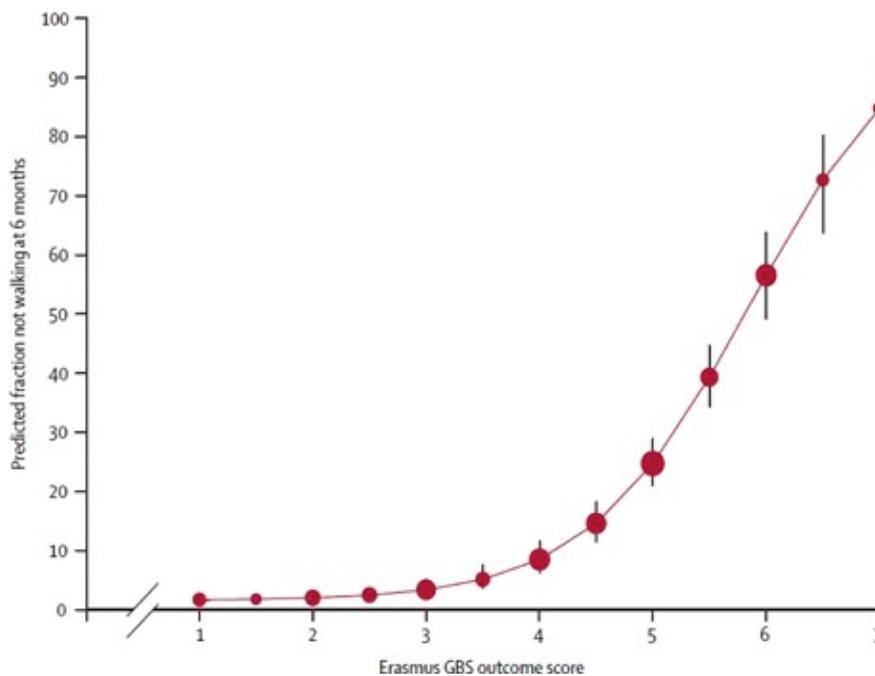


Figure 50.2 Predicted fraction of patients unable to walk independently at 6 months after randomization on the basis of the Erasmus GBS outcome score (n = 762). Vertical bars indicate 95% CI. Point sizes proportionate to the number of patients with a specific score. The probability of not walking independently at 6 months is given by the equation $1/(1+\exp[8.2-1.4 \times \text{EGOS}])$. Reproduced with permission from [11]

Walgaard C, et al. Prediction of respiratory insufficiency in Guillain-Barré syndrome. *Annals of Neurology*, 2010.

The previously described studies all tried to predict clinical outcome in terms of functional outcome after a longer period of time. In this study Walgaard and colleagues constructed a simple clinical model to predict respiratory insufficiency; the model can be applied at hospital admission and is therefore very useful in clinical practice [13] (Table 50.4). Patients with a high risk of respiratory insufficiency need to be monitored closely, possibly in an intensive care unit, to take precautions when the need for mechanical ventilation emerges. By now this model is used in many countries and included in many clinical guidelines on the management of GBS patients.

Fourrier F, et al. A simple functional marker to predict the need for prolonged mechanical ventilation in patients with Guillain-Barré syndrome. *Critical Care*, 2011

The duration of mechanical ventilation varies widely in GBS patients, ranging from a few days to months and even years. When a long duration of mechanical ventilation is expected, tracheotomy should be considered. Fourrier and colleagues analysed 40 mechanically ventilated GBS patients and found that the lack of foot flexion after immunotherapy combined with sciatic motor conduction block is strongly predictive of prolonged mechanical ventilation and tracheotomy should be considered [14].

Future Perspectives

In the last 100 years a lot has been learned about the prognosis of GBS patients and it is now possible to predict long-term outcome and other sequelae of the disease quite well in general perspectives. However, much is not known or not confirmed yet. Patients and their families have a lot of questions (Will I get rid of this awful tiredness? When will I be able to work again?) that are not easy to answer despite all the knowledge we have gathered, nor is it possible to tailor treatment personally for individual patients. So Mark Twain is still right in his quote and inspires professors today to conduct large studies. The International GBS Outcome Study (IGOS), a large international prospective study set up by the International Neuropathy Consortium included > 1,000 GBS patients worldwide (www.gbsstudies.org). Final goals of the IGOS are to be able to better inform patients and relatives about the prognosis of GBS, to understand the mechanism of disease progression and recovery and to conduct selective therapeutic trials to improve outcome in patients with poor prognosis.

Table 50.4 Erasmus GBS Respiratory Insufficiency Score (EGRIS)

| | Categories | Score |
|---|------------|-------|
| Days between onset of weakness and hospital admission | > 7 days | 0 |
| | 4–7 days | 1 |
| | ≤ 3 days | 2 |
| Facial and/or bulbar weakness at hospital admission | Absence | 0 |
| | Presence | 1 |
| MRC sum score at hospital admission | 60–51 | 0 |
| | 50–41 | 1 |
| | 40–31 | 2 |
| | 30–21 | 3 |
| | ≤ 20 | 4 |
| EGRIS | | 0–7 |

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Outcome Measures in Guillain-Barré Syndrome: From a Plummer's Tool to Interval-Based Metrics

Ingemar S.J. Merkies, Mariëlle H.J. Pruppers and Catharina G. Faber

Introduction

Change has a considerable psychological impact on the human mind

To the **fearful ones** it is threatening because it means that things may get worse ...

To the **hopeful** it is encouraging because things may get better ...

To the **confident** it is inspiring because the challenge exists to make things better ...

Whitney Young, 1921–1971

In a recent paper it was stated that the development of outcome measures in inflammatory neuropathies has received little attention when compared to the efforts taken in understanding of the many facets of inflammatory neuropathies [1]. Capturing the history of scoring in neurology brings us to Dr Henry Plummer, who developed an outcome measure to 'objectively and reliably' measure neuromuscular weakness in patients [2]. It is surprising to see that this tool is still broadly being used. Plummer's idea could presumably be considered as a strong geological phenomenon resembling a monolith, such as the Rock of Gibraltar. We can imagine Dr Plummer being very proud that his idea, captured in its beauty of simplicity, went 'viral' decades before the Internet was launched.

It is also amazing to note that we as 'modern' physicians are still stuck in Plummer's non-algorithmic way of describing ordinal-based observations, despite the known constraints of ordinal-based data. We still consider the findings at neurological examination as real measurements, which in essence they are not [3,4,5]. 'Quantitative' (e.g. neurological) observations are based on counting observed events, while meaningful measurements are based on the arithmetical properties of interval or ratio measures [3,6]. Observations, like muscle strength assessment at bedside using Plummer's modified tool, provide a good description of the clinical question of interest. The problem arises when we create sum scores from these observations, treating these scores as linear, and make assumptions from the findings [4,7]. We, as physicians, tend to hold on to the 'known truth' and provide information regarding 'the best way' to assess clinical changes in a particular illness of interest, thereby assuming, often incorrectly, that we have the proper knowledge to do so [8]. What we 'know' often tends to limit our ability to look at the same construct from a different perspective. We irrationally apply fixed decision-making to our use of outcome measures in the belief that what we 'know' reflects real 'knowledge'. And yet that belief is not necessarily 'true' [9].

In the following, we describe eight papers that reflect the evolution of outcome measures assessment

in Guillain-Barré syndrome (GBS), highlighting briefly their content, main strengths and weaknesses.

Dyck PJ, et al. History of standard scoring, notation, and summation of neuromuscular signs. *Journal of the Peripheral Nervous System*, 2005

This is a must-read ‘historical essay’ for all researchers in the field of inflammatory neuropathies and for neurologists in general [2]. The history of scoring neurological observations like muscle weakness, sensory deficit, and tendon reflexes are being highlighted in an elegant way in this paper, starting from the 19th century when Mitchel and Lewis initiated the practice of alphanumeric scoring of neurological signs [10]. The historical links between Plummer’s tool, the introduction of the Medical Research Council (MRC) grading system and the motor subset of the neurological impairment scoring (NIS) system are also addressed. The strength of this paper is captured in the historical findings which shaped our neurological observations of today and suggesting standardisation of the various parts to increase consistency, as was recently demonstrated in an additional paper [11].

Its weakness relates to the relatively ‘unknown’. As an example, the NIS, introduced in 1991, is a composite score of various neurological observations such as muscle weakness, sensory deficit and changes in tendon reflexes, and has been the primary outcome measure in a large number of neuromuscular studies [12,13]. Despite its broad use, the obtained scores from the NIS are used as if these observations represent a ruler with a fixed unit, which is highly unlikely. Others have suggested using great caution when interpreting changes in scores coming from ordinal-based scales like the NIS [14,4,3]. There is a risk of drawing incorrect inferences using ordinal-based composite measures: positive trials may be falsely positive and as a consequence we may expose patients to interventions that might be harmful, and negative trials might be falsely negative, denying proper therapy to patients [3,14]. These methodological inconsistencies as part of modern scientific assessments are not addressed in this paper and will be the focus throughout this chapter.

Hughes RA, et al. Controlled trial prednisolone in acute polyneuropathy. *The Lancet*, 1978

This is the first randomized trial of prednisolone versus placebo in patients with GBS [15]. A total of 40 patients entered the trial and the primary outcome measure, the Functional-score was introduced that ranged from 0 (healthy) to 6 (dead). The authors concluded that steroid treatment is not beneficial and can even be detrimental in GBS. The functional score (F-score), a ranked ordinal-based scale, has been slightly modified through the years, introducing the ability to walk 5 or 10 meters and was later named the GBS-disability score. This score was used in subsequent trials in GBS as the primary outcome: only patients who were not able to walk 5 to 10 meters independently were eligible for randomization and the primary objective was often addressed as the proportion of patients that improved one grade or more on the GBS-disability score, thus reflecting the ability to walk 5 to 10 meters independently [16,17,18]. In the 1984 trial, Greenwood and colleagues stated the F-scores “were easy to assess and not usually subjected to observer variability” [16].

Simplification of assessment may indeed enhance consistency, and the GBS-disability score has also been incorporated in prognostic models providing evidence for ‘good recovery’ at the six-month follow-up [19]. However, in a recently presented study as part of the Peripheral Neuropathy Outcome Measures Standardisation Study (PeriNomS), it was demonstrated that having the ability to walk independently did

not adequately reflect the full scope of activity limitations and participation restrictions as perceived by patients using the inflammatory Rasch-built overall disability scale (I-RODS), an interval ruler that assesses a larger array of functionality [20,21]. The patients with GBS may indeed improve to the ability of walking 5 to 10 meters independently, thus ‘doing better’, but they are not necessarily doing well [22]. This observation implies that, in performing randomized trials including only those patients who are not able to walk independently, we might bypass those who still experience substantial functional limitations despite their ability to walk independently.

Kleyweg RP, et al. Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barré syndrome. *Muscle & Nerve*, 1991

In this study, Kleyweg and colleagues state that the level of interobserver agreement using the GBS-disability score “is not known and that this method seems to be insensitive when applied to bedridden and artificially ventilated patients” [23]. Therefore, they developed an additional score, the MRC sum score, which ranges from 0 (total paralysis) to 60 (normal). The authors reported kappa coefficients that were “almost perfect” and also analysed the range of MRC sum score in relation to the GBS-disability scores. The results showed functional groups 1–3 having limited variation in their MRC sum scores, whereas in functional groups 4 and 5, the MRC sum score ranged from 0 to 55. Deterioration as well as recovery was better monitored by the MRC sum scores.

Since then, the MRC sum score has been used in several trials in GBS and has been incorporated as part of the daily routine examination. However, there are severe methodological limitations to the use of a composite measure like the MRC sum score: the MRC grades, introduced in 1943, are ordinal-based and creating such a sum score only reflects counting observed events rather than creating a ruler with a fixed unit, thus having no intrinsic numerical value [24]. Hence, this brings us back to the concept of type of data and how to deal with these [5]. This will be thoroughly discussed in the section about Vanhoutte, Faber, Merkies and the PeriNomS study group article, below.

Vanhoutte EK, et al. Modifying the Medical Research Council grading system through Rasch analyses. *Brain*, 2012

The MRC grading system is the most commonly used method of evaluating muscle strength clinically [2,24]. Despite the power of its simplicity, with great reliability scores reported, this method has been criticized due to the unequal width of its ordinal constructed categories. In particular, the inequality of the MRC grades (grades 1, 2 and 3 being too narrow, and 4 being too broad) has been extensively discussed by comparison with a dynamometer (data at the ratio level) for assessing strength [25,26,27,28,29]. Attempts to modify the scale even further have been reported [26,30,31,32]. The 2012 study by Vanhoutte and colleagues [25] investigated whether clinicians in the neuromuscular field could properly differentiate between patients with various neuromuscular disorders and degrees of muscle weakness using a Rasch method [33]. The results demonstrated disordered thresholds in 80% of the muscles examined according to the MRC grades in 1,065 patients (n = 480 had GBS) [34,35]. Physicians (whether senior experts or residents) were unable to differentiate between the grades 1 through 4. Most thresholds were restored after rescoring the original 6 response options to 4 categories. The MRC grading system, although in use for 7 decades, failed to meet Rasch model’s expectations. A modification to a 4 response

carrying out certain duties/responsibilities. The top graph shows the inability of patients to discriminate among the 7 response options using Rasch analyses. The bottom graph illustrates the rescoring of the response options, hereby creating an ideal picture of an ordered threshold. Reproduced with permission from [20].

van Nes SI, et al. Rasch-built Overall Disability Scale (R-ODS) for immune-mediated peripheral neuropathies. *Neurology*, 2011

The van Nes and colleagues' E-ODS study presents the development of a new scale based on Rasch methodology that challenges the outcome measurement belief we have acknowledged for decades [1,33]. The paper also aims to teach the basic principles of types of data and their requirements, and highlights the steps needed to transform ordinal-obtained data into interval measures using the Rasch technique, aiming to simplify its mathematical background to more digestible pieces. A comprehensive educational paper on Rasch's background, specifically for neurologists, was also recently published [44]. The inflammatory RODS (I-RODS) is the first disease-specific interval measure constructed for patients with inflammatory neuropathies. I-RODS was built after completion of its preliminary form by 294 patients (174 with GBS), hence capturing and centralizing the voice of patients and fulfilling all the Rasch model expectations, including high validity and reliability scores. Through comparative responsiveness studies, the PeriNomS study group subsequently succeeded in demonstrating the superiority of the I-RODS over the ordinal-based outcome measures used thus far in inflammatory neuropathies [45]. Despite these steps forward, I-RODS needs further evaluation, particularly regarding its cross-cultural validity.

Vanhoutte EK, et al. 196th ENMC international workshop: Outcome measures in inflammatory peripheral neuropathies. *Neuromuscular Disorder*, 2013

At the 3rd European Neuromuscular Centre (ENMC) workshop the PeriNomS study group produced a paper on outcome measures in inflammatory neuropathies [46,47,48]. In this workshop, 20 neuromuscular researchers from various countries and a patient representative of the GBS CIDP Foundation International discussed the longitudinally obtained responsiveness results for selected outcome measures comparison and strove for consensus on a specific core set of scales for future clinical studies in various forms of inflammatory neuropathies. The workshop highlighted themes like the historical background of assessment, various trial design aspects, traditional versus modern requirements for evaluation and construction of (new) outcome measures and the concept of minimum clinically important difference (MCID). Finally, all serially obtained comparative data were presented.

Recommendations were ultimately provided: for future trials in GBS, the consensus was to adopt the I-RODS as the primary outcome, since this outcome measure has demonstrated significantly higher (heuristic and statistical MCID related to varying standard errors (SE) across the metric; MCID-SE) responsiveness. In addition, the following outcome measures were suggested as part of the minimum core set of scales for future GBS studies: grip strength (Martin Vigorimeter), the Rasch-transformed modified INCAT sensory sum score, being ventilated (yes/no), duration of respiratory ventilation, and the GBS disability scale (for historical purposes). Recommendations to also focus on pain and fatigue were given. Measuring strength at bedside needed further exploration, since there was no consensus on how, and if, strength should be measured.

Vanhoutte EK, et al. Rasch-ionale for neurologists. *Journal of the Peripheral Nervous System*, 2015

Various educational papers have been written on the Rasch method some of which are referred to here [49,50]. We strongly recommend physicians in the research field of GBS read these papers as well as the recently published “Rasch-ionale for neurologists” [44]. In the latter paper, the PeriNomS study group aimed to systematically address the concept of types of scales based on types of data (nominal, ordinal, interval, ratio) collected, discussing differences between classical versus modern test theories, emphasizing the (dis)advantages of both streams, highlighting and simplifying the various steps needed in the evaluation and construction of (new) outcome measures using the Rasch method, and striving to increase the knowledge and utility of this technique. The background of the Rasch modelling is discussed, providing simple graphical examples to improve its understanding. In addition, various steps are addressed like statistical model fit requirements, having (or not) ordered thresholds, differential item functioning, local dependency, uni-dimensionality, and other requirements like items’ weights and persons’ location reliability, as well as various definitions of being a responder, emphasizing the use of MCID-SE. All these steps are graphically explained, highlighting potential pitfalls and ways to handle and improve the data. It is argued that Rasch-built outcome measures should be used for future studies in neuromuscular disorders and their method of construction could easily be extrapolated to other neurological illnesses.

Epilogue

Future studies embracing the unmet needs highlighted by the PeriNomS group should be imbedded in the ongoing registries in GBS and other inflammatory neuropathies. Validation of new outcome measures using larger samples of collected data from independent cohorts, potential relation to biomarkers, development of new prognostic models to predict clinical course, and examining cross-cultural validation of selected outcome measures like the I-RODS are just some of the essentials that require further investigation. We believe that the lessons learned, particularly from the PeriNomS studies, should help us to utilize and focus more on outcome measures that are preferably at the interval and ratio level of assessment.

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Evidence-Based Practice in Rehabilitation for Guillain-Barré Syndrome

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Introduction

Guillain-Barré Syndrome (GBS) or acute inflammatory demyelinating polyneuropathy (AIDP) is a monophasic immune-mediated disorder due to inflammation of peripheral nerves and nerve roots [1]. It presents as an evolving sensorimotor polyneuropathy of varying severity, which leads to rapidly developing motor deficits (symmetrical ascending paralysis), autonomic dysfunction, sensory deficits and respiratory failure [2,3,4]. The annual incidence is estimated to be 1–2 per 100,000 worldwide and affects both sexes equally [5,6]. It can occur at any age, but has a reported preponderance between ages 30 and 50 (approximately 30% of cases are under 20 years old) [7,8]. GBS can be described as a heterogeneous syndrome with several variants and as a collection of clinical syndromes, the most common type being AIDP [4].

With advances in medical management, the incidence of GBS has been stable in the majority of developing countries and mortality has been reduced to 2–3% (but is higher in the developing world) [6,9]. In general, mean time to the clinical function nadir is 12 days, with 98% of patients reaching a nadir within 4 weeks from onset [8]. Overall mortality from GBS is low; approximately 5–10% of patients may die of complications in the acute phase of GBS [8,9,10]. The progressive phase of GBS typically is limited to 4 weeks, and the majority of patients make a good physical recovery and are ambulant within 6 months from onset of symptoms [11]. However, just under two-thirds (30%) of patients may have rapid progression, requiring artificial ventilation within a couple of days, due to involvement of respiratory and bulbar muscles [2]. Approximately 20% of patients may have residual, permanent, severe disability, with deficits in ambulation, or require ventilator assistance 12 months later [1]. Autonomic dysfunction (sinus tachycardia or bradycardia, fluctuating hypertension or hypotension, flushing of the face, loss of or excessive sweating) can occur in up to 70% of patients; this can be associated with sudden death [3]. Facial weakness and cranial nerve involvement occurs in more than half of patients [2]. A number of factors, including preceding diarrhoea, older age, rapid progression, disability at nadir and specific neurophysiological parameters have been associated with poor outcome [12,13].

Long-Term Disabilities in Persons with GBS

There is limited information on longer-term sequelae of GBS and their impact on everyday life. A longitudinal study (N = 76 patients) reported that despite good functional recovery up to 14 years post-

GBS (median 6 years, range 1–14), 16% of patients continued to report moderate to extreme impact on work, family and social activities; and 22% reported ongoing substantial impact on mood, confidence and ability to live independently [14]. Other studies show that psychosocial performance does not necessarily correlate with the severity of impairment in GBS, but may be explained by poor conditioning and fatigue [15,16]. Approximately 40% of all cases require intensive inpatient rehabilitation [10] and can present to rehabilitation settings with a wide array of physical, emotional, psychosocial and/or environmental difficulties. These disabilities can have a cumulative effect over time and cause considerable distress to GBS survivors (and their families), and reduce their quality of life (QoL) [14,17]. These can limit their function and participation with a high impact on daily activities, vocational activities and social activities (work, family and intimate relationships, and community/social activities).

The World Health Organization, International Classification of Functioning, Disability and Health (ICF) [18], provides a standard framework for disability and participation in various health conditions, including contextual factors. Rehabilitative care uses the terminology of this classification system to describe the impact of the disease at different levels. A simulated case example of the ICF model related to GBS is given in [Figure 52.1](#).

A list of ICF categories (in all domains) relevant to the care of pwGBS was developed through a rigorous, multi-method, scientific process, incorporating patient and multidisciplinary perspectives [19]. The GBS ‘core set’ represents a selection of ICF categories/items for a minimal standard of reporting in clinical settings (brief core set) or comprehensive assessment (comprehensive core set) [19]. It is envisaged that these core sets describe specific biopsychosocial issues; provide a solid and stable reference point; test the current and future health status measures; and can be used for defining ‘what should be measured’ [20].

An integrated approach was used, which included a comprehensive review of literature (peer review and grey literature) documenting rehabilitation interventions currently used in management of a person with GBS (pwGBS). A comprehensive search of the literature published up to December 2015 was undertaken using the Medline, Embase, PubMed and Cochrane Library databases. The search strategy included interventional studies investigating rehabilitative management of pwGBS, using combinations of multiple search terms for 3 themes: GBS, rehabilitation interventions and patient outcomes. Medical subject heading (MeSH) search terms were used for all databases, and a keyword search was used if the MeSH term was not available. Bibliographies of identified articles were scrutinised for additional references and a manual search of relevant journals was undertaken. A grey literature search using different Internet search engines and websites such as System for Information on Grey Literature in Europe, New York Academy of Medicine Grey Literature Collection, and Google Scholar, was also undertaken. Additional searches of the websites of prominent national and international organisations associated with GBS management were conducted to identify relevant reports, health technology assessments or other related materials. This review excluded pharmacological agents and other alternative/conservative interventions, which do not typically form part of the rehabilitation process.

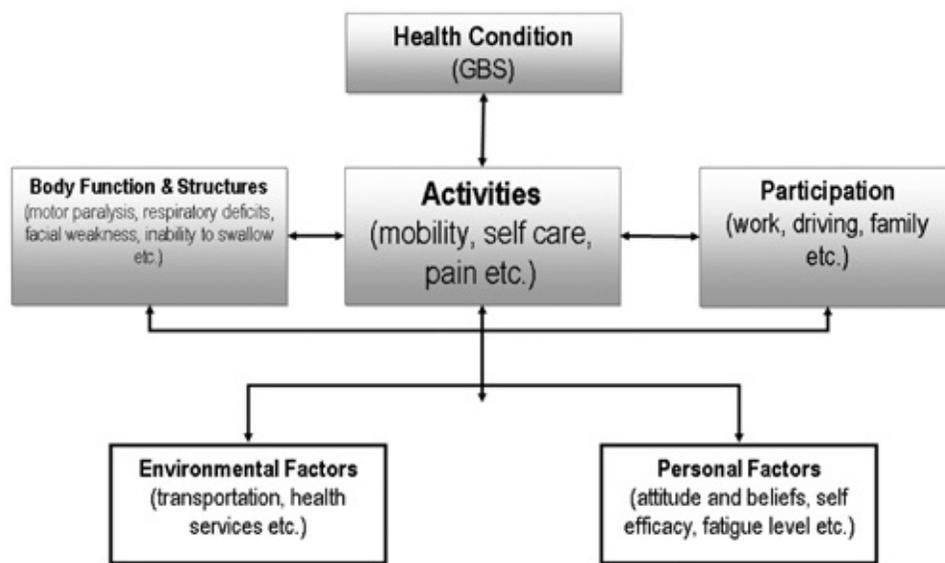


Figure 52.1 ICF model with case example for GBS

Systematic reviews, meta-analyses (MA), and randomised controlled trials (RCT) were given high priority, and where high-level evidence was not available lower levels of evidence were reviewed (such as observational studies, narrative reviews etc.).

Evidence for GBS Rehabilitation

The majority of GBS survivors are young; hence, the emphasis should be on the provision of long-term integrated care [17]. Rehabilitation is an integral component in overall management [17]. The main focus of rehabilitation in pwGBS is on reducing symptoms and limitations at the level of a person’s activity and participation (including personal and environmental factors). It extends beyond acute management, to restoration of patient’s previous daily activities and reintegration into the home and community [1,17]. It is estimated that over one-third of all GBS patients require inpatient rehabilitation, particularly those who are more severely disabled with an extended period of disease nadir [1]. Earlier reports suggest that inpatient rehabilitation should be initiated earliest possible and continued for 3–6 weeks, followed by a community and home-based rehabilitation program for 3–4 months [10]. Existing GBS clinical guidelines and frameworks recommend comprehensive, flexible interdisciplinary coordinated care with appropriate follow-up, education and support for patients (and carers) [21,22].

A rehabilitation approach to GBS includes a wide spectrum of treatments and use of diverse interventions. The existing best-evidence synthesis for specific rehabilitation interventions in GBS are summarised below, based on compilation of most recently published studies. (See [Table 52.1](#).)

Multidisciplinary Rehabilitation

Multidisciplinary (MD) rehabilitation is defined as the “co-ordinated delivery of intervention by two or more disciplines (physiotherapy, occupational therapy, social work, psychologist and other allied health, nursing), under medical supervision (neurologist, rehabilitation physician)” [23]. It is designed to be patient-centred, time-based and functionally oriented, and aims to maximise activity and participation (social integration) using a biopsychosocial model [20].

Table 52.1 Summary of studies evaluating rehabilitation intervention in GBS.

| Study (author, year, country); | Design, participants (N) | Main findings |
|---|--|---|
| <i>Multidisciplinary rehabilitation</i> | | |
| Khan et al., 2010; Australia | Systematic review (included studies = 3 observational studies) | MD rehabilitation improved disability (function) in the short term (less than 6 months) and QoL |
| Khan et al., 2011; Australia | RCT (N = 79) | High-intensity MD rehabilitation effective in reducing disability, improved function compared with low-intensity rehabilitation |
| <i>Physical therapy</i> | | |
| El Mhandi et al., 2007; France | Prospective cohort study without controls (N=6) | Individualized physical therapy programme based on muscular reinforcement and active mobilization increased isometric and isokinetic strength |
| Garssen et al., 2004; Netherlands | Prospective cohort study without controls (N = 20) | Structured 12-week bicycle training beneficial for physical fitness, function and QoL |
| Bussmann et al., 2007 Netherlands | Case series (N = 20, including 6 with CIDP) | Physical exercise improved fitness in severely fatigued pwGBS |
| <i>TENS</i> | | |
| Bokhari and Zahid, 2010; Pakistan | Case report (N = 2) | TENS contributed in marked improvement in function and pain reduction |
| <i>Other interventions</i> | | |
| Orthoses and ambulatory aids | No studies | Expert opinion |
| Occupational therapy | No studies | Expert opinion |
| Speech therapy | No studies | Expert opinion |
| Nutritional interventions | No studies | Expert opinion |
| Cognitive/psychological interventions | No studies | Expert opinion |
| Bowel bladder intervention | No studies | Expert opinion |
| CIDP = chronic inflammatory demyelinating polyneuropathy; pwGBS = person with Guillain-Barré syndrome; MD = multidisciplinary; N = total number; QoL = quality of life; TENS = transcutaneous electro neuro stimulation | | |

Khan and colleagues conducted a comprehensive systematic review to evaluate the effectiveness of MD care in adults with GBS, especially the types of approaches that are effective (settings, intensity) and the outcomes that are affected [17]. This review did not identify any RCTs or CCTs, and evidence were summarised based on 3 observational studies, which supported the effectiveness of MD rehabilitation programs in inpatient settings in terms of improvements in activity (disability) and participation for up to 6 months [17]. These 3 studies [24,25,26] included a total of 128 participants and all were rated as ‘very low’ quality using the grades of recommendation, assessment, development and evaluation (GRADE) approach [27]. All studies showed a consistent result with an improvement in disability from the time of inpatient rehabilitation admission to discharge in a timeframe shorter than 12 months. There was no conclusive evidence in regards to improvement in QoL during or after rehabilitation. Overall

generalisation of the results was limited, as the 3 studies included patients with severe GBS with high levels of physical dependency. This highlighted a need for future research using robust study designs, appropriate outcome measures and optimal intensity/modality of rehabilitation therapy [17].

Khan and colleagues subsequently conducted a RCT (N = 79) to evaluate the effectiveness of MD ambulatory rehabilitation in pwGBS after the initial post-acute phase (median 6.5 years since diagnosis) [23]. The authors compared a high-intensity MD rehabilitation program with a low-intensity rehabilitation program over 12 months. The intensive rehabilitation program included individualized, functional, goal-oriented MD treatment comprising half-hour blocks of therapy sessions from a MD team (medical, occupational, social, psychology, speech and physiotherapist), 2 to 3 times per week for up to 12 weeks. The therapeutic model included physiotherapy for strengthening, endurance and gait training; occupational therapy to improve everyday function (domestic, community tasks), driving and return to work; and clinical psychology for counselling and support, and medical input from rehabilitation consultants as required. The findings demonstrated that high-intensity rehabilitation programs were effective in reducing motor disability (mobility, self-care, continence program) and improving participation (relationships) [23].

Specific Rehabilitation Interventions

A variety of rehabilitative interventions have been trialled in pwGBS; there is, however, a dearth of research evaluating these interventions. Moreover, many of these proposed interventions are yet to be integrated into comprehensive MD rehabilitation programs, and few studies show its implementation. The existing evidence for various specific rehabilitation interventions in GBS are summarised below.

Physical Therapeutic Modalities

Improving or restoring physical abilities is a key issue in rehabilitation of pwGBS. Physical therapeutic modalities may involve a graduated mobility program, which includes maintenance of posture and alignment, maintaining joint range of motion, provision of orthotics, endurance and muscle strengthening, and progressive ambulation program using adaptive gait aids [21]. The literature investigating effectiveness of physical therapeutic modalities is sparse.

Physical Therapy/Exercise

The review did not identify any systematic reviews or RCTs evaluating physical therapy in persons with GBS. The best evidence are summarised below from a few observational studies.

El Mhandi and colleagues conducted a prospective cohort study (N = 6) to evaluate individualized physical therapy programs based on muscular reinforcement and active mobilization (average 2–3 weekly sessions) for pwGBS [28]. The results demonstrated significant muscle strength improvement using dynamometric measures and all patients satisfied the criteria for a full recovery at 18 months. At 6 months, manual muscle testing and functional independence motor total scores were close to normal levels. Compared with matched healthy controls, isometric and isokinetic strength increased significantly during the first 6 months, though muscle strength increased less rapidly between 6 and 18 months [28].

Garssen and colleagues, in a prospective cohort study, evaluated the effectiveness of a 12-week bicycle exercise training in 20 patients with polyneuropathy (including 16 with GBS) and severe fatigue [29]. The findings showed significant beneficial effect of bicycle training on physical fitness, functional outcome and QoL of these patients. There was also a significant improvement in the post-intervention

fatigue scores (decrease by 20%). Further, all participants tolerated intervention well and the majority (80%) were motivated to continue with regular training activities [29].

Bussmann and colleagues, in a case series report, explored the effects of physical exercise in severely fatigued pwGBS and chronic inflammatory demyelinating polyneuropathy patients [30]. The authors reported that physical exercise did not influence changes in fatigue, actual mobility and perceived functioning, but did improve fitness. Significant relationships were found between the domains: perceived mental functioning and actual mobility, perceived mental functioning and perceived physical functioning, and fatigue and perceived physical functioning.

Other physical modalities, such as digital gait analysis [31], partial body weight support systems [32], percutaneous kyphoplasty [33] and podiatrons (mechanized rotating platforms) [34] were explored in pwGBS, but their effectiveness is yet to be determined. Earlier reports suggest that care should be taken not to overwork muscle groups [35] as this can lead to paradoxical weakening [36]. Patients also can develop tightness of muscles, rather than joint contractures [1,8]. It is advocated that exercise programs should initially be non-fatiguing and strengthening exercises can be applied as muscles regain greater antigavity strength [37]. More evidence is needed for the use of physical therapeutic modalities.

Transcutaneous Electrical Nerve Stimulation

There is conflicting evidence in regards to the beneficial effect of transcutaneous electrical nerve stimulation (TENS) in pwGBS. Evidence from 2 case reports suggests that the application of TENS may be an effective treatment for pain in patients with peripheral neuropathy [38,39]. The authors concluded that further research is needed to use TENS in routine management of pain in GBS.

Orthoses and Ambulatory Aids

A high proportion of pwGBS experience mobility problems, muscle weakness, paralysis, balance impairment and/or fatigue, which can be alleviated by using assistive devices [21]. To date there are no studies evaluating the effectiveness of these devices in persons with GBS. Current guidelines and reports recommend that mobility assistive devices such as ankle-foot orthoses, canes, crutches, walkers and wheelchairs should be prescribed for proper positioning and optimising residual motor function [1,21]. Patients with prolonged residual weakness of calf (for example anterior compartment musculature) benefit from devices such as ankle-foot orthosis, modified shoes with a broadened heel and good stabilization around the ankle joint [21,40].

Occupational and Recreational Therapy

Many pwGBS may have prolonged neurologic deficits, subsequently limiting their function, that require occupational restoration and maintenance of functional independence skills in everyday activities [8,41]. There is lack of studies evaluating OT in persons with GBS. Published reports recommend OT to promote activities in pwGBS to facilitate functional self-care, which may include task reacquisition, use of adaptive equipment, and the modification of environment for personal, domestic and community tasks [8,21,24,41]. The introduction of recreational therapy may also support a patient's adjustment to disability and improve social reintegration [41].

Speech Therapy

Severe cases of GBS can be associated with cranial nerve involvement leading to dysphagia and dysarthria, necessitating a speech therapy program to promote communication and safe swallowing skills

[40,41]. Speech therapy programs may include proper positioning, head control, oral motor coordination and conscious swallowing techniques (thicken fluids progressively depending on patient response) [41]. More specific communication strategy may be required for ventilator-dependent patients and tracheostomies [41]. Clinical trials evaluating speech therapy programs in pwGBS are lacking.

Nutritional Interventions

Malnutrition in pwGBS can be caused by immobility, decreased gastric motility, dysphagia and psychological symptoms (such as depression) [42]. Many patients tend to lose weight in the acute phase; therefore, routine assessment of nutritional status is important [43]. Those with malnutrition may require enteric or parenteral nutrition and high protein, high-energy enteral diets [43,44]. There are no studies evaluating nutritional status in GBS.

Cognitive and Psychological Interventions

Cognitive problems have been reported in pwGBS, especially in the acute phase and in those with extended ICU stay [8,17,40]. Anxiety and fear are common due to the sudden onset of symptoms and may accompany with depression in many [40]. Psychotic symptoms (hallucinations, delusions, incoherence) have been reported in more severe patients [40]. There is a lack of studies addressing psychosocial and vocational outcomes in GBS. Expert opinion recommends early cognitive screening and implementation of psychological interventions, such as good communication with patients and/or their families regarding prognosis and treatment plan, involvement in decision-making and early counselling from experts [8,40,42].

Interventions for Respiratory Complications

Respiratory dysfunction is common in one-third of pwGBS, which can lead to serious complications such as incomplete respiratory recovery including chronic obstructive pulmonary disease, restrictive respiratory disease (pulmonary scarring, pneumonia), tracheitis from chronic intubation and respiratory muscle insufficiency [4,22,45]. A few expert opinions suggest initiation of physical therapy measures (chest percussion, breathing exercises, resistive inspiratory training) to clear respiratory secretions and, for more severe patients with tracheostomy, a special weaning protocol to prevent over-fatigue of respiratory muscles [1,8].

Bladder/Bowel Intervention

Autonomic dysfunction in GBS, including urinary and bowel dysfunction, have been reported in pwGBS [21,46]. Bladder dysfunction may include detrusor acontractility, disturbed bladder sensation and non-relaxing urethral sphincter, causing symptoms such as voiding difficulty, urinary retention, frequency and urge incontinence [47,48]. There is lack of studies in GBS addressing bowel/bladder dysfunction in pwGBS, expert opinion suggests individualised management programs may include: timed voiding, intermittent catheterization and anticholinergic medication [35]. Pelvic floor muscle training with or without biofeedback or electrical stimulation, are also commonly used in women with stress leakage and mixed urinary incontinence [35,41]. Similarly, effective bowel management program includes an appropriate diet, adequate fluids, scheduled bowel care, and laxatives for those with bowel dysfunction [4,35].

Discussion

This narrative review provides an evidence-based overview of the effectiveness of rehabilitation intervention in pwGBS. A limited number of studies evaluate the effectiveness of rehabilitation intervention, and ‘best’ evidence to date is based upon expert opinions and observational studies. Despite recommendations for many rehabilitation interventions, there is a paucity of information on effectiveness of these treatments. The ‘best’ available evidence to date is for studies evaluating MD rehabilitation which provide some support in producing longer-term gains in the levels of activity (disability) and participation of pwGBS. The evidence for most uni-disciplinary rehabilitation interventions remains limited and/or poor and is based upon ‘low quality’ observational studies (using the GRADE methodology for bias) or inferred from other neurological conditions. Adequate descriptions of what comprises a ‘black box of rehabilitation’ (therapy intensity, duration, modalities) in GBS are lacking.

GBS is a complex condition with marked clinical heterogeneity and a varied level of disability, requiring an individualised approach to rehabilitation. The clinical decision-making process can be subjective and biased, and clinicians may not always agree with one another [49]. GBS survivors need regular evaluation for persisting disability and psychological sequelae (especially over time), and clinicians should incorporate the patient perspective on functioning and health [19]. The ICF, a useful tool in the rehabilitation context, can describe the patients’ experience by emphasizing the complex ways in which the ‘condition’ and contextual (environmental or personal) factors (Figure 52.1) may modify outcomes [17]. It lists information for clinicians about domains that are considered important by the pwGBS and provides a common language for more effective communication and agreement amongst the treating MD clinicians [19].

Difficulties in assimilation of data are further compounded by a wide range of rehabilitation interventions proposed for the pwGBS and diverse outcomes used [17]. Integrated GBS services are needed to address issues not only in acute settings, but also over the longer-term care [17]. Further, a national registry and or formal data collection process (nationally and internationally) are needed to gather information on variability in the types of rehabilitation programs available and their outcomes in GBS survivors. An analysis of the National Rehabilitation Dataset reviewed outcomes of inpatient rehabilitation for pwGBS (N = 572) from 162 accredited rehabilitation facilities across Australia and New Zealand, and showed improved clinical efficiency of inpatient rehabilitation, reductions in hospital length of stay and increased discharge of these persons back to community [50]. Such analyses assist in reviewing rehabilitation outcomes to identify future clinical needs for planning health service provision [50].

Conclusions

GBS is a complex and challenging condition, with many survivors experience residual neurological and neuropsychological sequelae over the long term. This paper highlights the current gap in evidence for rehabilitation intervention in persons with GBS. However, a gap in current research should not be interpreted as an ineffectiveness of rehabilitative intervention. Integrated, holistic, multidisciplinary rehabilitative care with integrated long-term care of these persons is recommended to address various medical and physical problems and issues of participatory restriction. Education and support for pwGBS (and their carers), and the treating multidisciplinary teams should be incorporated into future management models. More research is needed to support specific rehabilitative interventions in GBS.

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Physiotherapy in Guillain-Barré Syndrome: Developing the Evidence over the Years

Claire White

Introduction

It is not easy to show the value of physiotherapy in the management of GBS; the condition is relatively rare and there is limited previous research. There are currently no comprehensive, evidence-based guidelines for physiotherapy, and practice has been based on experience from other neurological conditions.

The following compilation of my Top 10 is a reflection on the history of physiotherapy in the United Kingdom, the role of physiotherapy within the multidisciplinary management of GBS and the potential for a better understanding of any unique, effective contribution of physiotherapy to patient outcomes.

The History of Physiotherapy in the United Kingdom

Nicholls DA, Cheek J. Physiotherapy and the shadow of prostitution: the Society of Trained Masseuses and the massage scandals of 1894. *Social Science and Medicine*, 2006; Øvretveit J. Medical dominance and the development of professional autonomy in physiotherapy. *Sociology of Health & Illness*, 1985

Nicholls and Cheek provide a commentary on the emergence of new forms of physiotherapy practice against the social and political background of the late Victorian era [1]. The period was influenced by the industrial revolution, urban overcrowding as the population moved from the country to the cities, associated public health developments and the greater emancipation of women. The authors note that it was becoming more acceptable for educated women to take up professional roles not only within the previously accepted female professions of nursing and teaching but also in new areas. One area that burgeoned during this period was the use of massage and the movement to complement nursing. This became so popular that soon the market for masseurs and masseuses was overstocked with variably trained individuals and an editorial in the *British Medical Journal* (1894) described concerns regarding the practices of less reputable massage establishments. This led to the birth of physiotherapy, when in the same year the Society of Trained Masseuses (STM) was formed by 4 nurses and midwives in a response to the massage scandals. They sought to regulate the education, training, registration and practice of massage by establishing one of their founding rules of the society that “no massage be undertaken except under medical direction”. Whilst this achieved the aim of distancing therapeutic massage from moral

outrage, it was a rule that the profession subsequently fought long and hard to remove to establish professional independence.

Health services organisational research in the 1980s [2] further documents the development of the profession as the STM became chartered in 1920 and was renamed the Chartered Society of Physiotherapy (CSP) in 1944. Later, following the creation of the Council for the Professions Supplementary to Medicine in 1960, where membership of the board was still one-third medical, the CSP was awarded the right to monitor its own standards and quality of education before finally achieving autonomous practitioner status in 1977. Since that time physiotherapists have furthered their clinical autonomy by advancing methods of clinical reasoning, increasing specialisation and becoming more theoretically oriented with greater involvement in research activity.

Learning from Poliomyelitis: A role for Physiotherapy in the Management of GBS

Cooksey FS. The role of physiotherapy in the treatment of poliomyelitis. *Proceedings of the Royal Society of Medicine*, 1948

Guillain-Barré syndrome (GBS) and poliomyelitis (polio) share some characteristics of both presentation and clinical course with initial acute flaccid paralysis followed by a period of neurological recovery and later adaptation to long-term problems. In 1916, the same year as Guillain, Barré and Strohl first described the symptoms of acute ascending motor weakness and showed how signs from lumbar puncture could differentiate between the 2 conditions, there was also a major polio epidemic. During the epidemic physical remedies and therapeutic massage were already being used to treat polio sufferers. A description of physiotherapy as an important therapeutic approach for the management of polio was subsequently published in 1948 by Dr Frank Cooksey and his description compares well with current recommendations for the management of patients with GBS:

First the inflammatory phase ... when treatment is directed ... to relieve pain, and to prevent stiffness or deformity by prophylactic movements.... Treatment involving some disturbance of the patient is necessary to mitigate these secondary effects.

Secondly the stage of potential recovery ... when the purpose of treatment is to assist the recovery of paralysed or weak muscles within the limits determined by the permanent damage in the CNS.

Thirdly the stage of chronic disability when ... appliances, vocational training and the development of compensatory function in surviving muscles are re-employed ... suited to the residual capacity of the individual. [3]

Cooksey also identifies potential dangers of physiotherapy and highlights the need to evaluate interventions for fidelity, safety and efficacy.

The obvious danger is that physiotherapy will be too vigorous and meddlesome in the beginning when hope and enthusiasm run high, and ineffective at the end of a long period of treatment just when it might make all the difference in the final accommodation to a permanent disability.

Understanding Disablement and Functioning in GBS

Lennon SM, et al. Reasons for persistent disability in Guillain-Barré syndrome. *Clinical Rehabilitation*, 1993; World Health Organization. *International Classification of*

Several previous reports identified persistent motor weakness as the primary cause of long-term disability in people after GBS. However, Lennon and colleagues demonstrated that, rather than presuming a hierarchical relationship between impairment, disability and handicap, clinicians should undertake objective measurements of all 3 outcomes to evaluate the true impact of the condition after neurological recovery [4]. Indeed, the INCAT group later showed that significant and meaningful associations exist between impairment, disability and handicap [5]. They recommended wider consideration of the factors contributing to disablement, since only just over half of the variance in handicap could be explained by impairment scores.

This disconnect between impairment, function and societal impact is something that physiotherapists repeatedly encounter when working with clients, and physiotherapy education was a relatively early adopter of the International Classification of Functioning that was published by the World Health Organisation in 2001 [6]. The ICF superseded the previous classification of impairment, disability and handicap (ICIDH) [7] for use as the scientific standardization of data on health and disability worldwide. It incorporates both the medical and social models of disability and a role for contextual personal (psychological) and environmental (physical and social) factors in describing functioning that recognises

reductions in the incidence and severity of disability in a population can be brought about both by enhancing the functional capacity of the person and by improving performance by modifying features of the social and physical environment.

This acknowledges different perspectives of functioning and disablement and supports a multidisciplinary approach to management of disablement in GBS.

Multidisciplinary Care in GBS

Khan F, et al. Outcomes of high- and low-intensity rehabilitation programme for persons with chronic phase after Guillain-Barré syndrome: a randomized controlled trial. *Journal of Rehabilitation Medicine*, 2011

The first randomised controlled trial of a multidisciplinary intervention for people at least one year after recovery from GBS illustrates the challenges of implementing the MRC guidelines for evaluating complex interventions [8]. The meticulously described and comprehensive study evaluated outcome at the level of activity and participation, and reported both on intention to treat and on treatment analyses of all complete cases. The moderate to small effects of reduction in activity limitations of mobility, self-care and continence as well as a similar improvement in participation for 'personal relationships' suggests an important role for MDT rehabilitation but highlights the challenges associated with interpreting the findings for specific active components of the intervention. The intervention comprised individualised, goal-oriented treatment incorporating therapy sessions with relevant disciplines based on participant need. The clinical rationale for this form of tailored approach to MDT intervention is clear but in RCTs the impact of such an individualised approach means the role of specific therapeutic components including physiotherapy is hard to determine.

One uncontrolled prospective study [9] evaluated the feasibility and likely effect of unsupervised physiotherapist-prescribed exercise on disability for people following recovery from GBS and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). Improvements in the majority of outcomes were observed after the intervention, suggesting that exercise may be beneficial in ameliorating these

persistent problems. However, the study design means it is not possible to attribute observed improvements directly to the intervention. Therefore, as Khan recommends [8], future research should address the paucity of evidence with well-designed RCTs of rehabilitation for GBS that include quantifying the specific components of rehabilitation interventions.

What Research Questions Are Important to People with GBS?

Forsberg A, et al. Balancing everyday life two years after falling ill with Guillain-Barré Syndrome: a qualitative study. *Clinical Rehabilitation*, 2015

In order to ensure that research addresses important outcomes for patients, evidence from qualitative studies is essential. A recent qualitative interview study is one of only a few aimed at exploring the experience of people living with GBS [10]. Interviews were conducted with people at least 2 years after onset of GBS and reiterate the variability in recovery of impairment, activities and participation from observational studies. The study's main theme of 'Striving for balance in everyday life' highlights the impact of symptoms and psychological adaptation on how patients manage the recovery process. What is particularly interesting is not only the varied lived experience but that the extent to which interviewees were able to cope with or accept their situation was crucially important. Where people had felt able to cope with long-term limitations, they also expressed greater satisfaction with health care. Conversely, and more importantly, where people felt they had not been listened to by health care professionals, they felt vulnerable and had difficulty accepting the consequences of their illness. The fact that all participants, irrespective of their impairments, including fatigue, described prioritizing time to exercise as a way of feeling better suggests that listening to patients' needs and preferences to enable them to engage effectively in exercise or physical activity is likely to be important in promoting well-being.

Fatigue in GBS

Merkies IS, et al. Fatigue in immune mediated polyneuropathies. *Neurology*, 1999

Fatigue is a highly prevalent problem for people with GBS. Experienced fatigue is the subjective sensation of persistent feelings of overwhelming tiredness or fatigue unrelated to physical activity. This is in contrast to fatigability which is the observable change in physical performance or muscle fatigue that is associated with physical activity. Whilst both are likely to exist for some people with GBS, Ingemar Merkies and colleagues demonstrated that experienced fatigue was reported by 80% of people with GBS [11]. This fatigue was not associated with physical symptoms of strength, sensory disturbance or physical function but rather with social and emotional functioning. Severe fatigue, defined as a score of greater than 5 on the fatigue severity scale, reportedly occurs for between 35% and 80% of people with GBS in observational studies, although the relationship between fatigue and health status is somewhat unclear. However, one uncontrolled study of supervised exercise for people with severe fatigue as a result of GBS showed improvements in fatigue and quality of life associated with participation in regular exercise [12], but this remains to be confirmed in future RCTs.

Evaluating Complexity and Behaviour Change in Interventions for GBS

Campbell M, et al. Framework for design and evaluation of complex interventions to improve health. *BMJ*, 2000; Michie S, et al. The behavior change technique taxonomy (v1) of 93 hierarchically clustered techniques: building an international consensus for the reporting of behavior change interventions. *Annals of Behavioral Medicine*, 2013

In the *BMJ*, Campbell and colleagues describe the important contribution to health care research of the MRC guidelines for designing and evaluating complex interventions [13]. Multidisciplinary care for the management of GBS constitutes significant complexity at both disciplinary and organisational levels. Research into only a single MDT discipline can also present substantial complexity since a typical home exercise programme prescribed by a physiotherapist includes individualised assessment, prescription of exercise tailored to client clinical presentation, needs and preferences, compliance with and adherence to a programme of exercise, self-monitoring and progression of activity. This often requires considerable and sustained behaviour change on the part of the patient and it is therefore important to propose a clear mechanism for how an exercise intervention is likely to work at the outset.

If behaviour change is a desired outcome then one tool for achieving this is a recent classification of 93 behaviour change techniques (BCT) by Michie and colleagues [14]. The BCT taxonomy may aid in identifying components of physiotherapy or exercise prescription and progression alongside more conventional descriptions. The taxonomy includes groupings of techniques such as ‘goals and planning’ (problem solving) and ‘shaping knowledge’ (behavioural experiments). These could be used to explore whether exercise interventions may be more effective if patients are involved in problem-solving by identifying any potential barriers to exercise prior to setting specific exercise goals. In addition, exercise adherence may be enhanced if patients are able to self-monitor the outcomes of exercise, including in the form of behavioural experiments where they can be encouraged to test out any negative beliefs they may hold about exercise (e.g. ‘all exercise will increase my fatigue’, ‘people will stare at me if I go to the gym’). Behavioural experiments and graded activity permit patients to try out new behaviours and use the results to inform their ongoing exercise. Thus physiotherapists can use BCT to ensure that they listen effectively to patients’ experiences and incorporate them in a genuinely client-centred approach to prescribing exercise that is meaningful to patients with a fair chance of successful uptake. Our ongoing Home Exercise for Inflammatory Neuropathy Trial (HINT) includes the use of BCT as part of a theory-driven exercise intervention [15].

Conclusion

The patient-focussed approach of physiotherapy offers great possibilities in the management of people with GBS. I hope that this brief history of physiotherapy in relation to the condition shows that in order to improve outcomes for patients we need to conduct qualitative research in true collaboration with patients to identify meaningful questions. This will allow us develop rigorous randomised controlled trials, paying meticulous attention to components of both physiotherapy and wider multidisciplinary interventions.

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Fatal Guillain-Barré Syndrome

Bianca van den Berg and Bart C. Jacobs

When the paralysis reaches its maximum intensity the danger of asphyxia is always imminent. However in eight out of ten cases death was avoided either by skillful professional intervention or a spontaneous remission of this phase of the illness. In two cases death occurred at this stage.

Jean Baptiste Octave Landry, 1859 [translated, original version in French] [1]

Introduction

Landry already reported in 1859 that the disorder, later known as the Guillain-Barré syndrome (GBS), can be fatal [1]. Guillain, Barré and Strohl instead emphasized the spontaneous recovery and relatively good outcome of this syndrome, but GBS has been a life-threatening disorder ever since [2].

As a reference centre for GBS in the Netherlands, we are frequently consulted about the most severe cases of GBS. We would like to present 2 impressive cases that we have encountered in the last years showing that GBS still can be fatal and that the influence of medical care is substantial.

The first case was a child of 4 years old who was transferred to our centre from another hospital after a resuscitation and an emergency intubation. According to the treating paediatrician of that hospital the patient was admitted with pain in the neck, swallowing difficulty, drooling and frequent falling in the last days. The working diagnosis of the paediatrician was a tonsillitis/tonsillar tumour, and the patient was admitted to the children's ward and treated with antibiotics. The day after admission he became abruptly respiratory insufficient and just before intubation the patient had an asystole and required resuscitation. After transferal to our hospital we saw the child for the first time, who had a severe post-anoxic encephalopathy without showing any sign of neurological improvement in the next days. When we talked with the parents about the grave situation and the poor prognosis, they explained to us that in the last week the patient already had difficulty walking and the day before admission he was unable to lift his arms. Considering the diagnosis of GBS, nerve conduction studies showed a severe motor polyneuropathy, CSF an elevated protein level, with normal leukocyte count, and in serum high titres of IgG antibodies to the ganglioside GM1 were found. Patient died some days later. All findings were consistent with a rapidly progressive GBS followed by respiratory failure.[3]

A second case was in another hospital in one of the clinical studies coordinated by our centre. This patient was 84 years old and developed a severe form of GBS with tetraplegia, facial diplegia, bulbar weakness, ventilator dependency and many complications with autonomic dysfunction and even a short asystole. In addition there were severe decubital wounds for which necrotomy was necessary. The patient had already been ventilated for more than 3 months without any signs of recovery and had suffered from persistent and progressive pain. The treating neurologists asked for our advice, and although we

indicated that even severe cases of GBS may recover, the patient, his family and the local treating multidisciplinary team made a decision not to perform any life-prolonging treatments. The patient died one week later.

These 2 cases of fatal GBS not only illustrate the rapidity and severity of GBS, they also show the problems in clinical decision-making for the physicians. Although mortality from GBS is rare, it still exists in both developing and developed countries. For this chapter we have selected 10 studies that we found important for understanding the background of the mortality of GBS. In this chapter we describe the history of mortality and GBS, the mortality rate, the causes of death and risk factors of mortality in GBS, and give our personal view on how mortality may be reduced.

Mortality Rate of GBS

GBS has a variable mortality rate worldwide. Already many clinical cohort studies have been performed studying the mortality rate in GBS. [Table 54.1](#) gives a short overview of the mortality rate of the 10 articles included in this chapter. In 1991 Ropper, Wijdicks and Truax wrote a monumental book for the Contemporary Neurology Series [4]. In this book only a small section was dedicated to the mortality in GBS. An overview was given about the mortality in different series ranging from 1966 to 1988 with a mortality rate from 1% to 20%. The authors state that, due to improvements in respiratory and intensive care, the overall mortality from GBS decreased, although they show no data to illustrate this.

Table 54.1 Mortality in GBS

| Reference | Region/Country | Period | No. Patients | Mortality rate |
|--|---|-----------|--------------|----------------|
| Winer et al. 1988 | South-East England | 1983–1984 | 100 | 10% |
| Ropper et al. 1991 | Different GBS series from different countries | 1940–1988 | 100–302 | 1–18% |
| The Italian Guillain-Barré study group, 1996 | Italy | 1988–1993 | 297 | 11% |
| Lawn et al. 1998 | USA | 1976–1996 | 320 | 4% |
| Alsheklee et al. 2008 | USA | 2000–2004 | 4,954 | 2.58% |
| Dhar et al. 2008 | Canada | 1983–2003 | 76 | 6.5% |
| van den Berg et al. 2013 | Netherlands, Belgium, Germany | 1986–2008 | 527 | 2.8% |
| Ishaque et al. 2014 | Dhaka, Bangladesh | unknown | 491 | 12.4% |

In 1988 Winer, Hughes and Osmond published an influential prospective study of 100 patients with acute idiopathic neuropathy, compatible with a diagnosis of GBS [5]. After a 12-month follow-up, they found that 13 % of the patients had died. Ten of the 13 (77%) deaths probably could be attributed to GBS, indicating a GBS-related mortality rate of 10%. The Italian GBS study group found a similar mortality rate of 11% [6].

Lawn and Wijdicks were among the first to dedicate a whole article to mortality in GBS [7]. This study provides a nice overview of the studies investigating the mortality of GBS conducted between 1962 and 1995, with mortality rates ranging from 1% to 10%. They also reviewed the medical records of 320

patients with GBS admitted to Mayo Clinic-affiliated hospitals and reported a mortality rate of 4%.

More recent studies show a somewhat lower mortality rate. Alsheklee and colleagues used a Nationwide Inpatient Sample database (2000–2004) and searched for patients diagnosed with GBS using the ICD-9-CM to determine the in-hospital mortality rate and predictors of death of GBS in U.S. hospitals. The mortality rate was 2.58%. This study had, however, some important limitations, including a lack of case ascertainment [8].

In 2008 Dhar, Stitt and Hahn studied the morbidity and outcome of GBS patients admitted to the ICU and included 76 GBS patients. They found that the majority of GBS patients admitted to the ICU, although suffering from high morbidity, have a good outcome (ability to walk independently). The mortality rate they found of 6.5% is probably higher than in other recent studies due to the selection of severely affected GBS patients [9].

In our own study, published in 2013, we reviewed prospectively collected data of a cohort of 527 GBS patients from 1986 to 2008. We found a low mortality rate of 2.8% [10]. A reason for the lower mortality rate could be the improvements in standard and intensive care and the high proportion of patients receiving treatment with plasma exchange and intravenous immunoglobulins, 2 proven effective treatments for GBS.

Although these studies show a lower mortality rate in high-income countries, a much graver situation was evident in a presentation about the mortality of GBS in Bangladesh presented at the Inflammatory Neuropathy Consortium meeting of 2014 at Dusseldorf [11]. Ishaque and colleagues from the Emerging Diseases and Immunobiology research group at the International Centre for Diarrhoeal Disease Research in Bangladesh reported a high mortality rate in Bangladesh of 12.4% (61 out of 491). One of the most disturbing results is that 20% of the deceased GBS patients died because there was no mechanical ventilator available.

This study shows that there is a big difference between high-income and low-income countries, probably explained by the different standard in general, supportive and intensive care and the availability of treatment.

Death Causes in GBS

Winer and colleagues and the Italian GBS Study Group reported that the most common cause of death was acute cardiac arrest preceded by autonomic disturbances [5,6]. Ropper, Wijdicks and Truax reported that the causes of death in GBS are pneumonia, adult respiratory distress syndrome following aspiration, sepsis, myocardial infarction, pulmonary embolus and profound dysautonomia [4]. Furthermore, they stated that dysautonomia can be life threatening but only rarely leads directly to death. Lawn and Wijdicks reported ventilator-associated pneumonia as a leading cause of death, followed by cardiac arrest (in absence of dysautonomia) [7]. The most common causes of death found by van den Berg and colleagues were respiratory complications (pneumonia, respiratory failure), cardiovascular and autonomic complications [10].

Risk Factors of Mortality in GBS

Only a few studies have determined the risk factors of death in patients with GBS. All studies found that the most important risk factor for mortality from GBS is older age [6,7,8,9,10,11]. Most studies also found that GBS fatalities have more comorbidity, more often need mechanical ventilation and have more

severe weakness at entry to the hospital [6,7,8,9,10,11]. In a developing country like Bangladesh, the strongest risk factor for mortality was lack of ventilator support in patients with respiratory failure [11].

Timing of Death in GBS

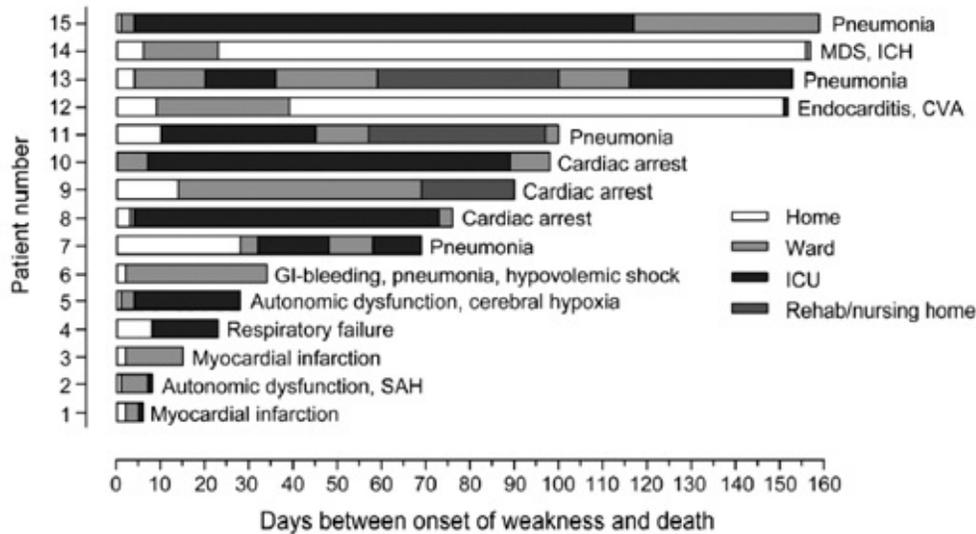
Winer and colleagues showed that the majority of deceased GBS patients died within an interval of 4 weeks [5]. Italian GBS study group confirmed this finding and stated that the majority of GBS patients died within the acute phase of the disease with dysautonomia and cardiac arrest as leading causes of death [6]. In contrast, Lawn and Wijdicks described that only a minority of the fatal GBS patients died within the first 28 days [7]. In our own study two-thirds of the fatal GBS patients died in the recovery phase [10]. In the acute phase the patient died of cardiovascular or autonomic complications, in contrast with patients who died in the recovery phase. These patients died mostly from pneumonia or cardiovascular complications [10]. (See [Figure 54.1](#).) Important for clinical practice is the finding that even in the recovery phase of GBS patients are at risk of dying. This shift from the majority of deaths occurring during the acute phase to the majority of deaths occurring in a later stage could be due to better monitoring of GBS patients in their acute phase of the disease at high care or intensive care units or frequent monitoring on a neurology ward.

How to Reduce the Mortality of GBS?

There is very little evidence for measures that can prevent patients from dying from GBS. It is clear, however, that GBS patients are at risk of dying in every phase of their disease, especially the elderly patient, patients with comorbidity or ventilated GBS patients. Physicians need to be aware that patients who start recovering are still at risk of dying. Some further recommendations may be helpful to reduce the mortality rate in GBS, although none of these have been evaluated in controlled studies.

- Standardized and routine monitoring of vital, respiratory function and weakness, in the acute phase every 2 to 4 hours
- Checking for autonomic dysfunction and swallowing disturbances
- Use of the Erasmus GBS Respiratory Insufficiency Score to predict the risk of respiratory insufficiency. This can help physicians decide whether the patient should be admitted to a general ward, high care or intensive care [12].
- Monitoring, prevention and early treatment of infection, decubitus, thrombosis and contractures, and when diagnosed treatment begun as soon as possible.
- Use of step-down units for patients with severe weakness or a trachea cannula

Figure Hospital admission and transfers in patients with fatal Guillain-Barré syndrome



The bars indicate the number of days at home between onset of weakness and hospital admission and of the various care facilities for each of the deceased patients. In addition, the death cause is specified. CVA = cerebrovascular accident; GI = gastrointestinal; ICH = intracerebral hemorrhage; ICU = intensive care unit; MDS = myelodysplastic syndrome; Rehab = rehabilitation center; SAH = subarachnoid hemorrhage.

Figure 54.1 Hospital admission and transfers in patients with fatal GBS. Reproduced with permission from [10].

Summary and Conclusions

Probably due to improvements in general and intensive care of GBS patients the mortality rate has decreased. Although dying from GBS is uncommon, GBS still is a life-threatening disease, especially in the elderly, in ventilated GBS patients, and in GBS patients with significant comorbidity. GBS patients can die in every phase of the disease. Every GBS patient deserves, therefore, intensive supportive multidisciplinary care, especially the patients at risk. There still is a higher mortality rate in developing countries compared to developed countries due to less general and intensive care.

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TREATMENT

55

Treatment of GBS: Times Are A-Changing: From Wait and See to Active Interference of the Immune System

Pieter A. van Doorn

Introduction

Over the past 100 years, Guillain-Barré syndrome (GBS) has become a treatable disease. Neurologists are traditionally good in diagnosing a disease, but unfortunately treatment for many neuromuscular diseases largely stays behind. This, however, is not the case for GBS! I can remember very well the year 1985, when the landmark paper of the North American GBS plasma exchange trial was published, just when I started my residency in neurology [1]. This changed the perspective on treating patients with GBS. Nonetheless, we all know that treatment for GBS is far from adequate and urgently needs to be improved. It is very challenging that the knowledge of pathophysiological processes leading to GBS has exploded over the past decade. This hopefully helps to better select drugs or any other treatment regimen to be tested. With the help of proper outcome measures and using up-to-date trial design, it is to be hoped that the future for GBS patients will be much better 120 years after the syndrome was first described. My personal Top 10 papers highlight the development towards a better treatment for GBS (see [Figure 55.1](#)).

Plasma Exchange Is the First Proven Effective Treatment for GBS

The Guillain-Barré Syndrome Study Group. Plasmapheresis and acute Guillain-Barré syndrome. *Neurology*, 1985

About 70 years after the famous publication by Guillain, Barré and Strohl [2], 2 small studies reported the positive effects of plasma exchange (PE) in patients with GBS [3,4]. The first large PE trial had been conducted in North America and Canada and was published in 1985 [1]. I can very well remember the publication of this landmark study. It showed that PE is the first proven effective treatment for GBS patients being unable to walk, when PE starts within the first 4 weeks after onset of disease. It was concluded that PE hastens recovery compared to supportive treatment alone and especially when it was started within the first 2 weeks. Later on a large PE trial from France showed similar results [5]. It was a bit surprising that no difference was found between fresh frozen plasma (FFP) compared to albumin as a replacement fluid for the PE procedure, because FFP is the source of IVIg, and FFP likely is also effective in CIDP [6]. The PE trials used some different exchange regimens. In patients unable to walk, a

regimen of five 50-ml/kg exchanges over 8–13 days seems appropriate. One other large PE trial from France showed that 2 PE sessions are better than no exchanges in GBS patients being able to walk [7].

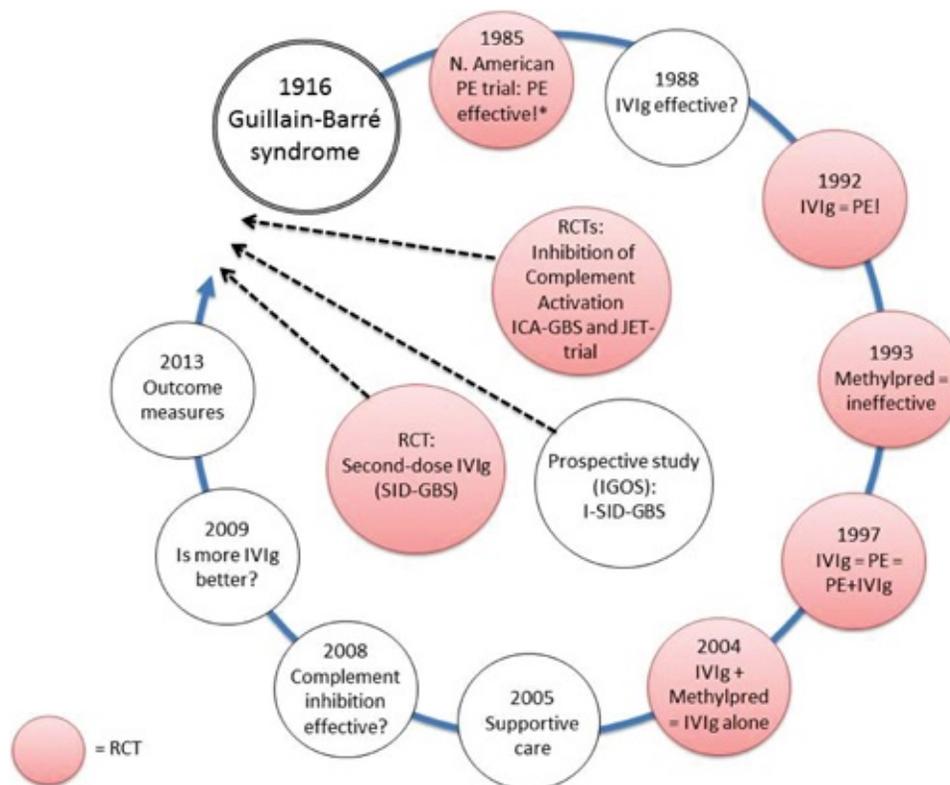


Figure 55.1 My personal highlights towards a better treatment for GBS patients.

IVIg = intravenous immunoglobulin; PE = plasma exchange; Methylpred = methylprednisolone; SID-GBS = Second IVIg dose randomized trial in GBS; I-SID-GBS = International second IVIg dose prospective study in GBS; ICA-GBS = inhibition of complement activation in GBS; JET = Japanese eculizumab trial

* = North American PE trial was followed by important French PE trials

We need to acknowledge that PE is not an attractive procedure to be performed in many groups of patients—for example young children—and that it is relatively contraindicated in individuals with autonomic failure. Although it generally is less expensive than IVIg, it remains a costly procedure and it requires special equipment and appropriately trained staff. Therefore, exchanging multiple small volumes of plasma without using a special apparatus potentially could be an attractive procedure for a large population living in low-income countries. Such a study is now under consideration (Badrul Islam, personal information). Currently, both the effect and the potential side effects first need to be studied.

First Paper on the Potential Effect of IVIg in GBS

Kleyweg RP, et al. Treatment of Guillain-Barré syndrome with high-dose gammaglobulin. *Neurology*, 1988

Already during the recruitment phase of the North American PE trial, there were some pioneers in the Netherlands who aimed to find another treatment for GBS and chronic inflammatory demyelinating polyneuropathy (CIDP) [6,8]. This was largely done because there were local logistical problems with conducting PE as a regular treatment in CIDP at that time. Here in Rotterdam, as far as I know, the first patients were treated with FFP instead of PE, and it appeared effective! Then a resident, I clearly

remember the first CIDP patients receiving their regular FFP infusions. This treatment was replaced by intravenous immunoglobulin (IVIg) when this product became available [6,9]. The beneficial therapeutic effect of IVIg in CIDP prompted an open study in GBS. The first study included 8 GBS patients. It was observed that IVIg seemed to be beneficial in patients with severe GBS [8]. It was concluded that the results should be conformed in a randomized trial.

IVIg Is Effective in GBS!

van der Meché FG and Schmitz PI. A randomized trial comparing intravenous immune globulin and plasma exchange in Guillain-Barré syndrome. *New England Journal of Medicine*, 1992

The possible effect of IVIg in GBS was investigated in a randomized controlled trial (RCT) that compared IVIg (0.4 g/kg bodyweight for 5 days) with standard PE treatment. The Dutch GBS study group led by Frans van der Meché started this RCT in June 1986 and the last patient entered the study by the end of 1989. After the inclusion of 150 patients and a follow-up of half a year, the study was terminated because strength had improved by one grade or more on the GBS disability grade after 4 weeks in 34% of the group treated with PE, compared to 53% in the group treated with IVIg ($p = 0.024$). The IVIg group additionally had significantly fewer complications and less need for artificial ventilation [10]. It was concluded that IVIg is a practical, safe and effective treatment for GBS. This was a major result and would finally change the world's policy to treat GBS! However, not right at that moment because there initially was a lot of debate whether IVIg really would be as effective as PE. One paper indicated that a proportion of GBS patients required a second course of IVIg because of a treatment-related fluctuation (TRF) [11]. In the IVIg/PE trial, the percentage of patients with a TRF, however, was about the same as for PE. Additional reports appeared [12–14]. Finally, other studies using IVIg in GBS were published. The Cochrane review concluded that IVIg started within 2 weeks from onset hastens recovery as much as PE, that adverse events were not significantly more or less with IVIg compared to PE, but that IVIg is significantly much more likely to be completed than PE [15–17].

IVIg currently is the preferred treatment for GBS. However, despite standard treatment with IVIg (0.4 g/kg bodyweight for 5 days), about 20% of patients require artificial ventilation, many patients still have severe pain for a prolonged period of time and about 20% are still unable to walk after half a year [18]. Therefore, treatment for patients with GBS urgently needs to be improved. Hopefully a better and more individualised treatment will become available in the coming years.

Steroids Are Surprisingly Ineffective in GBS

Guillain-Barré Syndrome Steroid Trial Group. Double-blind trial of intravenous methylprednisolone in Guillain-Barré syndrome. *The Lancet*, 1993

GBS is considered to be caused by an aberrant immune response often related to a preceding infection, that potentially could be treated with corticosteroids, like in CIDP. Initial reports on oral steroids in GBS were not very promising. However, there were methodological issues. This likely was the main reason to conduct a double-blind placebo-controlled RCT [19]. The 242 patients were randomized for steroids (500 mg methylprednisolone for 5 days) or placebo treatment. Some patients additionally received PE. It was a disappointment that the study did not show a beneficial effect of corticosteroids. The Cochrane

review concluded that corticosteroids given alone do not significantly hasten recovery from GBS or affect longterm outcome. Additionally, there is low-class evidence that oral corticosteroids even delay recovery [20]. An important question is now whether one should avoid steroids in GBS under all conditions, including when combined with IVIg. Fortunately, that was also investigated in a large RCT.

Ig and Steroids Are Likely Not More Effective Than Ig Alone

van Koningsveld R, et al. Effect of methylprednisolone when added to standard treatment with intravenous immunoglobulin for Guillain-Barré syndrome: randomised trial. *The Lancet*, 2004

Because we hoped that we could improve the outcome for GBS patients, we conducted an RCT in which we combined IVIg (0.4 g/kg bodyweight for 5 days) with methylprednisolone (500 mg/day for 5 days) or placebo treatment. We started that study before the results of the methylprednisolone study were known [21]. In the MP-IVIg trial, 225 were randomized. The primary outcome criterion was that the percentage of patients that improved at least one grade on the GBS disability scale 4 weeks after randomization was not significantly different between IVIg + MP and IVIg + placebo. After correction for known prognostic factors (a post-hoc analysis) it seems that there is a minor advantage for the combination IVIg and methylprednisolone, but only for an endpoint at 4 weeks. The overall conclusion was that the results of the trial were negative, but that a small, short-time benefit of the combination IVIg and methylprednisolone was not excluded [20]. All together, it seems that steroids, including high-dose steroids, are not effective in GBS. The ineffectiveness of steroids to treat GBS remains puzzling, because steroids are effective in CIDP.

Combination of PE Followed by IVIg Is Not Better

Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Randomised trial of plasma exchange, intravenous immunoglobulin, and combined treatments in Guillain-Barré syndrome. *The Lancet*, 1997

Because IVIg is insufficiently effective in a substantial portion of GBS patients, an international consortium lead by Professor Hughes conducted an RCT in 383 adult GBS patients who were randomized for IVIg, PE and PE followed by IVIg [22]. This trial unfortunately did not find significant differences between any of these treatment groups. This is a very important study because it shows that the combination of 2 effective treatments is not necessarily better than only one of these treatments alone. It remains a question whether this is due to the fact that the second treatment was started too late, when a lot of nerve damage had already occurred. It has been one of the reasons we took care in the design of the second dose IVIg trial (SID-GBS) since we decided to the start of the second IVIg course or placebo treatment one week after onset of the first standard course of IVIg.

Is More IVIg Better?

Kuitwaard K. Pharmacokinetics of intravenous immunoglobulin and outcome in Guillain-Barré syndrome. *Annals of Neurology*, 2009

The dose of IVIg in individual patients is currently based only upon bodyweight. There is still a debate about whether one course of IVIg (2 g/kg administered in 5 days) is adequate for all GBS patients. It would be preferable to treat individual patients more based upon their clinical or otherwise biological characteristics, or maybe based on biomarkers (if available). There are a couple of arguments suggesting that some patients may require a repeated or otherwise higher IVIg dosage: (A) about 10% of GBS patients have a secondary deterioration (TRF) that improves after a second IVIg course [24]; (B) one important study showed that GBS patients having a higher rise in their IgG levels 2 weeks after start of IVIg treatment (delta IgG) had a higher chance to improve better and faster [23]; (C) the results of a small open study suggested that a second IVIg course might have been beneficial in GBS patients who seemed unresponsive to a standard dosage of IVIg [25].

Partially based upon these arguments, we started the second dose IVIg randomized placebo-controlled trial (SID-GBS) in GBS patients with a poor prognosis as based upon the mEGOS prognostic model [26]. We expect that the results of this RCT will be available in 2018. The international version of this study (I-SID GBS) is conducted as part of the prospective International GBS Outcome Study (IGOS). With these 2 studies we aim to investigate whether a second course of IVIg is effective when started one week after the start of the first IVIg course, when irreversible nerve damage likely has not yet occurred. The first results of the prospective follow-up study are expected in 2017.

The Importance of Supportive Care

Hughes RA, et al. Supportive care for patients with Guillain-Barré syndrome. *Archives of Neurology*, 2005

This is a very important publication because it focuses just on supportive care for GBS patients [27]. We need to realize that good general medical care remains essential, irrespective of whatever immunological treatment is administered. I have also learned a lot from the books on GBS written by Richard Hughes, Eelco Wijdicks, Allan Ropper and Garreth Parry. I am sure that these books have largely contributed to the clinical knowledge and understanding of GBS of many neurologists, intensive care physicians and others who take care of these patients. Further reports on good medical care, like how to monitor patients in the progressive phase of the disease when admitted on a general neurological ward, when to admit patients on an intensive care unit, how to treat severe pain and many other aspects of giving better psychological support require a lot of attention and additional studies. Hopefully, this part of treating GBS will also be part of a future PNS/EAN guideline.

Complement Inhibition Can Be the Future for GBS

Halstead SK, et al. Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. *Brain*, 2008

In this amazing study, built upon the shoulders of a lot of other studies, in particular from the Willison group in Glasgow, it was shown that blockade of complement activation by eculizumab, a monoclonal antibody that prevents the formation of membrane attack complex (MAC), completely inhibits clinical disease activity in mice [28]. A clinical trial in humans led by Hugh Willison now seeks to answer the question of whether eculizumab given together with IVIg is of benefit to GBS patients. The results of the 'inhibition of complement activation in GBS' (ICA-GBS) are eagerly awaited. Another trial, the Japanese

eculizumab trial (JET) using the same protocol has started in Japan. Inhibition of complement activation may push the treatment of GBS to a new and higher level.

The Importance of Using Proper Outcome Measurements in Studying the Effect of Treatment

Vanhoutte EK, et al. Outcome measures in inflammatory peripheral neuropathies
Neuromuscular Disorders, 2013

The Peripheral Neuropathy outcome measures Standardisation (PeriNomS) study, is a large international study lead by Ingemar Merckies. It is in large part focussed on assessment scales in patients with inflammatory neuropathies, GBS in particular. Rasch-based new assessment scales will hopefully play an important role in new treatment trials in GBS and other immune-mediated neuropathies. It is hoped that modern clinimetrics using linear scales will finally help to assess GBS patients in a more proper way than we now generally do [29]. An example is the development of the I-RODS assessment scale [30].

Conclusion

Treatment trials studying new drugs, combinations of drugs or different dosing regimens, but also large prospective studies like the IGOS, led by Bart Jacobs are essential to the search for a better treatment for patients with GBS. Using modern statistics and validated assessment scales is extremely important for designing new treatment studies appropriately. Covariate adjustment and proportional odds analysis can potentially help us conduct trials in a more efficient manner, requiring fewer patients to be randomized. This will be done in the current SID-GBS trial. New treatment trials, including the studies assessing the effect of complement activation blockage (ICA-GBS and JET) are very important because, unfortunately, GBS is still a severe disease, 100 years after the publication by the 3 French giants [2]. Fortunately, there currently is substantial interest in researching inflammatory neuropathies, as demonstrated by the large numbers of delegates attending the Peripheral Nerve Society / Inflammatory Neuropathy Consortium (INC) meetings. With all these new developments, it seems not unlikely that the future for GBS patients and their relatives will be better in the next 10 to 20 years.

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Intravenous Immunoglobulin (IVIg) for Guillain-Barré Syndrome: The Journey and Current Practice

Angelika F. Hahn

Introduction

By the 1980s, it was generally accepted that GBS, the ‘acute inflammatory demyelinating polyradiculoneuropathy’ (AIDP), represented a post-infectious immune-mediated neuropathy. This hypothesis was supported by preceding infections and by the very early pathology showing extensive lymphocyte infiltrations in roots and peripheral nerves, oedema, deposition of complement on outer aspects of myelin sheaths followed by vesicular disruption, and macrophages infiltrating Schwann cells and acting as scavengers by engulfing the disintegrated myelin and leaving behind a segmentally denuded axon. Thus, the primary immune target epitope appeared to be localized in the outer Schwann cell membrane or myelin. Simultaneously, nodes of Ranvier became widened as myelin loops lifted off from their ‘paranodal’ attachments [1,2]. Degeneration of axons was considered a secondary event. The observations suggested a T cell-dependent, complement and macrophage-mediated aetiology. While target antigen(s) remained elusive, humoral factors seemed relevant.

In 1979, Saida and colleagues reported an EAN model in rabbits induced by sensitization with the myelin constituent galactocerebroside [3]. The animals developed flaccid paralyses, and their nerve pathology showed extensive multifocal demyelination with few infiltrating lymphocytes. Subsequently, the investigators injected serum from these rabbits intraneurally into the sciatic nerves of Wistar rats, which induced vesicular disruption of myelin that developed before the recruitment of macrophages [4]. A year later my colleagues and I confirmed the observation [5]. Using this paradigm, we and others observed that sera from a proportion (41–76%) of GBS patients, particularly when obtained within the first 3 weeks of the patients’ illness, produced focal demyelination and electro-physiological alterations in rat nerve, that correlated with disease severity [6,7,8]. These observations lend support to the role of humoral factors in the pathogenesis of AIDP and GBS.

Plasmapheresis Becomes Standard Therapy for GBS

The Guillain-Barré Syndrome Study Group. Plasmapheresis and acute Guillain-Barré syndrome. *Neurology*, 1985

Prior observations and anecdotal reports of patients with GBS who benefitted from therapeutic plasmapheresis (PE) led the way to the first large randomized controlled trial (RCT) comparing PE with supportive therapy in 245 acutely ill and severely affected GBS patients [9]. Results showed statistically significant differences in all predefined outcome measures in favour of PE. PE was particularly effective when started within 7 days of disease onset, and for patients who had required mechanical ventilation (MV) after randomization. In comparison with standard supportive care, PE hastened the recovery and shortened the duration of disability. Its use in severe GBS was endorsed by a NIH consensus conference as standard therapy [10], and was reaffirmed in 1986 by results of a second large French RCT [11].

IVIg Comes on the Scene

In 1980, Imbach and colleagues first successfully used IVIg at an empirical dose of 2g/kg in 13 children with immune-thrombocytopenia (ITP) [12]. Once these observations were confirmed in adult ITP patients [13], the way was clear to extend IVIg as immunomodulatory therapy to other autoimmune disorders. In 1985, Vermeulen and colleagues reported that 13 out of 17 patients with chronic inflammatory polyneuropathy (CIDP) had benefitted from infusions of fresh-frozen plasma (FFP). On average, improvements lasted 3 weeks, they were reproducible, and functions stabilized with repeat infusions. Similar improvements occurred in 8 out of 9 CIDP patients after infusions of polyclonal gammaglobulin, which accounts for the treatment effects of FFP [14]. In a pilot study gammaglobulin, prescribed to 8 patients with severe GBS, appeared to be beneficial [15].

Confirmation of the Therapeutic Efficacy of IVIg for GBS

van der Meché FG, et al. A randomized trial comparing immune globulin and plasma exchange in Guillain-Barré syndrome. *New England Journal of Medicine*, 1992

Encouraged by these observations, van der Meché and colleagues initiated an RCT comparing IVIg (0.4 g/kg daily for 5 days) with plasmapheresis (PE, 200–250 mL/kg in 5 sessions over 7 to 14 days) in 150 adult patients and children with severe GBS, treated within 7 days from onset [16]. Outcome was measured with the disability scale used in previous trials. Primary outcome measure (proportion of patients improved by one or more functional grades at 4 weeks) and secondary ones (time to improve one functional grade and time to recover unaided walking) were the same as those used for the North American plasmapheresis trial [9]. Ethical concerns ruled out a comparison with placebo. The van der Meché study was not blinded, leaving room for bias. Although the 2 study groups appeared appropriately matched, more patients in the PE group had required MV and for a longer time, and more had very low amplitudes of the distally evoked compound muscle action potentials (CMAPs), both shown previously to be associated with a worse prognosis.

According to predetermined rules, enrolment was stopped after the 150th patient as the interim analysis of the primary outcome measure significantly favoured IVIg. Results indicated that 53% patients treated with IVIg had improved by one or more functional grades at 4 weeks, compared to 34% patients treated with PE ($p = 0.024$). The analysis of secondary outcome measures equally favoured IVIg. Median times to improve one functional grade were 27 days for the IVIg group versus 41 days for the PE group ($p = 0.05$), and the median time to recover unaided walking was 55 days for the IVIg group versus 69 days for the PE group ($p = 0.07$). IVIg treatments were well tolerated and more likely to be completed, and associated medical complications were significantly less frequent.

The trial results were met with great interest, but also raised concerns as observations with PE differed substantially from those of the 2 prior large RCTs. Using the same outcome measures—‘proportion of patients improved by one or more functional grades at 4 weeks’—the earlier trials had documented a response rate with PE of 59% and 61%, respectively, differing from Dutch trial results. The issues were resolved by the landmark trial initiated and conducted by Dr Richard Hughes [17].

Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Randomised trial of plasma exchange, intravenous immune-globulin, and combined treatments in Guillain-Barré syndrome. *The Lancet*, 1997

The relative efficacy of plasma exchange (PE), intravenous immunoglobulin (Sandoglobulin) and the combined regimen of PE immediately followed by IVIg were compared in 383 adult patients with severe GBS, who were randomized within 14 days from disease onset and studied by an international, multicentre RCT [17]. The ‘disability at 4 weeks’—the primary endpoint—was assessed by an examiner who had no knowledge of the treatments, eliminating potential bias. The 3 study groups were evenly matched according to age and sex, functional impairments, delay of randomized treatments from onset of neuropathy, and baseline characteristics known to influence prognosis.

On analysis, there were no significant differences between the 3 groups in the major outcome criterion—‘mean disability grade improvement after 4 weeks’. The difference of improvement between the PE group and the IVIg group was 0.1 grade (95% CI –0.23 to 0.42), where by the pre-set criterion for equivalence of the 2 treatments was met. Moreover, differences of improvement between PE+ IVIg group and PE alone of 0.20 grade (95% CI –0.14 to 0.54) and IVIg alone 0.29 grade (95% CI –0.04 to 0.54) respectively, were not significant. Furthermore, there were no significant differences between the treatment groups in regards to the secondary outcome measures: time to recover unaided walking; median time to discontinue mechanical ventilation; and pattern of recovery over 48 weeks. At 48 weeks, 16% of patients in each group were left severely disabled and unable to walk unaided, and ~5% patients had died. The median times to hospital discharge and to return to work did not differ significantly between the treatment groups.

The trial results documented unequivocally that PE and IVIg had equivalent efficacy in the treatment of severe GBS, and that the combined treatment did not confer additional benefit. A regression analysis confirmed that older age, small distally evoked CMAP amplitudes, and prior gastrointestinal infections adversely affected the prognosis, irrespective of the treatment.

Adverse effects attributed to IVIg (malaise, nausea/vomiting, rigor, fever, flu-like symptoms, myalgia, chest pain, meningism, erythema at infusion site) were short lived and responded to symptomatic treatment. Complications with PE were potentially more serious, and 25% of planned PE treatments had been interrupted versus only 2.8% of IVIg infusions. Given the equivalent efficacy and the greater ease of application, IVIg was recommended as the preferred treatment.

Efficacy of IVIg in Children with GBS

Jones HR Childhood Guillain-Barré syndrome: clinical presentation, diagnosis, and therapy. *Journal of Child Neurology*, 1996

In childhood GBS is much less common but may affect children of all ages. The clinical presentation can be variable and a diagnostic challenge. Most children have a relatively limited clinical illness and remain

able to walk unaided. However, nearly 25% become severely ill with a rapidly ascending quadriplegia, facial and bulbar weakness requiring intubation, assisted ventilation and intensive monitoring. Death due to sepsis or autonomic failure is rare. The prognosis for children with GBS is excellent, as nearly all make a full recovery.

Most controlled RCTs evaluating the efficacy of PE and IVIg had excluded children. Hence, observations in severely affected adult GBS patients have been extrapolated to children. This may be justified, as a comparative study of 18 children and 50 adults from a single centre found no significant differences in disease severity, need for MV and duration of hospitalization [18]. Immunomodulatory treatments are used primarily for children who have lost independent ambulation.

Following the endorsement of PE as standard treatment for severe GBS [9], several anecdotal reports of small case series using historical controls documented that children with severe GBS benefitted from PE [19]. Results of this Dutch RCT, which had shown equivalence for IVIg and PE in hastening recovery from GBS, influenced the choice of therapy for children [20]. From then on, IVIg largely replaced PE, given the more convenient delivery. Reports of uncontrolled prospective case series documented benefit from IVIg in childhood GBS [21]. These observations were confirmed by the retrospective analysis of a larger study, which compared IVIg to supportive care in 75 children with GBS, and compared 2 dosing regimes of IVIg [22]. The study comprised 3 groups that were evenly matched in regards to disease severity. Twenty-four children received IVIg 1g/kg for 2 days, 23 children IVIg 0.4g/kg for 5 days, and 28 children received supportive care. The primary outcome measures were 'mean time to improve 1 disability grade', and 'mean grade change at 4 weeks'. On analysis, the mean time to improve 1 disability grade was significantly shorter with IVIg irrespective of dosing: 20.8 days with IVIg versus 62.4 days with supportive care ($P < 0.01$). Moreover, at 4 weeks improvement with IVIg was a mean 1.0 grade versus a mean 0.35 grade with supportive care ($P < 0.01$). While the study is limited by its retrospective nature, the observed large differences strongly suggest that IVIg benefits childhood GBS. A prospective randomized study compared IVIg with PE in 41 children with severe GBS requiring MV [23]. Twenty children randomized to IVIg and 21 children to PE treatment. The 2 groups were balanced in regards to baseline characteristics and motor dysfunctions. Primary outcomes measures were duration of MV, length of stay in the intensive care unit (ICU), and ability to walk within 4 weeks from discharge from the ICU. The open nature of the trial left room for bias. Results indicated that children treated with PE required MV for a median 11 days (IQR 11.0 to 13.0) versus those with IVIg for a median 13 days (IQR 11.3 to 14.5) $P = 0.037$. There were nonsignificant differences in the length of ICU stay and in the outcome at 4 weeks as 20 out of 21 children treated with PE and 18 out of 20 treated with IVIg had regained independent ambulation. The trial concluded that PE had a slight but significant advantage over IVIg in reducing the time of MV. The study was not blinded, which could have introduced bias.

A second prospective randomized multicentre study by Korinthenberg and colleagues sought to clarify whether early treatment with IVIg mitigates the subsequent disease severity [24]. Twenty-one children, who were able to walk unaided, were randomized to IVIg (1g/kg over 2 days; half the usual dose) or no treatment. Concealed randomization resulted in unequal allocation of 14 participants to IVIg and only 7 to no treatment. The 2 groups were otherwise matched. 'Degree of disability at nadir' was the primary outcome measure. Eleven of the 21 children progressed to losing the ability to walk unassisted, 7 out of 14 on IVIg and 4 out of 7 on no treatment, a nonsignificant difference ($P = 0.25$). However, the improvement in disability at 4 weeks was significantly greater in the group treated early than in the untreated participants (the mean difference (MD) -1.42 , 95% CI -2.57 to -0.27). The same investigators assessed the effect of IVIg dosing in a second trial involving 51 children who were unable to walk unaided: 25 participants randomized to IVIg 2g/kg over 2 days, and 26 to IVIg 2g/kg over 5 days. The

primary outcome measure was ‘days to regain unaided walking’. Results showed no significant differences in the time to regain unaided walking irrespective of IVIg dosing: median 19 days for IVIg 2 day group versus median 13 days for IVIg 5 day group (log-rank test $P = 0.94$). The median time to improve 1 point in disability score was 5 days in both groups. Multivariate analysis showed that ‘disease severity at nadir’ was the only prognostic determinant for recovery. Different IVIg dosing regimes did not influence recovery time or outcome. However, treatment-related fluctuations were significantly more frequent with treatment when IVIg 2g/kg was given over 2 days.

Despite efforts to arrive at objective guidance for the use of IVIg in childhood GBS, all studies suffered from small enrolment and variable potential for bias. Nonetheless, observations point consistently to a beneficial effect of IVIg in children. These underscore the current guidelines to prescribe immunomodulation with either IVIg or PE in children with severe GBS, unable to walk 10 meters without assistance. There is limited evidence that children with milder disease, able to walk 10 meters unassisted, would benefit from early IVIg treatment. In view of the natural speedy recovery and excellent outcome in childhood GBS, it would be justified to monitor such children closely without use of special treatments. Anecdotal evidence, cited below, suggests that children suffering from acute motor axonal neuropathy (AMAN) might benefit from IVIg [25].

Efficacy of IVIg in Axonal Forms of GBS

Shahrizaila N, et al. Antibodies to single glycolipids and glycolipid complexes in Guillain-Barré syndrome subtypes. *Neurology*, 2014; Susuki K, et al. Dysfunction of nodes of Ranvier: A mechanism of anti-ganglioside antibody-mediated neuropathies. *Experimental Neurology*, 2012; Kuwabara S, Yuki N. Axonal Guillain-Barré syndrome: concepts and controversies. *Lancet Neurology*, 2013.

The primary axonal autoimmune neuropathies constitute a spectrum of clinical disorders with a shared immunopathogenesis. The above papers by Shahrizaila, Susuki and Kuwabara and colleagues are landmark publications in establishing this concept [26,27,28]. Most often, the triggering infections are caused by strains of *C. jejuni* and *Haemophilus influenzae*, which express epitopes in lipooligosaccharides of their cell-surface that are homologous with gangliosides expressed on neurons. Given the molecular mimicry, immune responses in the host induce anti-ganglioside autoantibodies, which recognize ganglioside epitopes in the axolemma of nerve fibres most accessible at the nodes of Ranvier and nerve terminals. Binding of autoantibodies to their targets induces complement-mediated damage to nodal and paranodal molecular structures and to terminal axons [29]. Blockade or disruption of sodium channel clusters at the nodes of Ranvier causes impaired nerve excitability, conduction block (CB) and rapid loss of function. Depending on the degree of damage, CB may be reversible with resulting early clinical improvement. Alternatively, recruitment of macrophages and progression of complement-mediated pathology causes axonal degeneration and consequently protracted recovery [30].

The clinical manifestations of acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN), acute sensory ataxic neuropathy (ASAN), and the Fisher syndrome (FS) are each associated with distinct anti-ganglioside autoantibody profiles. These disorders extend the spectrum of GBS and make up between 30% and 67% of GBS cases in East Asia and in Central and South America, while they represent less than 10% of GBS in Europe and North America [31]. Previous RCTs that examined the efficacy of IVIg in GBS had enrolled participants from Europe and North America, most of whom had suffered from AIDP. There are no reported RCTs examining IVIg in axonal forms of GBS

and related conditions. Few retrospective analyses have explored the potential of benefit from IVIg in children and adults afflicted with AMAN or FS [32,33,34].

Observations in Acute Motor Axonal Neuropathy (AMAN)

This rapidly evolving, purely motor neuropathy, AMAN, is commonly preceded by a diarrhoeal illness caused by *C. jejuni* strains that induce IgG anti-ganglioside antibodies against GM1, GD1a, GalNAc-GD1a and GD1b, a pattern characteristic for AMAN. The clinical course is marked by an acute onset of flaccid weakness that initially affects the lower limbs, ascending upwards but often sparing cranial nerves. In contrast to AIDP, tendon reflexes may be preserved. Usually, patients will have reached their nadir within days, often at their first presentation to a physician. They may be able to ambulate or be bed-bound, and approximately 25% require assisted ventilation [35]. At times, recovery from paralysis in AMAN can be remarkably fast, within days from onset, which is due to resolution of conduction block at the nodes of Ranvier. Alternatively, recovery is more protracted because of more severe complement-mediated damage to motor axons with ensuing axonal degeneration [30,36]. Preceding diarrhoea is considered a prognostic marker of less favourable outcome, yet recovery in children with AMAN is usually speedy and good [37].

To determine incidence and prognosis of AIDP and AMAN in childhood GBS in Japan, data from 31 children with GBS were analysed retrospectively [33]. According to electrodiagnostic criteria they were classified as AIDP 35% (n = 11), AMAN 48% (n = 15), and undetermined cases 16% (n = 5). The 2 defined groups did not differ significantly in age, sex and median clinical disability at nadir (AIDP 4.0 (2–4) versus AMAN 4.0 (2–5)). Nearly half of the children with AMAN had suffered a preceding gastroenteritis, and 4 patients had required MV. Five patients in each group were only mildly affected and not treated. Six AIDP patients (55%) were treated with IVIg, and 8 AMAN patients (53%) received IVIg and 2 PE. At 6 months all children with AIDP and 80% AMAN children were able to walk unaided. By 2 years all but 1 child with AMAN (93%) had regained independent ambulation. Hence, the authors suggested, that the long-term prognosis for the 2 subtypes of childhood GBS may be similar.

The secondary analysis of participants in the original Dutch RCT of IVIg and PE in severe GBS identified 27 participants with characteristics of AMAN: 16 had randomized to IVIg and 11 to PE. On assessment at 6 months 14 out of 16 participants treated with IVIg had regained independent walking compared to only 5 out of 11 treated with PE (P = 0.02). The authors suggested that IVIg might be the preferred treatment for AMAN [38,39].

The retrospective analysis of 24 adult AMAN patients, who had been treated either with IVIg (n = 10) or PE (n = 14) came to the same conclusion [32]. The 2 groups did not differ significantly in age, sex, median disability grades, CMAP sum scores and frequency of *C. jejuni* infections. Those treated with IVIg had significantly lower disability scores at 1, 3 and 6 months from onset of treatment (P = 0.03), and they also had a higher probability to regain independent ambulation at 6 months (log rank P = 0.044). Moreover, 6 out of 10 patients treated with IVIg had shown markedly rapid improvements of 2 or more disability grades in the first 4 weeks compared to only 3 out of 14 in the PE group (P = 0.03). At 6 months, all IVIg-treated patients had regained independent ambulation while 4 out of 14 PE-treated patients had a very delayed recovery (p = 0.07). These observations, albeit derived from a small number of AMAN patients, suggest that IVIg may be the preferred treatment modality in AMAN.

Observations with IVIg in the Fisher Syndrome

Ito M, et al. Bickerstaff's brainstem encephalitis and Fisher syndrome form a continuous spectrum. Clinical analysis of 581 cases. *Journal of Neurology*, 2008

Although the Fisher syndrome (FS) is a unique entity, it is included in the wider spectrum of GBS because of its post-infectious aetiology, presence of pathogenic anti-ganglioside autoantibodies against GQ1b and GT1a in 85% of cases, and observations of clinical overlap with GBS in ~7% of FS patients. Such patients have a combined presentation of FS and AMAN, characterized by a descending pattern of paralyses, frequent need for MV and a less favourable prognosis [40].

In Japan, FS is more prevalent in middle-aged men (male to female ratio is 2:1). A review of 466 representative FS patients indicated that most patients recalled an infectious illness within the preceding week, causing either only upper respiratory symptoms (64%), or only diarrhoea (13%), or both (12%) [41]. Serologic and cultural evidence attested to a recent infection with *H. influenzae* or *C. jejuni* in some of the patients, yet for most the causative agent remained undefined. Initial symptoms were abrupt onset of diplopia or gait disturbance. All patients presented the classical triad of external ophthalmoplegia, ataxia, and hypo- or areflexia. Blepharoptosis, mydriasis and facial weakness were seen in one-third of patients, while mild bulbar and limb weakness were seen in one-quarter of patients. At nadir, reached within a median 4 days (range 1–20), nearly one-third of patients had complete ophthalmoplegia and one-third were unable to walk independently.

Although half of the patients reported mydriasis, loss of deep sensation was rare and sensory nerve conduction studies were normal. Results of a body-sway analysis indicated dysfunction of group 1a sensory neurons and proprioceptive afferents. Brain MRI studies were normal in all but 1% of the FS patients in whom minor abnormalities were found in the brainstem. CSF albuminocytological dissociation was present in 76% of patients, and anti-GQ1b IgG antibodies and anti-GT1a IgG antibodies were detected in 86% of FS patients [41]. The GQ1b epitope is expressed in the paranodal regions of human ocular motor nerves, in muscle spindles and in dorsal root ganglia, explaining the clinical presentation [42,43].

FS is a monophasic illness, characterized by spontaneous recovery and generally good prognosis. The natural history and course of recovery have been delineated from a study of 28 consecutive patients, who had not received immunotherapy and were followed for a median period of 4 months (range 1–185) [44]. Earliest signs of recovery of ataxia and ophthalmoplegia were noted at a median 2 weeks from onset of neurologic disease. Ataxia was fully reversed by a median 32 days (8 to 271), while the resolution of ophthalmoparesis required a median 88 days (29–165). By 6 months, almost all patients had made a full functional recovery.

Excellent natural outcome was no less than that of FS treated with IVIg or PE. A retrospective analysis of 92 FS patients compared outcomes for 28 patients treated with IVIg with those of 23 patients treated with PE, and those of 41 FS patients, who were left untreated [34]. The 3 groups were evenly matched with the exception of the frequency of complete ophthalmoplegia, which was significantly higher in the IVIg group ($p = 0.007$). The time from onset of ataxia and ophthalmoplegia to the earliest signs of improvement was significantly shorter in the IVIg treated group compared to the control group (ataxia $p = 0.027$; ophthalmoplegia $p = 0.04$). However, the times required for the full resolution of the symptoms were not significantly different between the 3 groups; this was also seen in a sub-analysis of the most severely affected patients. At one year after onset of the neurological symptoms in 96% FS patients had fully recovered. The study concluded that IVIg slightly hastened the recovery in FS patients but that did not affect the outcome. Interestingly, PE did not seem as effective in FS as had been observed in a previous study [45].

In view of the fairly rapid recovery and generally excellent prognosis of FS, there is consensus that IVIg treatments should be reserved for FS patients who are unable to ambulate or have overlapping presentations of either FS/GBS or of FS/Bickerstaff brainstem encephalitis.

Potential Mechanism of Action of IVIg in GBS

Buttman M, et al. Polyclonal immunoglobulin G for autoimmune demyelinating nervous system disorders. *Trends in Pharmacological Sciences*, 2013; Shahrizaila N, Yuki N. The role of immunotherapy in Guillain-Barré syndrome: understanding the mechanism of action. *Expert Opinion on Pharmacotherapy*, 2011; Hughes RA et al, Intravenous immunoglobulin for Guillain-Barré syndrome, *Cochrane Database Syst Rev*, 2014

Intravenous immunoglobulin (IVIg) preparations comprise the pooled fraction of serum IgG from thousands of healthy blood donors, generated by a multistep purification process of cryoprecipitation, followed by ethanol fractionation and/or chromatography. The large donor pool ensures a wide spectrum of natural and induced antibody activities, including antibodies directed against external antigens, autoantibodies and anti-idiotypes. This polyclonal antibody repertoire is likely important for the therapeutic effects of IVIg [46,47,48]. Various measures are taken for the inactivation of viruses and other infectious agents, and for the depletion of ABO antibodies, to reduce the risk of contamination and haemolytic reactions, respectively. Concentrations of IgA are kept to a minimum (< 2.5%) to avoid the risk for anaphylactic reactions in patients with IgA deficiency carrying anti-IgA antibodies. Care is taken to remove IgG aggregates to avoid cytokine release and overt immune stimulation. Thus, IVIg preparations contain ~95% monomeric IgG, with a distribution of IgG subclasses equalling those of normal serum. Most commercial IVIg preparations also include stabilizers, such as sorbitol, glycine, sucrose or L-proline, which may potentially contribute to the adverse effects, such as headache, nausea and fever. In general, IVIg infusions are well tolerated, with side effects occurring in less than 10% of patients. The various proprietary IVIg preparations are considered as therapeutically equivalent and comparable in safety and in cost.

Mechanisms by Which IVIg Exerts Anti-inflammatory and Immunomodulatory Effects

Therapeutically administered high-dose IVIg can interfere with the adaptive and innate immune systems via antigen-specific (mediated by the antigen-binding IgG Fab region), and non-antigen-dependent mechanisms (mediated via the IgG constant Fc region). Both domains participate in the anti-inflammatory and immunomodulatory activity of IVIg. It is likely that several mutually nonexclusive mechanisms contribute to the beneficial effects.

Commercial preparations of IVIg contain a multitude of highly specific antibodies generated by the adaptive immune system of contributing blood donors after exposure to infectious microbial organisms and external foreign antigens, as well as natural antibodies and anti-idiotypic antibodies. The latter are of particular importance as they recognize the antigen-detecting domains of other antibodies and are thus potentially able to block or neutralize pathogenic autoantibodies, thereby preventing the antibody-induced activation of the complement cascade. Natural self-reacting antibodies are an integral component of IVIg and are believed to contribute to immunoregulatory effects of IgG by establishing normal immune homeostasis [49]. IVIg also contains neutralizing antibodies against pro-inflammatory cytokines. In

parallel, Fc-mediated mechanisms play a major role. Saturation of neonatal Fc receptors (FcRns) by IgG may accelerate clearance of circulating autoantibodies. IgG-mediated blockade of activating Fc-gamma receptors (FcγRs) on macrophages may lead to decreased secretion in pro-inflammatory cytokines, as is found in serum of GBS patients. It also blocks the complement pathway and thereby prevents the C5b-9 membrane attack complex-mediated pathology. Moreover, IVIg has profound effects on both T and B lymphocytes, the key cellular components of the adaptive immune system, by suppressing pro-inflammatory T cells, inhibiting cytotoxic T cells, upregulating T regulatory cells, and by inducing a protective Th1/Th2 shift. Importantly, IgG-induced upregulation of the inhibitory FcγRIIB receptor on antigen presenting dendritic cells (DC) modulates T-cell activation. Furthermore, upregulation of FcγRIIB receptors on B cell surfaces results in diminished antigen-induced B cell proliferation and decreased antibody production. Moreover, IgG-mediated cross-linking of FcγRIIB receptors on B cells and plasma cells induces apoptosis, thereby maintaining B-cell and plasma-cell homeostasis. In addition, IgG exerts anti-inflammatory effects by downregulating the expression of adhesion molecules on endothelial cells, thereby reducing the migration of activated T cells into the endoneurium.

Despite solid evidence of the therapeutic efficacy of IVIg in GBS, it remains unclear which of the above outlined pleiotropic immunomodulatory mechanisms are operative and whether they apply to all subtypes of GBS. The rabbit model of AMAN serves the *in vivo* study of effects of IVIg in the axonal forms of GBS [50]. The mouse diaphragm nerve-muscle preparation serves the *ex vivo* study of effects of IVIg on the anti-GQ1b antibody-mediated injury at neuromuscular junctions in the FS [51].

IVIg in Evidence-Based and Individualized Care of GBS

van Doorn PA, et al. IVIg treatment and prognosis in Guillain-Barré syndrome. *Journal of Clinical Immunology*, 2010

Treatment of GBS combines multidisciplinary supportive medical care and immunotherapy [52]. Both IVIg and PE provide equivalent benefit to hasten the recovery and shorten the duration of disability in patients with severe GBS (updated evidence appears in *Cochrane Reviews*, 2014). IVIg is the generally preferred treatment because it is convenient and more available, and is more likely to be completed according to protocol. Immunotherapy is usually started when the patient has lost the ability to walk 10 meters without assistance (GBS disability grade ≥ 3). It is currently not known whether mild GBS would benefit from IVIg. However, one RCT of PE included a group of 91 patients with mild GBS (GBS disability grade 2) who were randomized to receive 2 PE exchanges or no treatment. Onset of recovery was significantly earlier in the treated group [53]. Although the number of participants in this comparison was small, the observations support early treatment.

Randomized controlled trials showed that IVIg 0.4g/kg given over 5 days was as effective as a course of 5 PE treatments spread over 2 weeks. IVIg is generally prescribed at an empiric dose of 0.4g/kg/day for 5 days, or else with an equivalent dose of 1g/kg for 2 days. According to small comparative studies, the recovery and outcome in children with GBS was comparable with the 2 dosing regimes [24]. The 2-day infusion regime is more convenient for children. In adult patients, particularly those with hemodynamic instability, cardiovascular risk factors, and compromised renal function, and in the elderly, one is advised to prescribe a slow infusion rate and delivery over 5 days. A randomized double-blind study of 39 GBS patients compared treatment with 0.4g/kg/day given for 3 days or 6 days. Patients treated for 6 days showed a trend towards better outcome, which became significant for ventilated patients [54].

Treatment-Related Fluctuations and Variation in Treatment Response

GBS has a highly variable presentation but follows a monophasic course. Disease nadir is reached within 2 weeks in 80%, within 4 weeks in 97%, and within 6 weeks in all [55]. After a period of stabilization or early improvement with IVIg, approximately 10% of patients experience a secondary deterioration, so-called treatment-related fluctuations (TRFs) [56,57,58]. A prospective study of 164 GBS patients identified 16 patients with GBS/TRFs. Relapses occurred at a median 18 days from onset of weakness; in fact the great majority of GBS/TRF patients relapsed within the first 4 weeks, and 5 (31%) experienced a second relapse. All TRFs responded to repeat courses of IVIg. GBS/TRF patients were severely affected and nearly half required MV. In parallel, the investigators identified 8 (5%) patients who represented acute-onset CIDP. A-CIDP could be differentiated from GBS/TRF. Patients with A-CIDP seldom had cranial nerve dysfunction, had less weakness and disability, did not require MV, and had more pronounced signs of demyelination on electrophysiological studies. In addition, they differed significantly by a later nadir, delayed first worsening at ~ 4.5 weeks, and by multiple exacerbations, all of which responded to IVIg. Their treatment was changed to a long-term CIDP protocol [58].

GBS/TRF patients likely have a longer-lasting active immune phase of their disease, and/or the standard IVIg infusions were less effective. Response to immunomodulatory therapy is variable and ~20% GBS patients have a protracted disease course and are left severely disabled. Studying the pharmacokinetics of IVIg in 174 GBS patients, comparing pretreatment serum IgG levels to those at 2 and 4 weeks, 3 months and 6 months after a standard IVIg dose, it was found that the increase in serum IgG at 2 weeks varied markedly between patients. Patients with a low increase in serum IgG recovered significantly more slowly and fewer could walk at 6 months. A low increase in serum IgG levels at 2 weeks was an independent prognostic marker for poor outcome [59]. This raised the question of whether such patients might benefit from a higher dose and/or second course of IVIg. Observations from a pilot study of 4 GBS patients showed this to be the case [60].

Prognostic Models of Outcome and Standardization of Outcome Scales

van Koningsveld R, et al. A clinical prognostic scoring system for Guillain-Barré syndrome. *The Lancet Neurology*, 2007

An individualized approach to therapy for GBS is highly desirable as responses to treatment as well as outcome vary markedly among patients. This can be achieved by accurate prediction of short and long-term outcome at first presentation. Moreover, by predicting risks for respiratory insufficiency (Erasmus GBS Respiratory Insufficiency Score [EGRIS]) [61] and cardiac arrhythmias, anticipatory care can avoid added complications. To this end, validated prognostic models (Erasmus GBS Outcome Score [EGOS]) [62] have been introduced into clinical care, which are derived from readily available clinical parameters and are easily and quickly applied at the bedside. As adjustment of treatment to the individual patient's need will be most important in the early phase of the illness, the prognostic model was further modified [m EGOS] to be used at admission and at day 7. It allows the accurate prediction of poor outcome at 4 weeks, 3 months and 6 months of GBS with high discriminate value [63]. These approaches are being used in clinical trials and will eventually make it possible to tailor therapy according to the needs of individual patients.

SID-GBS Trial

A placebo-controlled RCT compares the effects of a second course of IVIg given shortly after the first course in GBS patients with early projected poor outcome (defined as an mEGOS score of 6–12 at 1 week) and was launched by the Inflammatory Neuropathy Consortium in 2009 and is ongoing.

Newly introduced Rasch-transformed outcome scales for disability, the Inflammatory Rasch-built Overall Disability Scale, or I-RODS, and for impairment, the Rasch-transformed MRC sum score, or RT-MRC, with proven greater precision will be used in future therapeutic trials. Moreover, the significance of outcomes will be calculated and expressed as minimal clinically important difference (MCID), which reflects clinically relevant changes at the individual-person level [64].

Adverse Reactions and Risks of IVIg

Lünemann JD, et al. Intravenous immunoglobulin in neurology—mode of action and clinical efficacy. *Nature Reviews Neurology*, 2015

IVIg is usually well tolerated, with adverse reactions occurring in no more than 10% of patients. [65] In the largest RCT of GBS 15 out of 258 (5.8%) patients treated with Sandoglobulin experienced side effects, which led to early termination of the infusion in 7 patients = 2.8% (PSGBST group 1997). In a small open trial of IVIg in young children with GBS, treatment-related adverse events occurred in 4 out of 11 children, which included aseptic meningitis (1), recurrent fever (1) and reversible laboratory abnormalities (2) [21].

Common Infusion-Related Reactions

Evidence-based guidelines for the use of IVIg in neuromuscular disorders (Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology) list the prevalence of common reported adverse events in therapeutic trials as follows: headaches (16.1%), fever (6.6%), mild hypertension (4.6%), chills (3.3%), nausea (3.2%), asthenia (1.4%), arthralgia (1.3%), anorexia (1.1%), dizziness (1.1%) and malaise (1.1%) [66]. Most adverse events are short-lived and respond to symptom therapy. Chest and back pain, or shortness of breath occurring during the infusion, resolve by stopping the infusion for 30 minutes and reducing the infusion rate. A slower rate of infusion is advised in older patients and in those with compromised cardiovascular functions, as the excess fluid load may precipitate congestive heart failure. Post-infusion fatigue, fever and nausea may last up to 24 hours.

Rare, More Serious Reactions

Migraine headache and aseptic meningitis can be triggered by IVIg in the predisposed individual. Symptoms of meningeal irritation usually subside within 48 hours, yet hospitalization and strong analgesia are often required. The diagnosis is made clinically and no further tests are necessary. Pretreatment with steroids or nonsteroidal anti-inflammatory drugs may lessen the recurrence with repeat IVIg treatments. Immobilized individuals or those prone to thrombophlebitis are at higher risk to develop a deep-vein thrombosis. Thromboembolic events, such as strokes, pulmonary embolism or myocardial infarction are reported but are rare. The main causative factor is an increase in serum viscosity by IVIg. Skin reactions such as urticarial, lichenoid lesions, eczema, pruritus of the palms and petechiae of the extremities can develop 2–5 days after the infusion and last for up to a month.

Most serious anaphylactic reactions occur in patients with IgA deficiency (prevalence 1:1000

population), of whom ~30% have anti-IgA antibodies that can cross-react with infused IgA contaminants in the IVIg preparation, and can lead to the formation of macromolecular complexes. Fortunately the complication is rare. The potential risk should be considered at the start of each first infusion. Rarely, haemolytic anaemia may be provoked by haemolytic antibody contaminants in IVIg preparations. Acute renal tubular necrosis with resulting acute renal failure can occur in patients with pre-existing kidney disease, especially diabetic nephropathy.

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Guillain-Barré Syndrome, Respiration and ITU

Robin S. Howard and Nicholas P. Hirsch

Introduction

Aristotle (384–322 BC), reflecting the poetry of the physician Empedoches (500–430 BC), linked respiration to inward and outward movement of air through small pores in the skin. The concept of alternating repulsion and attraction between elements was dominant in Plato's (427–327 BC) dialogue, the *Timaeus*. In his system the diaphragm separated the uppermost, 'better soul' (responsible for courage and spirit) from the lower, base soul responsible for our animal appetites for food, drink and other wants. Aristotle did not link respiration to a particular organ and a specific movement of the thorax. He maintained that the 'use' of respiration was to cool the innate heat of the body generated by the heart and to keep it from becoming excessive. He considered the lungs expanded because of the heat generated by the heart and that the cooling caused by air entering led to the lungs shrinking. The diaphragm had no role in respiration and the thorax moved only because the lung expanded and deflated [1,2].

Recognition that the diaphragm was a muscle and that muscles were under nervous control is attributed to the Alexandrian physicians Herophilus and Erasistramus (approx. 300 BC) who based their observations on the first animal experiments; indeed, they even taught that the diaphragm was the main muscle of respiration. Their work was later described in the writings of Galen (AD 130–200) in Pergamum and Rome but it is to Galen himself that we owe the first clear description of the workings of the respiratory pump. He undertook a meticulous description of experiments demonstrating that the diaphragm was innervated by the upper cervical cord and continued to move the rib cage in response to spinal section below this level, but that if the phrenic nerve is sectioned above this level no diaphragm excursion occurs. It is said that Galen was a skilled clinician who taught physicians to observe the motion of the chest wall to see which respiratory muscles were being used. Derenne and colleagues [1] recount the history of an athlete named Secundus, with a weak diaphragm, whose breathing Galen improved by putting a girdle around the hypochondrium.

Guillain-Barré syndrome (GBS) has been known to cause weakness of the respiratory muscles since the original eponymous description by Landry in 1859 in which he described involvement of the respiratory muscles and the eventual danger of asphyxia when the paralysis reached its maximum intensity. Richard Hughes [3] draws attention to Landry's prescient recognition of the presence of diaphragmatic weakness by observing paradoxical movement of the abdomen. Landry observed: "On the day before dying of asphyxia the patient was lying quietly on his back and there was hollowing of the abdomen during inspiration and outward movement during expiration. This paradoxical movement was much less evident when the patient was sitting up".

Osler (1892) also recognised that 'acute ascending paralysis' was associated with respiratory

involvement and that some of the patients died from this, within a week to 10 days [4]. Gordon Holmes (1917) described 12 cases of ‘acute febrile polyneuropathy’ which was seen at Queen Square and on the World War I battlefields in France [5]. Although the cases were all associated with pyrexia at onset, the progressive motor and sensory impairments were characteristic of Guillain-Barré syndrome and he recognised that “in two cases which died from pulmonary complications the paresis of the respiratory movements was probably a contributory factor to the fatal termination”.

Pinckney (1936) describes 3 similar cases in which diaphragm weakness or paralysis occurred, although only one of the patients died [6]. By 1943 the diagnosis was more clearly understood and a case of relapsing GBS is described in a *New England Journal of Medicine* (NEJM) clinicopathological conference [7]. Prior to death, the patient is reported to be “unable to raise secretions or to swallow, or to breathe outside the respirator”. We can find no earlier reference to respiratory support for this condition, although the first NICU was said to have been established by Walter Dandy at Johns Hopkins Hospital in 1932 [8]. The NEJM report is also striking for being the first observation of a critically important sign of incipient respiratory muscle insufficiency: “the fact that, in this case, the shoulders were severely involved almost from the start made me a little cautious about the prognosis because the lesion was getting closer to the fourth cervical segment, where the phrenic nerve comes off”. This remains an important observation in routine clinical practice because shoulder weakness indicates involvement of C3/4 innervation of the trapezius, which often precedes phrenic nerve impairment.

The major stimulus to the development of intensive care came from the poliomyelitis epidemic in Copenhagen in 1952 and a later one in London. Lassen reviewed his experience in Denmark noting that “during the 11 years, 1934–44 respirator treatment was used in 76 cases with a mean mortality rate of 80%. Only cuirass respirators were used” [9]. When the epidemic occurred available ventilatory support was completely inadequate. In consultation with Dr Bjorn Ibsen, a Copenhagen anaesthetist, Lassen undertook tracheostomy in those patients who could not maintain their airway because of excessive secretions, generally due to acute bulbar poliomyelitis. Positive pressure ventilation was then delivered manually with a rubber bag. At the height of the epidemic over 300 such patients were admitted and treated each week with continuous manual ventilation being provided by teams of medical and dental students. The mortality fell from over 80% to 40%. This experience led not only to the introduction of intermittent positive pressure ventilation (IPPV) but also to the idea of caring for all sick patients in a dedicated ward in which each patient could have their own nurse. Thus, it might be considered, that in December 1953 the specialty of intensive care medicine was born [10].

The polio epidemic was also severe in London in 1952. This led to the development of several respiratory isolation units across the city. In October 1953 an intensive care unit was established at Queen Square by Dr Michael Kremer, and this continued to function after the polio epidemic. The unit was subsequently led by Professor John Marshall and Dr Atwood ‘Bobby’ Beaver. Marshall described the development of neurological intensive care to encompass the management of temporary neuromuscular paralysis [11]. He described the ICU management of 229 patients over 6 years, including 39 with polyneuritis. He notes “polyneuritis is in many ways a more gratifying condition to treat [than poliomyelitis], because in the vast majority of cases there is no residual disability”. This important paper is remarkable in recognising many issues concerning the introduction of ventilatory care which have become increasingly apparent over the intervening years. In particular Marshall draws attention to the rapid introduction of intermittent positive pressure respiration (IPPR), the importance of early tracheostomy undertaken by a skilled operator, early recognition of CO₂ retention and incipient ventilatory failure, the importance of early intervention with ventilatory support and of good supportive management including fluid and nutritional intake, metabolic, bowel and bladder care, the risk of

atelectasis and infection and the importance of regular nursing and physiotherapy support. This important paper also describes the difficulties in providing respiratory support in patients with bulbar weakness using a cabinet (negative pressure) respirator and recognises that IPPR overcame these difficulties and facilitated nursing and physiotherapy.

In 1960, Osler and Sidell reviewed GBS but made only passing reference to death from respiratory failure, describing a single patient who was a 'respiratory case' from admission but died after a few days [12]. Marshall described the Queen Square experience in *Brain*, noting 4 of 35 patients died, 3 from respiratory failure and one from cardiac arrest, but there is no description of those who received respiratory support [13]. The rapid development of intensive care through the 1960s and 1970s is been described in a number of papers, including those by Pontopodou, Hilberman, McCleave and, more recently, Widjicks [8,14,15,16].

The intensive care management of acute Guillain-Barré syndrome has evolved with the introduction of new modes of ventilation, better techniques of supportive care and the widespread availability of intravenous immunoglobulin as a more convenient form of immunomodulatory treatment than plasma exchange [17]. In the UK, highly specialised neurological ICU has become easier to access with the development of neuroscience units, although there is considerable variation in the provision of neurological support for patients admitted to general ICUs. It remains uncertain whether these changes have led to a significant change in the pattern of referral for specialist care or an improvement in the management and outcome of GBS.

In a previous report from Queen Square ITU, the overall mortality in 79 patients with acute GBS was 5.1% although 15% remain severely disabled at 6 months and 10% at 1 year [18]. Several large series of patients treated for GBS on ICU have subsequently been published. The mortality has varied from 6.5% to 12.2% but there are significant differences in the severity and clinical pattern of the cases seen and treated [19,20]. Most patients died from complications of intensive care and prolonged immobility, including sepsis and pulmonary emboli. Major complications, including pneumonia, sepsis, pulmonary embolism and gastrointestinal bleeding, develop in 60% of intubated patients [21].

We undertook a retrospective review of 110 patients with acute GBS admitted to the medical ICU at Queen Square over 25 years to observe any change in the patterns of referral and care [22]. The series showed that patients referred over the last 15 years, when compared to the earlier cohort, were of a similar age and sex, had a similar incidence and range of ICU complications and a comparable range of immunomodulation treatment. However, patients admitted more recently were transferred to the ICU later, required mechanical ventilation for longer periods and required longer ICU and hospital stays. In the more recent group there was a much higher incidence of axonal neuropathy (51% > 24%) but the increased duration of ventilation and length of stay applied whether the primary neuropathy was demyelinating or axonal. Despite the delay in referral and the severity of the underlying condition, the mortality was 3 out of 58 (5.2%) compared to 7 out of 52 (13.5%) and the rehabilitation outcomes were similar. There was no late mortality after 'step down' to ward neuro-rehabilitation or discharge to home or the referring hospital.

The results suggest a change in the pattern of referral, at least to this specialised neurological ICU, indicating that patients with acute GBS are now referred later and with more prolonged and severe disease, reflected in a higher proportion of severe axonal neuropathy requiring longer ICU and hospital stay. The mortality has reduced but the outcome is similar. The reasons for this apparent change in practice are unclear but are likely to reflect easier access to long-term care in ICU beds in the admitting hospital and to immunomodulatory treatment.

Respiratory insufficiency occurs in 25% of patients with GBS and major complications are common.

In a recent review undertaken in collaboration with the Intensive Care National Audit and Research Centre (ICNARC) [23] we showed that, in the UK, the mortality rate in ICU is 7.7% but the in-hospital mortality rate after ICU step-down is 16.7%. Others have described similarly high early mortality after ICU discharge [19,24,25,26]. The cause of this alarming mortality rate is unclear, but poor outcome does seem to be associated with delayed weaning and long-term ventilatory impairment. Weaning and step-down care are critically important in determining long-term outcome after severe GBS. Prolonged weaning requires specialist techniques of care and general medical ICUs do not always possess the expertise or facilities for weaning patients with prolonged ventilatory problems secondary to severe GBS.

To study these issues further we reviewed 20 patients with GBS, managed over 12 years in The Lane Fox Unit at St. Thomas' Hospital, a specialist tertiary referral respiratory care and weaning centre [27]. The mean age of patients was 59.3 years with a marked male predominance. All had tracheostomy at the time of transfer. The duration of mechanical ventilation between the onset and the time of transfer varied between 45 and 489 days. The time from admission to removal of the tracheostomy varied from 1 to 118 days. 19 patients were successfully weaned from invasive ventilatory support, 5 required nocturnal noninvasive ventilatory (NIV) support for 10 to 225 days, 1 continues to use nocturnal NIV and 1 patient died. Nineteen patients were successfully discharged. This study emphasises that, with appropriate management, many patients with severe and prolonged residual disability due to GBS can make an excellent or good recovery of respiratory function with meticulous ongoing care and rehabilitation, often despite severe residual limb weakness and long periods of dependency. This surprisingly high rate of recovery of respiratory and bulbar functional following acute GBS, even with severe residual weakness, remains a curious and unexplained phenomenon of the condition.

There is debate about the role of highly specialised neurological intensive care and this discussion has obvious relevance to the future care of GBS throughout a world in which facilities and resources vary enormously. It is uncertain if their primary role should lie in managing patients with common presentations of acute neurological disorders or if the scarce resources should be focused on the specialised care of tertiary referrals of the most complex and difficult management problems, which often demand extensive time and resource input to achieve the best outcomes in relatively small numbers of patients. If this is the case, it will be impossible to prove such units improve the mortality and morbidity rate of neurological disorders. However, they will have an important role as centres of last resort and in teaching, research and establishing guidelines of care.

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The Other Syndrome of Guillain-Barré Syndrome: Dysautonomia and Systemic Effects

Eelco F. M. Wijdicks

Introduction

I suspect the neurologists Georges Guillain and Jean-Alexandre Barré did not consider involvement of the autonomic nerves or even systemic effects; at least, when reading their seminal paper or later publications on variants it does not jump off the page. Why would they? Professor Guillain strongly felt the syndrome he described with Barré (and with some assistance from André Strohl) was utterly unique because the course was benign and their patients fully recuperated. His syndrome (GBS) was much different from the fatal Landry's ascending paralysis syndrome and he was unshakable in that conviction—no need to ponder a more serious clinical picture.

GBS with its weakness variants is now 100 years old, but dysautonomia has not even reached the age of 50. First, there was the typical disconnect with the proverbial left hand (read clinician) not knowing what the right hand (read pathologist) was doing. One of the first autopsy cases showed the sympathetic chain was involved (“myelin fragments in ballooned Schwann cells ... Scattered ganglion cells were in a disintegrated state”) but there was no clinicopathologic correlation with a patient dying from aspiration pneumonia and sepsis [1]. Perhaps it started with Mitchell and Meilman, who concluded there was sympathetic hyperactivity on the basis of a high excretion of urinary catecholamines in a markedly hypertensive patient with GBS [2]. Perhaps the observation that tweaked their syndrome (GBS) by adding involvement of the autonomic nervous system came with Lichtenfeld's paper [3].

Now we know from case reports and case series—some informative, others not—that GBS may have many other manifestations and vital organs can become compromised [4,5]. We also know that some are therapy related (e.g. IVIg), that others are rare but pop up in the literature occasionally (e.g. membranous nephropathy) or that manifestations are nonspecific and a result of failure to recognize and treat dysautonomia (e.g. posterior reversible encephalopathy syndrome in dysautonomia -associated hypertension). There is a good argument to be made that in the more severe cases of GBS there is probably another ‘hidden’ syndrome ([Figure 58.1](#)).

Here, I will interpret a collection of articles published over the years, but others have summarized the material in comprehensive topic reviews [6,7,8]. One can say these clinical manifestations are a less appreciated part of this acute immune mediated inflammatory disorder.

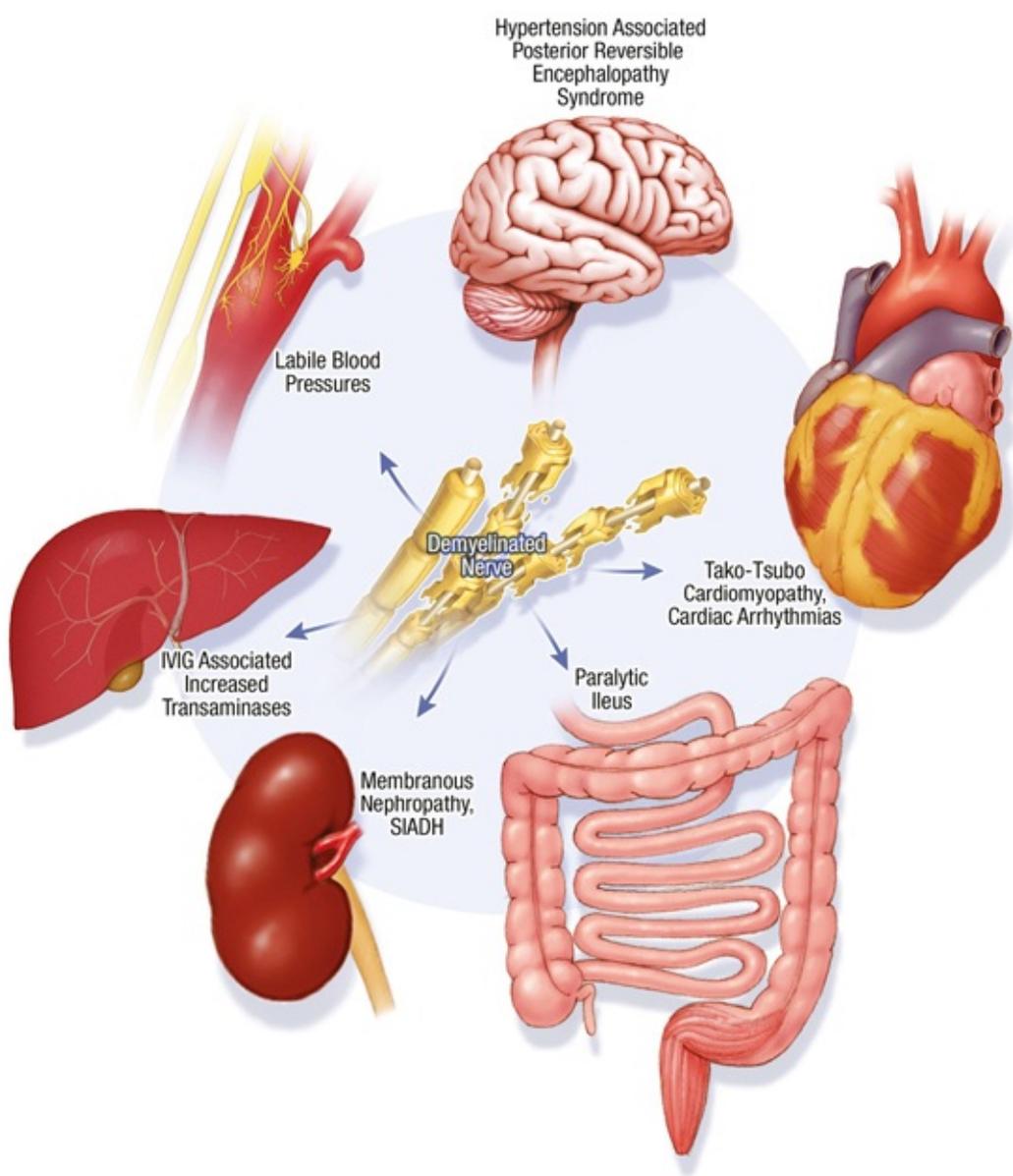


Figure 58.1 Systemic effects of Guillain-Barré syndrome. © 2016 Mayo Foundation for Medical Education and Research

The Many Facets of Dysautonomia

One of the earlier astute observations was by neurologist Peter Lichtenfeld from Mount Sinai Hospital, New York in 1971 and he attributed fatality in GBS to dysautonomia. In his study he describes how 4 of 28 patients died “during or immediately after episodes of severe autonomic dysfunction” as a result of “cardiac arrest following several hours of rapidly fluctuating autonomic status” or were “found dead after extremely high blood pressure recordings although paralysis was not severe” or “died suddenly after the development of a cardiac arrhythmia preceded by electrographic abnormalities” [3]. His sound advice included that “patients with inadequate sympathetic responsiveness must be positioned carefully, straining at bowel movements must be avoided, respiratory pressures deliberately set and at the first sign of autonomic dysfunction a cardiac monitor employed”.

Dysautonomia in GBS is recognized by blood pressure fluctuations and exaggerated drug responses, cardiac arrhythmias, hypersecretions, gastrointestinal dysfunction and bladder dysfunction. Curiously profound flushing and sweating had some clinicians considering a coexisting pheochromocytoma, and of course, in many patients urinary catecholamines were increased. Paroxysmal or sustained hypertension is seen in nearly 1 in 4 patients with GBS but not always the more severely affected. Systolic blood

pressures can become substantially elevated and reach values that may not only cause the left ventricle to acutely strain, but can even predispose the patient to develop posterior reversible encephalopathy syndrome. Encephalopathy with new onset seizures or visual disturbances is totally unexpected in a patient with GBS and therefore an MRI of the brain is essential to find its characteristic white matter vasogenic oedema.

Why these blood pressure fluctuations occur is not entirely known, but a baroreflex abnormality has been postulated [9]. Baroreceptor sensitivity might be altered as a result of vagal nerve demyelination and because when sympathetic nerves have less myelin, it results in a sympathetic overdrive. Dysfunction of afferent input from atrial stretch receptors could also play a role in the origin of blood pressure swings [10].

These blood pressure elevations require treatment, but treatment might lead to a marked hypotension due to exaggerated drug sensitivity. Clonidine, sodium nitroprusside, or a calcium channel blocker such as nifedipine have been used to treat severe hypertension, but simply controlling these responses with multiple doses of IV morphine is just as effective and perhaps safer.

The whole gamut of cardiac arrhythmias can be seen in GBS, including complete heart block [11,12]. Sinus tachycardia and so-called vagal bradycardia spells are most frequent in patients with GBS. Persistent sinus tachycardia may appear at any time during the illness and generally is not associated with hypotension or chest pain but slowing of rate is indicated with signs of myocardial ischemia on EKG. In patients with hypotension, echocardiography is needed to look for stress cardiomyopathy [13]. Vagal spells are brief salvos of bradycardia or sinus arrest, and nursing staff know that tracheal suctioning is a common trigger. Vagal spells are usually a feature seen in the worsening and plateau phase but may extend into the recovery phase. These bradycardic spells may be so severe that they can lead to a brief pause. A pacemaker may be considered if these episodes are symptomatic and recurrent [14]. In some patients, atrioventricular block or other more benign arrhythmias (e.g. bigeminy) become apparent.

Bronchial function is also likely impaired in Guillain-Barré syndrome, because bronchoconstriction and bronchodilatation are under the control of vagal and sympathetic innervation. There is some evidence that impaired bronchoconstriction and dilation due to abnormal innervation of bronchial smooth muscle can lead to profound impairment of clearing of already increased secretions and, in turn, lead to atelectasis of large lung segments.

As part of the screening for dysautonomia, patients should also be carefully examined for development of adynamic ileus. This occurs in about 1 in 10 patients with severe GBS and is recognized by loss of abdominal sounds, expansion of the abdominal girth, and enlarged colonic loops on abdominal x-ray. Perforation of the colon is a major complication which can substantially change the outcome of a recoverable neurologic illness. The treatment of patients with severe adynamic ileus is rectal and oral suction tubes, and a therapeutic decompressive colonoscopy. Opiates should be stopped. Peripherally acting mu-opioid receptor antagonists in the future may offer the reversal of ileus without loss of pain relief. The use of erythromycin, metoclopramide or neostigmine might be considered, but side effects (cardiac arrhythmias) may make it a much less favourable choice. In our series of patients with severe GBS, adynamic ileus developed in 15%, but only a few instances seemed correlated with dysautonomia. Pre-existing conditions, such as prior abdominal surgery, and incremental doses of opioids for pain management, were dominant causes [15].

Fatality from dysautonomia is difficult to prove. We, and particularly the Massachusetts General Hospital retrospective and prospective series [16], found none including among the more severely affected patients who were ICU admissions. The Queen Square series mentioned in this book (see Howard and Hirsh chapter) did not mention a single fatal case. Still, a report from the Dutch GBS group

with a large number of patients retrospectively reviewed between 1986 and 2008 noted 2 of 527 patients died after severe autonomic dysfunction, one patient with severe hypertension and subarachnoid haemorrhage and one patient whose care was de-escalated after hypoxic-ischemic encephalopathy. Three additional patients died unexpectedly of cardiac arrest during the recovery phase [17].

Organ Dysfunction

Any organ system is challenged in a patient with severe GBS maintained for some time on a ventilator. For sure any organ system can become critically involved when sepsis emerges. Some organs are involved (e.g. liver) because an infection preceded GBS (e.g. cytomegalovirus). But organ systems may potentially be injured as part of the immune target, and in some inflammatory lesions have been found.

Cardiac injury is likely secondary to dysautonomia. The mechanism of stress cardiomyopathy in GBS could be explained by sympathetic overdrive resulting in marked reduction in ejection fraction from sudden ventricular strain with hypertension. Morphologic EKG abnormalities are uncommon and nonspecific in GBS, but when they are present, ST-segment abnormalities are frequent. It is uncertain whether they represent myocardial damage. Myocarditis has been found in fatal cases that went to autopsy but this entity remains poorly understood. It might be difficult to sort it out from a co-existing viral infection affecting the heart. When I read through many of these cases the full ICU course is not fully known and little has been published lately.

Most fascinating is a membranous nephropathy causing in some patients a mild nephrotic syndrome and pitting oedema. The cases are detected if attentive physicians note the urinalysis results with marked proteinuria and microscopic haematuria [5]. Reported renal biopsies (including one case of subacute GBS I remember clearly) have been unquestionably confirmative, but not all cases in the literature may be GBS and an underlying systemic disorder such as lymphoma or vasculitis should be considered.

Transient abnormalities of transaminases (rarely double from baseline) have been found with no evidence of viral or toxic hepatitis other than an association with IVIg [18]. Sucrose containing IVIg is more likely to cause these largely clinically insignificant changes, and liver function abnormalities may coincide with worsening renal function but resolution is seen in 2 weeks. Immunoglobulins acting as immune complexes is a speculative explanation for increased transaminases. In most practices liver function abnormalities are not a concern with IVIg.

Other systemic disorders—not explained by dysautonomia—have been reported over the years and cases have included SIADH, hyperthyroidism and Addison's disease [19]. Each of these manifestations remains questionably associated with GBS.

On a Personal Note

The early observations on dysautonomia in the 1960s were far from alarmist, and I have seen impressive manifestations and mostly spontaneous wild blood pressure swings—one moment in frank shock then markedly hypertensive after being placed in the Trendelenburg position. Although I have personally not encountered fatality or cardiac arrest associated with dysautonomia and I have not seen a patient who needed a pacemaker, I still feel very uncomfortable with keeping patients with GBS and dysautonomia on the ward. The last patient I have seen at the time of this writing was improving from GBS but had a sustained sinus tachycardia for several weeks (Figure 58.2) and no other explanation. In severe GBS a neurointensivist will have to differentiate a veritable dysautonomia from early sepsis, pulmonary

embolus, aspiration pneumonia, opioid overuse and other guises, including urethra obstruction. Another practical problem is that drugs to treat dysautonomia may worsen dysautonomia (glycopyrrolate for increased secretions, neostigmine for ileus, beta blockers for tachycardia) and there is no good solution. Acute autonomic failure in GBS usually resolves before full improvement in motor function. Marked orthostatic hypotension may persist during the recovery phase. Whether this is due to persistent autonomic failure or a result of long-standing bed rest is undetermined. Dysautonomia and organ dysfunction plays no small part in the syndrome of severely affected patients but all in all it is reassuring to know it disappears and commonly leaves no trace.

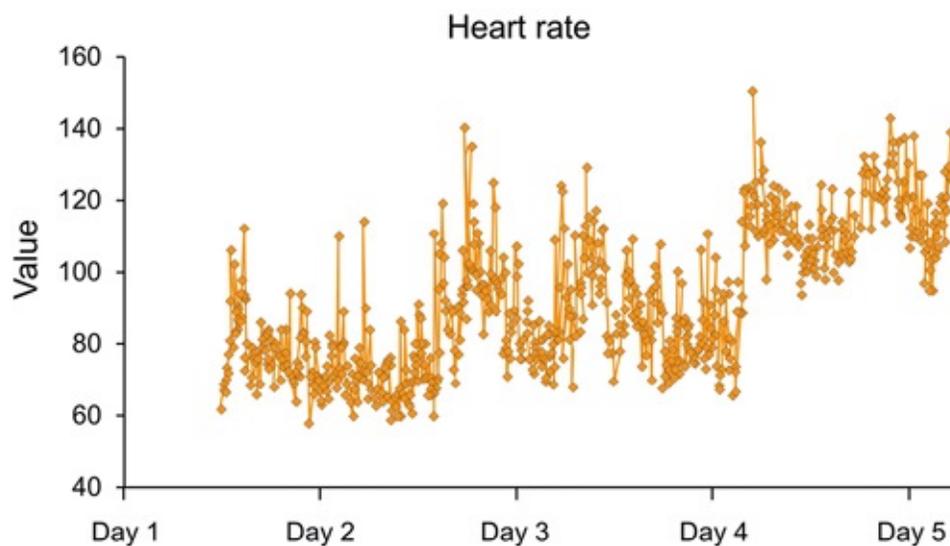


Figure 58.2 Asymptomatic sinus tachycardia as a single clinical recognizable sign of dysautonomia in Guillain-Barré syndrome. © 2016 Mayo Foundation for Medical Education and Research

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GENERAL

Guillain-Barré Syndrome: The Patient's Perspective

E. Benson, J. Baer, P. Bloomquist, L. Butler, G. Sanders, T. Sammut, M. Tedesco and C.L. Koski

As physicians, we approach a patient in terms of diagnosis, treatments available and eventual outcome. Guillain-Barré syndrome (GBS) was first reported in the early 19th century as a self-limiting, progressive weakness with preserved cognition, normal bowel and bladder function [1], loss of deep-tendon reflexes (DTRs) and abnormally elevated spinal fluid protein with a normal white blood cell count [2]. Other variants, such as the Miller Fisher syndrome of external ophthalmoplegia, sluggish pupil reflex and DTR loss [3], as well as pure sensory, pure motor, autonomic and multiple cranial nerve presentations, were subsequently identified. Most research suggests that the aetiology of this syndrome is complement-fixing antibody (Ab) with specificity for elements of peripheral nerve myelin or axolemma [4–6]. Treatment with plasma exchange or intravenous immunoglobulin shortens the clinical course and reduces the time on the ventilator, the time in the ICU and the time to independent walking [7]. This is particularly true in patients with high-risk factors including older age (> 60 years), ventilator dependence within a week of onset, and preceding diarrheal illness [8,9]. The patient's perspective, although appreciative of these advances, includes issues of communication; isolation; availability of accurate information; quality of care; ability to participate in work, family and social activities; and their ultimate outcome, as demonstrated in the following series of patient stories and memories.

Bob Benson (Written by Estelle Benson)

In November 1979 51-year-old Bob Benson, USA had an upper respiratory tract infection which developed into pneumonia. Within 2 weeks, he had trouble walking, holding a briefcase and turning a key to start his car. His signature was a straight line. In the emergency room (ER) a spinal tap supported a diagnosis of GBS. Bob was in an intensive care unit (ICU) for four weeks and treated with prednisone. To help him sleep and ease the pain, he had wine at bedtime. His course was complicated by hallucinations. From ICU Bob was transferred to a step-down unit and subsequently a rehabilitation facility. With hard work and a great attitude, he 'walked' out (barely) after four months.

We had no idea what this condition was or if he would ever get better. No literature, no one to talk to, no Internet! We vowed to do something about this. One year later, eight people including patients and doctors met around our dining room table in the first meeting of what would become the GBS/CIDP Foundation International! I started with a one-page newsletter and more people came. Dr Arthur Asbury, University of Pennsylvania, expert neurologist, mentored me. For one of the meetings (all at my home) he sent two of his neurology residents to speak—David Cornblath and Gareth Parry! Thirty-five years later, look where we are now with more than 150 chapters worldwide!!!

Glennys Sanders

In August 1981, at age 37, while on holiday, I, Glennys Sanders, UK, experienced fatigue, neck and back ache, inability to open bottles and climb stairs and generalized weakness. Overnight I could not turn over in bed, walk to the toilet or use my hands, and was hospitalized. I was intubated day 2 of a 6-week ICU stay prior to transfer to a hospital closer to home for another 3 months. Although GBS was suspected, neither I nor my family knew the diagnosis or prognosis until weeks later. Throughout, a dedicated and caring expert staff made my time in ICU as pleasant as possible, despite my pneumonia and 5 near-death situations. The ventilator was noisy. I was manually bagged during turning and bed linen changes. I was frightened, reliant on human expertise and nasogastric tube feeding, could not communicate despite an alphabet chart (only movement was to blink my eyes), and had concurrent hallucinations. No audio books, radio or television were available. I was unable to see my two young sons because of ICU policy. Insertion of a speaking trachea tube resulted in enormous joy to be able to communicate. After Christmas, I transferred to Rehab. Hydrotherapy was immensely beneficial. I relearned to swim, beginning with 13 floats attached. After 4 months, when I was able to independently turn over in bed, I was discharged to outpatient care. The Rehabilitation Centre was 4 1/2 hours by car from home. Within 6 weeks I learned to drive with adaptations. I felt independent for the first time in many months. Now 35 years on, I live a relatively normal life, albeit slower than most people my age. I am unable to climb stairs but run a home, play golf, swim, drive and travel alone. For 30 years I have voluntarily developed the GBS/CIDP British Support Group. In the beginning, Prof. Richard A.C. Hughes mentored me, and gradually chapters throughout the UK were formed and I became the International Director of the GBS/CIDP Foundation International. For my efforts Queen Elizabeth II awarded me the Member of British Empire medal.

Patricia Bloomquist

In 1990, I Patricia Bloomquist, 31 years old, Netherlands, experienced acute onset of severe lower back pain, oral numbness, and total malaise. I was hospitalized 5 days later and a diagnosis of GBS confirmed by spinal tap and nerve conduction studies. A course of IVIg was given, but deterioration continued, requiring intubation and mechanical ventilation for 35 days; a 2-month ICU stay was complicated with pneumonia, deep-vein thrombosis and hallucinations; and a 4.5-month further hospitalization was followed by outpatient rehabilitation. Six to eight weeks prior to my neurological symptoms I was given a tetanus immunization and developed swollen lymph nodes after 4 weeks. While in ICU I was visited by a recovered GBS patient found by my parents. This visit inspired me to start a support group, eventually leading to my involvement with the GBS/CIDP Foundation (liaison, regional director, board member, secretary of the board and currently vice president).

GBS has had a great impact on my life. Obviously, the first years (and sometimes still) it has given me a total different outlook on life. After 25 years, GBS still is a big part of my life, but in a good way. My 'work' for the Foundation and the Dutch support group is very rewarding and fulfilling. And there has been another bonus: because of GBS I've met so many wonderful people that I wouldn't have otherwise met.

All 3 of these patients noted hallucinations and abnormal dreams that have been described in as many as 19% of GBS ICU patients, which is higher than the 3.6% noted in other ICU patients. This phenomenon is linked to abnormalities in REM sleep, autonomic dysfunction and decreased levels of hypothalamic hypocretin-1 [10], suggesting involvement of the central nervous system.

Josua Baer

In 1996, I, Josua Baer, 11 years old, USA, following a flu-like illness, developed paraesthesia and mild weakness in my legs. The family doctor, anticipating recovery, approved a family vacation to Israel. On the first day, I was unable to drink from a straw, developed an inability to whistle and fully to open or close my eyelids. I progressed to generalized weakness and unsteady gait, prompting hospitalization and diagnostic studies in Jerusalem that supported a diagnosis of Miller Fisher syndrome with GBS overlap reflecting diffuse motor weakness. Following a course of IVIg, I was flown home to the United States unable to walk, with persistent double vision and loss of deep tendon reflexes, the last of which never returned. I lost 20% of my body weight and was in physical therapy for 10 months. I very gradually regained strength,

flexibility and stamina. Support and encouragement from my family played a large part in the recovery. Attendance in school in the fall for a half day was complicated by weakness and fatigue. I did not fit in with my class and was not able to make new friends because of abnormal thinness, frequently not being able to attend a full day of school and an inability to play sports. I was able to participate full time the second half of the year. With hard work I achieved full recovery.

Stuart Butler (Written by Lisa Butler, Mother)

In 2001, Stuart, USA, was a 5-year-old who had a viral infection on a cruise. Four weeks later he fell on the playground and started to limp. Over 5 days the unsteadiness progressed. He was unable to walk 40 feet without support. He was hospitalized for ataxia. An examination showed an inability to raise his arms over shoulder height or raise his legs against gravity. The weakness and associated loss of DTRs throughout resulted in a diagnosis of GBS, supported by an elevated spinal fluid protein. He was treated with IVIg over 4 days and released from hospital to home in a wheelchair. Over the next two weeks mild improvement was followed by a relapse, and nerve conductions showed a primary axonal variant of GBS. He was retreated with IVIg 2 months after initial symptoms and showed rapid improvement. He walked his first steps independently within a month. Physical therapy including aqua therapy was continued over a further 4 months. He currently is an active teenager on his way to college and plays competitive water polo.

Michael Tedesco

I, Michael Tedesco, 32 years old, USA, developed flu-like symptoms in March of 2003 and within a week had back pain with difficulty standing. Nerve conduction studies showed prolonged distal latencies and spinal fluid findings supported a diagnosis of GBS. During hospitalization, increasing weakness and difficulty breathing prompted my transfer to the ICU and intubation. A month and a half later, following two courses of IVIg, I developed some movement in the neck and trunk, followed by movement in my hands, arms and eventually legs. After 3 months I was weaned from the ventilator and moved to a rehab unit for another 2 months. I stood for the first time at 4 months. At my weakest I had lost a third of my body weight. I was discharged 5 months into my course, largely confined to a wheelchair. Gradually, over several months of outpatient rehab, 3 days per week, I discontinued my wheelchair, progressed to a walker, a cane and finally no support. I believe my mental and physical commitment to a full recovery was instrumental in my outcome. I have returned to my investment practice and enjoy many outdoor physical activities including surfing ([figure 59.1](#)).



Figure 59.1 Michael surfing in Fiji 2012

Trevor Sammut

Trevor Sammut, Republic of Malta. In November of 2011, as a 30-year-old man, I was struck down with food poisoning associated with vomiting, abdominal pain and runny diarrhoea that was treated with two different courses of antibiotics. Midway through the second week I was unable to form a scoop with the fingers of my left hand. I had fatigue, cramping and 'laziness' in my legs. I could not write despite resolution of the gastroenteritis. Difficulty standing up from a sofa and climbing up and down one flight of stairs prompted an emergency room visit. I entered the hospital limping at 9 pm on Sunday 20th November 2011 and was wheelchair-dependent within 6 hours. I had good sensation but prominent distal weakness in both upper and lower extremities. A diagnosis of GBS was verified with a lumbar puncture and NCSs. IVIg initially stabilized the downward course and after 10 days allowed me to begin to walk a few steps independently. After a 20-day hospitalization, I had distal atrophy and shortened ankle tendons. I underwent physical and occupational therapy. I psychologically fought real hard not to let GBS win over my morale and mind. At times GBS did win, as I definitely imagined myself permanently crippled at moments of weakness. I imagined myself secretly wheeling myself to a high bridge or a high location—to take a good view of course. My recovery was complicated by shock-like pain at night originating from my hips down my legs. Seven months later, with much determination and effort, I can open a garage-door lock, button a shirt and pants, separate my fingers from one another and walk independently. I am able to work full time and serve as a liaison for the GBS/CIDP FI. I enjoy my life and my family, not excluding my cat.

The overall incidence of GBS, estimated to be 1.1/100,000 to 1.8/100,000 a year, is increased after 50 years of age to 1.7/100,000 to 3.3/100,000 a year [11]. In this study, up to 70% of cases reported a preceding infection, either a URI or diarrheal illness, as was the case in all of the above series of patients. Only 1 of this series was in their sixth decade of age, most were in the fourth decade and 2 were children ages 5 and 11. Though not perhaps a representative sample age-wise of the GBS spectrum, where the peak incidence is between 50 and 60 years of age, all of these patients ultimately did well and currently enjoy an independent life. Two were empirically treated with a second course of IVIg because of relapse or lack of initial improvement. The use of a second course in patients with risk factors for a poor outcome is currently being studied in a randomized controlled trial in the Netherlands. Some of these patients participated in rehabilitation longer than others and one had significant neurological deficit even after 3 decades. Nonetheless, the overall response was good and 2 currently participate in competitive sports requiring good balance, strength and coordination.

Many cited the importance of the support by their family. I feel that the physician's role is not only to diagnose and provide appropriate treatment but also to follow the patient during rehabilitation. Documentation of the patient's improvement provides a different type of support and encouragement. It also identifies any relapse that might require additional retreatment. Knowing and working with more than 300 GBS patients in the past several years was perhaps the most rewarding experience in my entire neurological practice.

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Our Top 10: Medical Students' Perspectives on Guillain-Barré Syndrome

Eunice J.H. Goh, Odelia S.Q. Koh, Joshua S.W. Lee, Zongbin Li, Christen S.J. Lim, Tammy Y.Y. Tsang and Tham Sai Leong

Introduction

We are a group of medical students from Singapore in our third to final years of medical school researching Guillain-Barré syndrome (GBS) under the supervision of Dr Umapathi. We maintain a GBS database and work on projects that cover clinical, electrophysiological, laboratory and serological aspects of different GBS subtypes. Here we share our perspectives on this disorder that we have come to better understand in the course of our research.

Our First Encounter with the Enemy

Our first encounter with GBS was in a textbook, in a short paragraph buried amongst other medical conditions. Descriptors like 'uncommon' and 'autoimmune' prompted us to subconsciously relegate GBS to 'a good-to-know, but not a must-know' condition for the examinations. During tutorials on the approach to patients with flaccid weakness, GBS would be briefly mentioned as a differential diagnosis with minimal elaboration, some even using the terms 'acute inflammatory demyelinating neuropathy' (AIDP) and 'GBS' interchangeably. As we first embarked on our research journey, we learnt the global importance of this most common cause of acute flaccid paralysis. However, it was only after our encounter with Mr T that we appreciated the tremendous impact GBS had on patients.

A GBS Patient's Experience

"It began when I suddenly lost my appetite, felt bloated and unwell, but couldn't figure out what was happening." Such was Mr T's first encounter with something that would soon change his life forever.

At 60 years old, Mr T led an active life, worked as a construction company's technical officer and was happily married with children and grandchildren. When his vague symptoms failed to resolve, he consulted a general practitioner and was treated for indigestion. He did not get better and instead started finding it difficult to walk steadily and perform simple tasks like picking up a cup. His hands and feet were numb. This prompted a visit to the emergency department, whereupon he was admitted. He soon developed dysphagia and respiratory muscle paralysis, landing him in the neurology intensive care unit

(NICU) for 7 weeks. This was a frightening period as he could not comprehend what was happening to him. He was eventually diagnosed with GBS. The medical team provided some information about GBS to him, but much remained incomprehensible. Elderly people were supposed to, at some point in time, develop heart failure, strokes or cancer, but GBS? None of his friends had the disease. He had never heard of GBS before, nor of the nerve conduction studies (NCS) nor intravenous immunoglobulins (IVIg) which were soon to follow. Mr T remained hospitalised for 2 months and spent a further 3 at a rehabilitation hospital.

Mr T battled with GBS on multiple fronts: physically, psychologically, socially and financially. The disease robbed him of his livelihood and he grappled with the loss of gainful employment. The residual weakness and unsteadiness made it too dangerous for him to continue his physically demanding work inspecting construction sites. At his age, finding a new job would be an uphill struggle. Hefty medical bills further compounded the stress, and the previously self-sufficient Mr T had to seek financial support from his children.

An active and avid basketball player prior to his illness, it was devastating for Mr T when he lost his ability to walk, stand and sit upright over a few days. Having to rely on family members and nurses for simple activities like dressing and bathing was humiliating. It was no mean feat to relearn to walk independently, but he succeeded after persevering through intense rehabilitation.

To Mr T, this remains an incomplete victory. He counts himself lucky to have survived and regained some functional independence; however, he poignantly points out that going back to his previous pursuits remains impossible. Mr T hopes doctors will continue their research to find a cure for this debilitating condition. He still dreams of being able to play basketball again; to do so would be, as he puts it, 'better than winning the lottery'.

GBS through the Caregiver's Eyes

"It was one of the hardest and most emotionally draining times of my life" recounted Mdm T, wife and sole caregiver of Mr T. 'My heart ached when I saw him in the NICU with tubes and machines all over. He was so thin, weak and ill, I was afraid he would never open his eyes again'. Mdm T had many fears about her husband's condition. She felt helpless seeing his difficulty coping with activities of daily living (ADLs), but stoically batted away tears to maintain a strong front for her husband.

Mdm T had been through a lot even before this incident. After her mother-in-law suffered a stroke 31 years ago, Mdm T quit her job to be her full-time caregiver as well as to raise her young children. Although it was a trying time, she attributes her ability to tide through Mr T's illness to the 'training' she got when caring for her mother-in-law and young children simultaneously. This time, she had to care for both Mr T and her 2 grandchildren as she shuttled between the kindergarten, hospital and home every single day.

The ordeal continued even after Mr T was sent home from the rehabilitation hospital. Petite Mdm T struggled to support him as he walked and to deal with her husband's frustration over his inability to perform simple tasks such as bathing independently, something they took for granted prior to his illness.

Reflecting on the entire experience, Mdm T explained, "There will always be difficult times in life but the important thing is to remain strong and keep pushing forward. Indulging in self-pity will not help matters."

Mdm T is extremely thankful for her husband's recovery, knowing some patients are not so fortunate. Mr T is now able to walk independently and even squat down to carry his grandchildren! He is able to perform most ADLs independently, albeit with some limitation of movement in his upper limbs. Mdm T

smiled fondly when she recounted how the medical team supported her during those difficult weeks, thanking them for their care, comfort and concern which made the experience more bearable.

How Do Clinicians Diagnose GBS?

When we first started learning to diagnose patients with GBS, it felt as if we were running through a checklist. Reflexes decreased? Check. Ascending pattern of sensory loss? Check. Power decreased distally more than proximally? Check. Recent onset of symptoms and a flu a few weeks prior? Splendid, diagnosis of GBS made! Please wait while I order the appropriate tests to confirm the diagnosis and admit you to the ward for appropriate treatment. Next patient please!

However, as we delved deeper into our research, we encountered patients with a myriad of clinical presentations. With time, we began to appreciate the approach employed by clinicians. Not all symptoms or signs are equal, and we learnt that blindly following a checklist leaves us vulnerable to misdiagnosing mimics such as thyrotoxic hypokalaemic periodic paralysis, acute myelopathy and acute beriberi neuropathy as GBS.

We also began to appreciate the value of the various investigations involved. These include electrophysiological studies, spinal fluid examination and anti-ganglioside antibodies. While we learnt the utility of these tests in our textbooks, we appreciated their limitations in practice. This made us realise the significance of research in advancing knowledge of the disease at an electrophysiological, serological and even molecular level. Confining ourselves to a checklist leaves us blinkered and does a disservice to those whose symptoms are still evolving or lie beyond the fringes of our existing knowledge.

Is GBS a Single Entity or a Spectrum of Diseases?

Mr T's illness wore many masks. He initially presented with what appeared to be Miller Fisher syndrome (MFS) but unexpectedly progressed to develop additional features. He was later diagnosed to have acute motor and sensory axonal neuropathy (AMSAN). As we looked around, we found other patients similarly labelled GBS with very different clinical manifestations; some had paraesthesias, others even had pain. Through our database, we also encountered patients with central nervous system involvement namely Bickerstaff brainstem encephalitis (BBE).

Now, having been exposed to a myriad of presentations, we have learned to think of GBS as a spectrum of subtypes ranging from acute inflammatory demyelinating polyradiculoneuropathy (AIDP) to those with a more restricted neurological involvement such as MFS.

How Do We Sieve through the Different Subtypes of GBS?

With such a spectrum of presentations, how does one differentiate the various subtypes of GBS? Through our observations of clinicians at work and our research, we found that there are several factors useful in sorting this: demographics, antecedent infection, careful delineation of physical findings, serology and electrodiagnosis, especially serial studies [1].

Some forms of GBS can be distinguished on clinical grounds from 'classic' GBS, such as BBE, MFS and the pharyngeal-cervical-brachial variant. However, subtypes within classic GBS, namely AMAN and AIDP, are clinically indistinct. Serial NCS is the current gold standard [2,3,4]. GBS, being an autoimmune disease, also has a serological footprint. Research in this area has shed light on both the

identification of subtypes and on underlying pathogenesis at a molecular level. For instance, anti-GQ1b anti-ganglioside antibodies are present in majority of patients afflicted with MFS; these patients often have ophthalmoplegia, and it has been found that GQ1b gangliosides are concentrated in the oculomotor, trochlear and abducens nerves. Likewise, antibodies to GM1, GM1b, GD1a and GalNac-GD1a are raised in AMAN, and these have been implicated in paranodal injury. Mr T's serial nerve conduction study showed predominantly axonal changes and he had raised antibodies against GQ1b and GT1a. He was therefore assigned the subtype of AMSAN.

Despite many years of experience, clinicians and researchers still encounter difficulties in subtyping. To us novices in the world of GBS, watching our mentors think of novel solutions to these challenges has inspired us to press on and think of alternate solutions when faced with a scientific or clinical problem. We also learnt that dealing with complexity is one of the skills of an astute clinician. Our study of GBS has honed our clinical judgement, matured our thinking processes and enabled us to feel more confident in dealing with complex multi-system disorders. We have trained our minds to pick out the defining hallmark of each condition: the 'black-white stripes that differentiate the zebra from a horse,' as our mentor often quips.

Why Subtype GBS at All?

Does it really matter to patients like Mr T if he has AIDP or AMAN? After all, the mainstay of treatment remains the same, with the focus on supportive care and either IVIg or plasmapheresis to minimise the extent of autoimmune-mediated damage to peripheral nerve. Beyond the utility in prognosticating the individual patient's expected clinical course, the distinction does not generally affect clinical practice. This question kept recurring during the many late nights we spent trying to make sense of our research data and derive better ways of subtyping GBS. We learnt that our current methods, while being constantly improved, are still unable to consistently distinguish between subtypes. We encountered patients, undifferentiated at presentation, who were later reclassified as our research entailed serial NCS and long-term follow-ups. However, repeat NCS and serologies are not feasible for patients in underserved parts of the world.

Nevertheless, questioning the purpose of our research is just as important, if not more so, than the research itself. As we tried to derive a greater meaning from our work, we eventually came to the understanding that although management currently remains the same, the pursuit of knowledge is an end in its own right, and subsequent generations would recoup the benefits of our current work.

The Research Process: Entering Uncharted Waters

For most of us, this was our maiden foray into research (we now realise we might have entered the pool at the deep end). Before this, our experience with research was mainly limited to reading journal articles and making sense of published work. As we marvelled at the intellectual discussions at conferences by the giants of GBS research, whom we had only read about previously, a flame of inspiration was ignited within all of us to aspire to make a contribution to the understanding of GBS.

We were very fortunate to be exposed to both research and clinical work early in our medical school years. Clinical work laid the foundation of our understanding of GBS, allowing us to see how it played out in real life, while research armed us with tools to delve deeper. On the other hand, research was not a bed of roses as our idealistic selves soon realised; there were so many aspects we had not considered,

from fastidiously maintaining a database to securing adequate funding. We also learnt valuable skills such as manuscript preparation and cogent presentation of data. We have not figured it all out yet (and perhaps never will), but the seeds of a future in research have been planted.

The Future of GBS Treatment

We hold the same hope as Mr T to find a cure for GBS and read with excitement some of the developments in GBS. We now know that neuronal injury is a complement-mediated process [5] with immunologic targets differing amongst subtypes, such as Schwann cells in AIDP and nodal and paranodal axolemma in AMAN [6]. Further, histological-serological-molecular correlation may facilitate the development of treatment options, beyond IVIg and plasmapheresis. It is with great anticipation that we look towards a future where more targeted treatment can help patients like Mr T recover faster and better; and we hope we may be able to play a role in making this dream a reality.

Our Takeaways

The conundrum that is GBS is a lesson in humility and patience for us. It has taught us respect for the scientific method. Our initial lofty aspirations of making the next breakthrough in GBS by the end of medical school evolved into smaller, more deliberate steps at improving understanding of this disease by working with the global community of GBS researchers. The immediate benefits of our work may not yet be clear, but it is our hope that fellow medical students, doctors and scientists around the world, who share this common passion for GBS research, are encouraged that their efforts are never in vain.

It is also important we do not lose sight of our goal of research: to alleviate the suffering of patients like Mr T. We have learnt the importance of establishing prompt diagnoses, subtyping accurately, applying current evidence-based therapeutic and supportive protocols, deepening our understanding of the immunopathology of GBS and designing new, inexpensive, accessible and more effective therapeutic approaches. What we know of GBS in the future will likely be different from what we believe we now know; the last 100 years is proof of that. With so much still a work in progress, we realise it is important to keep an open mind to new solutions, yet not forget the art of applying our currently imperfect knowledge in clinical practice.

Finally, it has been our greatest privilege to have been mentored and taught by experts in the field which, coupled with the lessons Mr T has imparted to us, continue to guide us in our journey in understanding GBS.

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Top 10 Moments in Research: A PhD Student's Journey

Madeleine E. Cunningham

Introduction

As I am sure is the case for many young researchers entering the field of Guillain-Barré research, when the day came to start my PhD I had not even heard of a ganglioside and had only a vague recollection of a lecture mentioning Guillain-Barré syndrome (GBS). So I had a lot of reading to do. Some papers became staple reading material, necessary both to learning the general nature of my subject matter, but also to refer to for the nitty-gritty details needed to make sense of my own data. Here, I have compiled a list of my top 10 favourite papers, some of which helped me understand the world of GBS research and some of which helped me understand my own PhD. All of them are wonderful examples of how different areas of research have combined to forward our understanding of GBS over the last century.

McKhann GM, et al. Clinical and electrophysiological aspects of acute paralytic disease of children and young adults in northern China. *The Lancet*, 1991

My thesis focused on acute motor axonal neuropathy (AMAN); therefore one of my first looked-to papers was that of McKhann and colleagues' clinical and electrophysiological description of axonal GBS in children [1]. Until their ground-breaking work, GBS was thought of as demyelinating, synonymous with what is now referred to as acute inflammatory demyelinating polyneuropathy (AIDP). This paper described a paralytic syndrome which occurred in rural China, similar to GBS in all features with the exception of the electrophysiological tests [1]. These tests indicated not a demyelinating disease, but one which appeared to affect motor neuron axons. This syndrome was also seasonal, a feature not believed to be commonly seen with GBS at the time. This was the first indication not only that GBS could also be axonal, but also that it can be linked to seasonal outbreaks of illness.

Ilyas AA, et al. Serum antibodies to gangliosides in Guillain-Barré syndrome. *Annals of Neurology*, 1988

One of the first things I was ever told about GBS is that it is an autoimmune disease, caused by antibodies targeting peripheral nerves. Of course this only begins to scratch the surface of GBS pathogenesis.

However, until the work of Ilyas and colleagues in 1988 the target for this autoimmune attack was unknown. They were the first to demonstrate that antibodies against peripheral nerve gangliosides were present in the serum of GBS patients [2]. Since then, many other studies have confirmed this using various solid-phase assays. Some subtypes of GBS were identified as being associated with specific ganglioside antibodies, building the case for these antibodies being biomarkers for disease. The antigen for AIDP, however, still remains elusive.

Yuki N, et al. Animal model of axonal Guillain-Barré syndrome induced by sensitization with GM1 ganglioside. *Annals of Neurology*, 2001

While anti-ganglioside antibodies were associated with GBS patients, before 2001 it was still debated whether the antibodies were pathogenic or simply biomarkers of disease. Evidence in support of the former came with the development of the first animal model of AMAN. When Japanese white rabbits were inoculated with GM1 ganglioside, they began to develop limb weakness or flaccid paralysis, accompanied by an anti-GM1 antibody response in their serum [3]. Animal models are an essential step in understanding molecular pathogenesis of any disease and in this particular case they served to confirm the importance of an autoimmune response to ganglioside as a triggering step in the process.

Takamiya K, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proceedings of the National Academy of Sciences USA*, 1996

The generation of mice lacking the enzymes responsible for synthesizing ganglioside plays an essential role in our story. GalNAcT^{-/-} mice generated by Takamiya and colleagues lack all complex gangliosides, a subset of gangliosides whose production is dependent on the GalNAcT enzyme [4]. The generation of these mice, who develop an age-dependent degenerative phenotype, led to further understanding of the roles of gangliosides in nervous system maintenance. On a personal level they have served me faithfully as negative controls in many of my studies which require an interaction between antibody and ganglioside. One of their most useful roles, however, has been their involvement in ganglioside immunisation studies.

Bowes T, et al. Tolerance to self gangliosides is the major factor restricting the antibody response to lipopolysaccharide core oligosaccharides in *Campylobacter jejuni* strains associated with Guillain-Barré syndrome. *Infection and Immunity*, 2002

While antibodies against gangliosides had been generated previously in wild type mice, GalNAcT^{-/-} mice produce a much greater anti-ganglioside antibody response than their wild type counterparts [5]. For our lab, this has led to the use of these mice as hosts for generating many of our in-house anti-ganglioside antibodies. The incorporation of liposomes into our immunisations, as described by Bowes and colleagues, have proved to be an effective way of introducing gangliosides into the mice in a way which at least in part mimics the way gangliosides are presented in the membrane, especially when some ova protein is introduced to elicit T cell help [6]. This protocol has been used to make many in-house

monoclonal antibodies against gangliosides, and, more recently, antibodies to other lipids.

Greenshields KN, et al. The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice. *Journal of Clinical Investigation*, 2009

The discovery that antibodies against gangliosides are often found in patients' sera was only the beginning of a very complex (no pun intended) story. It has become increasingly clear that the organisation of gangliosides in the plasma membrane is incredibly important in GBS. Kaida and colleagues demonstrated that a proportion of GBS patients had antibodies against neoepitopes formed by a combination (or complex) of ganglioside antigens, an observation Alan Pestronk previously made about GM1 and other lipids in patients with multifocal motor neuropathy [7,8]. The studies by Greenshields and co-workers showed that despite binding to GM1 in solid-phase assays, surrounding gangliosides such as GD1a may block the binding of certain GM1 antibodies in the living plasma membrane, preventing injury [9]. These studies, particularly appropriate to this chapter as they were done during her PhD, demonstrated that this organisation of gangliosides is incredibly important for pathogenicity.

Yao D, et al. Neuronal expression of GalNAc transferase is sufficient to prevent the age-related neurodegenerative phenotype of complex ganglioside-deficient mice. *Journal of Neuroscience*, 2014

A development which greatly served my PhD was the generation of new transgenic mice, which only expressed the usually ubiquitous gangliosides on neuronal or glial membranes [10]. These mice are not only useful for the study of ganglioside function, they are great tools for the study of GBS. For unknown reasons, antibodies directed against gangliosides only result in injury to peripheral nerves, despite the fact that gangliosides are expressed on most cell types in the body. Therefore the production of these mice can allow more effective targeting of anti-ganglioside antibody-mediated attack to disease-relevant sites. For me, whose focus was the neuromuscular junction, this allowed the comparison of binding and injury at the perisynaptic Schwann cells or the motor nerve terminal. It also meant restricting in vivo binding of anti-ganglioside antibody to neuronal or glial sites.

Daniotti JL and Iglesias-Bartolome R. Metabolic pathways and intracellular trafficking of gangliosides. *International Union of Biochemistry and Molecular Biology Life*, 2011

Gangliosides have very dynamic existences. They are formed in the Golgi complex and transported to the outer leaflet of the plasma membrane, where they are thought to exist in the already-dynamic lipid rafts. However, they also can be re-endocytosed and post-modified or even degraded into their constituent parts [11]. This review details the comings and goings of gangliosides and how their endocytic pathways can change in different cell types and in response to different ligands, including toxins and antibodies. The movements of gangliosides in this way led to the idea that the anti-ganglioside antibodies, so relevant to patient disease, may be internalised along with the gangliosides.

Fewou SN, et al. Anti-ganglioside antibody internalization attenuates motor nerve terminal injury in a mouse model of acute motor axonal neuropathy. *Journal of Clinical Investigation*, 2012

As we come further down the list, the papers are particularly personal choices, whose work either has directly influenced the background or was essential to the methods employed during my PhD. My next choice heavily influenced the direction which my PhD studies took. This paper was the first to show explicitly that antibodies against gangliosides can bind the axonal component of the neuromuscular junction and become internalised at this site [12]. This observation is one which has a lot of potential implications. First of all, it provides an explanation as to why the motor nerve terminal is relatively spared in patient disease, when there is such a wealth of evidence showing its destruction in ex vivo tissue in response to anti-ganglioside antibody and complement. Second, since it seems to act as a vacuum for anti-ganglioside antibody, could this be enough to affect the amount of antibody which is circulating? That was the question I wished to answer in my PhD.

Fabian RH. Retrograde axonal transport and transcytosis of immunoglobulins: implications for the pathogenesis of autoimmune motor neuron disease. *Advances in Neurology*, 1991

While the observation of peripheral antibody being taken up at the nerve terminal is not a new one, it has never been considered as an important factor in the field of GBS research. Previous studies which looked at antibodies against nonspecific synaptic membrane components indicated that this uptake at the nerve terminal is not the end of the road for these antibodies. In fact, immunoglobulins which are taken up at the motor endplate may be retrogradely transported to the spinal cord [13]. Does this happen with anti-ganglioside antibodies as well? If so, what influence could those antibodies have once in the CNS? These were further questions which I sought to answer within the constraints of my 3-year PhD. Some have been answered in part and some remain unanswered.

Conclusion

From starting out my project, completely overwhelmed by the expanse of new information I needed to retain, eventually finding my niche within the field, and coming out the other end with a PhD, it has been a long journey. That journey is reflected in my top 10 papers which helped me build my knowledge and shape the direction of my studies. You may have noticed that 4/10 of my papers have a distinct Glaswegian quality about them. From this you may have guessed that my PhD was carried out in Glasgow in the lab of Professor Hugh Willison. It is not that I am biased (well maybe a little), just simply that these papers are very personal to my journey, often describing methods or resources critical to my PhD, or work which I took forward in my own research.

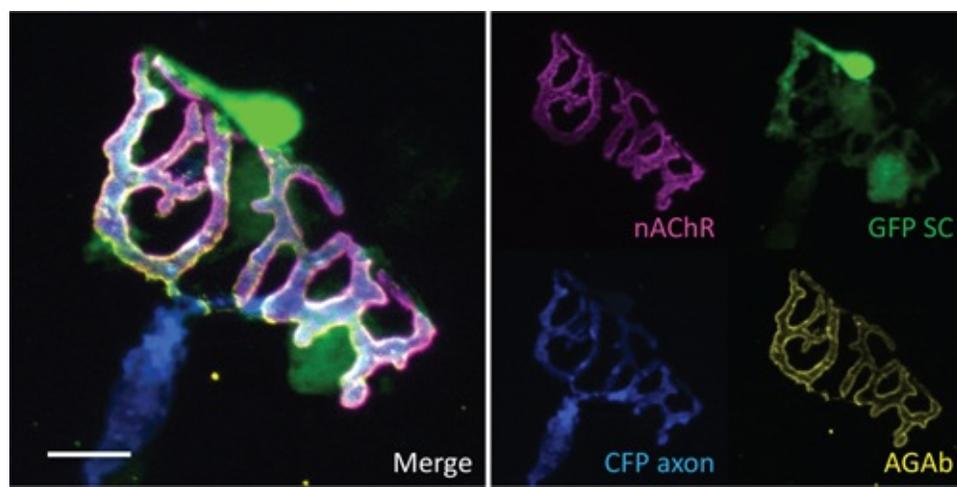


Figure 61.1 Neuromuscular junction labelled with anti-ganglioside antibody. α -Bungarotoxin labels the post-synaptic nicotinic acetylcholine receptors (nAChRs), while the axon and Schwann cells (SCs) express CFP and GFP, respectively.

While any PhD attempts to address several questions, it will inevitably throw up several more which remain unanswered when the time comes to submit. These questions are left to future PhD students who will find their own top 10 papers in their own personal journey in GBS research. I leave you with an image of my wee pal, the NMJ, taken during my PhD (figure 61.1). I must say it was difficult to choose only one.

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A Bibliometric Assessment of Guillain-Barré Syndrome: A Librarian's Perspective

Susan Ashworth

Introduction

Citation analysis is not an exact science and what is presented here is one interpretation of the top research and researchers in Guillain-Barré syndrome. There will be other interpretations depending on the tool or the methodology used to derive the citation analysis. There are a number of different tools that can be used; the tools which are commonly available in higher education are Web of Science (WoS), Scopus and Google Scholar. These databases each give different citation counts for the same article (WoS and Scopus may not differ significantly; Google Scholar usually returns a higher number of citations). This begs the question as to whether every citation is equal. WoS and Scopus have strict inclusion criteria for journals in their database, and citations to articles come from the same range of high-quality journals, arguably presenting a clearer picture of academic impact; Google Scholar includes citations from a much wider range of sources, for example from student dissertations, so arguably academic impact is diluted.

In this case, the Web of Science Core Collection (the citation indexes) has been used as the basis for analysis. WoS is an online, multidisciplinary indexing and abstracting database which also indexes the citations within and to articles.

Earliest Publications

The earliest paper related to GBS which is indexed in WoS was published in the *Journal of Nervous and Mental Disease* in 1900 (alongside 2 other papers in the same volume).

Knapp PC, Thomas JJ. Landry's paralysis. *Journal of Nervous and Mental Disease*, 1900

This paper has been cited just 4 times, and early publications in WoS on Landry's paralysis received very few citations. There were far fewer journal articles published in the early 20th century than today. Bornmann and Mutz (2014) have identified that global scientific publication is growing at a rate of around 3% every year and that the volume of publication approximately doubles every 24 years [1].

Top Publications

The search conducted on WoS was for the keyword 'Guillain', which returned 9,190 results^a, and papers

were listed in order of times cited from highest to lowest. This search was chosen as the most appropriate based on tests using a number of different variations—for example, searches for Guillain-Barré missed those articles where Barré is spelt as Barr or Barri. ‘Guillain’ is a broad search term and some of the papers found are peripheral to GBS. I consulted with staff from the Institute of Infection, Immunity and Inflammation at the University of Glasgow to identify these peripheral papers and, after consultation, the following papers are my Top 10.

All Publications

1. van der Meché FG, Schmitz PI (1992) A randomized trial comparing intravenous immune globulin and plasma-exchange in Guillain-Barré syndrome. *N Engl J Med* **326**(17): 1123–29. (10.1056/NEJM199204233261705)
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7. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I (1993) Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. *Neurology* **43**(10): 1911–17. (10.1212/WNL.43.10.1911)
8. Ho TW, Mishu B, Li CY, Gao CY, Cornblath DR, Griffin JW, Asbury AK, Blaser MJ, McKhann GM (1995) Guillain-Barré syndrome in Northern China: relationship to *Campylobacter jejuni* infection and anti-glycolipid antibodies. *Brain* **118**(3): 597–605. (10.1093/brain/118.3.597)
9. Rees JH, Soudain SE, Gregson NA, Hughes RA (1995) *Campylobacter-jejuni* infection and Guillain-Barré syndrome. *N Engl J Med* **333**(21): 1374–79. (10.1056/NEJM199511233332102)
10. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, Keenlyside RA, Ziegler DW, Retailiau HF, Eddins DL, Bryan JA (1979) Guillain-Barré syndrome following vaccination in the National Influenza Immunization Program, United States, 1976–1977. *Am J Epidemiol* **110**(2): 105–123.

All Publications by Authors

The ‘analyse results’ feature in WoS allows analysis of the 9,190 results returned by a search for Guillain; this includes an analysis by authors; the top 10 most prolific authors are listed in [Table 62.1](#).

The ‘create citation report’ feature in WoS then enables ranking of the top 10 most published authors by H-Index (the point at which the author has published n publications, each of which has been cited at least n times). See [Table 62.2](#).

All Publications by Citation

WoS provides the sum of the times cited for each author's papers and provides the sum of the times cited without self-citation. This enables us to see which authors have cited themselves most often (there could be very good reasons for this, for example, a researcher building on previous knowledge; the researcher's work is at the cutting edge of their field, and so forth [2]). See [Table 62.3](#).

Table 62.1 Top 10 most prolific authors

| Author | Number of Papers | % of 9,190 total |
|---------------|------------------|------------------|
| Yuki, N | 345 | 3.75 |
| Jacobs, BC | 190 | 2.07 |
| van Doorn, PA | 176 | 1.92 |
| Hughes, RAC | 157 | 1.71 |
| Hartung, HP | 152 | 1.65 |
| Willison, HJ | 127 | 1.38 |
| Kusunoki, S | 124 | 1.35 |
| Hirata, K | 101 | 1.1 |
| Kuwabara, S | 94 | 1.02 |
| Toyka, KV | 92 | 1 |

Table 62.2 Top 10 most published authors by H-Index

| Author | Percentage of self-citations |
|---------------|------------------------------|
| Yuki, N | 25.9 |
| Willison, HJ | 15.1 |
| Kusunoki, S | 14.4 |
| van Doorn, PA | 14.3 |
| Jacobs, BC | 14.2 |
| Kuwabara, S | 11.4 |
| Hartung, HP | 10.7 |
| Hirata, K | 8.4 |
| Toyka, KV | 7.9 |
| Hughes, RAC | 6.2 |

Table 62.3 Top authors by self-citation

| Author | No. of papers | Average cites per item | H-Index |
|---------------|---------------|------------------------|---------|
| Yuki, N | 345 | 23.95 | 49 |
| Hartung, HP | 152 | 39.07 | 45 |
| Hughes, RAC | 157 | 41.36 | 42 |
| Willison, HJ | 127 | 36.92 | 40 |
| Toyka, KV | 92 | 45.9 | 35 |
| van Doorn, PA | 176 | 21.12 | 33 |
| Kuwabara, S | 94 | 25.86 | 32 |
| Hirata, K | 101 | 28.16 | 31 |
| Jacobs, BC | 190 | 20.57 | 31 |
| Kusunoki, S | 124 | 20.11 | 24 |

Top 10, Articles Only

The search for ‘Guillain’ includes a wide range of document types; WoS allows searches to be refined by document type such as articles, review articles, meeting abstracts, letters, editorial material, proceedings papers, book chapters, etc.

Refining the search to include only articles gives 5,657 articles. Again I consulted with academic colleagues to determine which articles are peripheral to Guillain-Barré syndrome and produced my Top 10 articles:

Articles

1. van der Meché FG, Schmitz PI (1992) A randomized trial comparing intravenous immune globulin and plasma-exchange in Guillain-Barré syndrome. *N Engl J Med* **326**(17): 1123–29. (10.1056/NEJM199204233261705).
2. Plasma Exchange/Sandoglobulin GBS Trial Group. (1997) Randomised trial of plasma exchange, intravenous immunoglobulin, and combined treatments in Guillain-Barré syndrome. Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. *Lancet* **349**(9047): 225–30. (10.1016/S0140-6736(96)09095-2)
3. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I (1993) Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. *Neurology* **43**(10): 1911–17. (10.1212/WNL.43.10.1911)
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5. Rees JH, Soudain SE, Gregson NA, Hughes RAC (1995) *Campylobacter-jejuni* infection and Guillain-Barré syndrome. *N Engl J Med* **333**(21): 1374–79. (10.1056/NEJM199511233332102)
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7. Feasby TE, Gilbert JJ, Brown WF, Bolton CF, Hahn AF, Koopman WF, Zochodne DW (1986) An acute axonal form of Guillain-Barré polyneuropathy. *Brain* **109**(6): 1115–26. (10.1093/brain/109.6.1115)
8. McKhann GM, Cornblath DR, Griffin JW, Ho TW, Li CY, Jiang Z, Wu HS, Zhaori G, Liu Y, Jou LP, et al. (1993) Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. *Ann Neurol* **33**(4): 333–42. (10.1002/ana.410330402).
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10. Jacobs BC, Rothbarth PH, van der Meché FG, Herbrink P, Schmitz PI, de Klerk MA, van Doorn PA (1998) The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology* **51**(4): 1110–15. (10.1212/WNL.51.4.1110)

Articles Only, Publications by Authors

For the 10 most prolific authors, articles only, see [Table 62.4](#). To see these ranked by H-Index, see [Table 62.5](#). To see them ranked by self-citation, see [Table 62.6](#).

Top Review Articles

Limiting the ‘Guillain’ search to reviews gives 1,028 reviews. My Top 10 most highly cited reviews (again after academic consultation) are as follows.

1. Hughes RAC, Cornblath DR (2005) Guillain-Barré syndrome. *Lancet* **366**(9497): 1653–66. (10.1016/S0140-6736(05)67665-9)
2. Haymaker W, Kernohan JW (1949) The Landry-Guillain-Barré syndrome: a clinicopathologic report of 50 fatal cases and a critique of the literature. *Medicine* **28**(1): 59–141. (10.1097/00005792-194902010-00003)^b
3. Ropper AH (1992) The Guillain-Barré-syndrome. *N Engl J Med* **326**(17): 1130–36. (10.1056/NEJM199204233261706)
4. Willison HJ, Yuki N (2002) Peripheral neuropathies and anti-glycolipid antibodies. *Brain* **125**(12): 2591–25. (10.1093/brain/awf272)
5. Nachamkin I, Allos BM, Ho T (1998) *Campylobacter* species and Guillain-Barré syndrome. *Clin Microbiol Rev* **11**(3): 555–67.
6. Hartung HP, Pollard JD, Harvey GK, Toyka KV (1995) Immunopathogenesis and treatment of the Guillain-Barré syndrome. *Muscle Nerve* **18**(2): 137–53. (10.1002/mus.880180202)
7. Hughes RAC, Hadden RD, Gregson NA, Smith KJ (1999) Pathogenesis of Guillain-Barré syndrome. *J Neuroimmunol* **100**(1–2): 74–97. (10.1016/S0165-5728(99)00195-2)
8. Van Doorn PA, Ruts L, Jacobs BC (2008) Clinical features, pathogenesis, and treatment of Guillain-Barré syndrome. *Lancet Neurol* **7**(10): 939–50. (10.1016/S1474-4422(08)70215-1)
9. Kiefer R, Kieseier BC, Stoll G, Hartung HP (2001) The role of macrophages in immune-mediated damage to the peripheral nervous system. *Prog Neurobiol* **64**(2): 109–27. (10.1016/S0301-0082(00)00060-5)
10. Dalakas MC (2004) Intravenous immunoglobulin in autoimmune neuromuscular diseases. *JAMA* **291**(19): 2367–75. (10.1001/jama.291.19.2367)

Table 62.4 Top 10 most published authors, articles only

| Author | Number of papers | % of 5657 |
|---------------|------------------|-----------|
| Yuki, N | 200 | 3.54 |
| Jacobs, BC | 103 | 1.82 |
| van Doorn, PA | 89 | 1.57 |
| Hartung, HP | 87 | 1.54 |
| Willison, HJ | 87 | 1.54 |
| Hirata, K | 80 | 1.41 |
| Hughes, RAC | 79 | 1.39 |
| Koga, M | 77 | 1.36 |
| Kuwabara, S | 71 | 1.25 |
| Kusunoki, S | 69 | 1.22 |

Table 62.5 Top 10 most published authors by H-Index, articles only

| Author | No. of papers | Average cites per item | H-Index |
|---------------|---------------|------------------------|---------|
| Yuki, N | 200 | 30.95 | 44 |
| Willison, HJ | 87 | 41.38 | 36 |
| Hughes, RAC | 79 | 60.03 | 35 |
| Hartung, HP | 87 | 45.48 | 33 |
| Kuwabara, S | 71 | 33.13 | 32 |
| van Doorn, PA | 89 | 36.32 | 32 |
| Hirata, K | 80 | 34.29 | 30 |
| Jacobs, BC | 103 | 33.78 | 30 |
| Koga, M | 77 | 35.62 | 30 |
| Kusunoki, S | 69 | 29.74 | 24 |

Table 62.6 Top authors by self-citation, articles only

| Author | Percentage of self-citations |
|---------------|------------------------------|
| Yuki, N | 17.3 |
| Willison, HJ | 13.4 |
| Jacobs, BC | 11.6 |
| Kusunoki, S. | 11.1 |
| Koga, M | 10.4 |
| van Doorn, PA | 9.4 |
| Hirata, K | 8.1 |
| Kuwabara, S | 8.2 |
| Hartung, HP | 4.5 |
| Hughes, RAC | 3.6 |

The top review article authors, as measured with the H-Index is shown in [Table 62.7](#).

The top review article authors, by self-citation, appears in [Table 62.8](#).

Table 62.7 Top review article authors by H-Index

| Author | No. of papers | Average cites per item | H-Index |
|---------------|---------------|------------------------|---------|
| Hartung, HP | 35 | 49.89 | 24 |
| Hughes, RAC | 26 | 53.81 | 14 |
| Shoenfeld, Y | 18 | 36.5 | 14 |
| Yuki, N | 27 | 34.52 | 13 |
| Gold, R | 19 | 33.95 | 11 |
| Kieseier, BC | 16 | 49 | 11 |
| van Doorn, PA | 16 | 26.19 | 10 |
| Willison, HJ | 11 | 68.91 | 10 |
| Toyka, KV | 16 | 41.25 | 9 |
| Dalakas, MC | 10 | 57.2 | 7 |

Table 62.8 Top review article authors by self-citation

| Author | Percentage of self-citations |
|---------------|------------------------------|
| Kieseier, BC | 4.5 |
| Hartung, HP | 4.4 |
| van Doorn, PA | 3.8 |
| Yuki, N | 3.3 |
| Gold, R | 2.5 |
| Dalakas, MC | 2.4 |
| Hughes, RAC | 2.4 |
| Toyka, KV | 2.3 |
| Shoenfeld, Y | 2.1 |
| Willison, HJ | 1.6 |

Normalisation

There has not been any normalisation applied to take account of date of publication (the longer a paper has been published the more time it has to accrue citations). Reviews also tend to attract more citations than original research. All of the papers I have selected have been cited over a hundred times, most of them several hundred times.

Impact

Citations are one measure of impact, which is largely academic. Governments and research funders are increasingly interested in wider impacts such as the impact of research on the economy, or on society more generally, or on the culture. There are a new group of metrics which are designed to try to measure this wider impact; these are referred to as altmetrics or alternative metrics. Altmetrics are seen as complementary to traditional citation analysis and include article views, downloads, and mentions in social media, news media and policy documents. Altmetrics can provide real-time and immediate feedback on the attention being paid to scholarly content and can be very useful in particular for early career researchers whose research may not yet be cited. One example of the use of altmetrics is the University of Glasgow's institutional repository, Enlighten. The altmetrics.com API tool has been embedded into the repository and uses a journal article's DOI or PubMed ID to provide a 'donut' visualisation of sources discussing papers published by University of Glasgow authors, with a link to further details available.

^a All searches were conducted on 8th December 2015.

^b This paper is categorised as a review in WoS but appears to be an original article.

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2. Hyland K (2003) Self-citation and self-reference: credibility and promotion in academic publication, *J Amer Soc Info Sci Tech* **54**(3): 251–59. (10.1002/asi.10204)

63

Ten Papers That Set the Future

Simon Rinaldi

An Introduction to the Future

It is notoriously difficult to make predictions, especially about the future, as Niels Bohr (may have) previously noted. Nevertheless, this final chapter speculates on the directions GBS research and treatment might take in its second 100 years, drawing on some ground-breaking approaches and observations described in the existing literature. I await with interest the publication of GBS200 as the gold standard test for the clarity of my crystal ball.

Take Care of the Chickens—Prevention Is Better Than Cure

Baker MG, et al. Declining Guillain-Barré syndrome after campylobacteriosis control, New Zealand, 1988–2010. *Emerging Infectious Diseases*, 2012

It may seem curious to start this chapter with a paper that reports on nothing more cutting edge than epidemiological incidence data, yet this 2012 publication by Baker and colleagues [1] in Wellington, New Zealand, highlights the way in which existing knowledge might be used with substantial effect in the future. It has long been appreciated that *Campylobacter jejuni* is the most commonly identified prodromal infection in GBS. This study simply showed that population-level efforts to reduce *Campylobacter* contamination of poultry meat have a significant effect on preventing GBS, reducing its incidence by 13%. Likewise, influenza immunisation programmes have been shown to have a longer-term benefit in preventing GBS, which outweighs the smaller, short-term risk of disease induction [2]. Moving forward, the combination of an increased understanding of environmental risk factors, greater international collaboration (catalysed by the success of the International GBS Outcome Study, IGOS) [3], improved statistical rigour, and evidence-based public health policies offer a clear hope of substantially reducing the incidence of GBS. Much as the improvements in sanitation at the start of the last century, rather than the later development of antibiotics, contributed most to the reduction in death from infectious diseases, simple population-level measures may offer as much as personalised, targeted immunotherapies in the second century of GBS.

Gen and Other Omics

International Human Genome Sequencing Consortium. A physical map of the human genome. *Nature*, 2001

It is clear (at least to me!) that in addition to environmental factors, genetic influences must also be involved in GBS susceptibility. This is supported by the observations that the disease recurs at a rate much higher than expected by chance, but often with different serological findings, and that even with exposure to identical infectious agents, only a small proportion of patients will subsequently develop GBS. As an autoimmune condition, the usual candidate genes, and some more unusual immune-associated targets, have been investigated, but no consistent disease associations have been identified [4]. Does this mean that the search for genetic factors will be abandoned? It seems unlikely. The continuing evolution of genetic (and bio-informatics) techniques, boosted significantly during the completion of the Human Genome Project [5], even now facilitate much more extensive studies. It is already possible to perform genome-wide association studies looking for single nucleotide polymorphisms (SNPs) across many thousands of genes, and indeed, this is one of the proposed facets of the current IGOS project. Similar studies have already benefited from the SNP datasets made freely available via the internationally collaborative HapMap Project. The observation that large proportions of SNP hits occur in noncoding regions surely foreshadows an increasing awareness and understanding of their importance in disease. With the decreasing cost, and increasing speed, of whole exome and even whole genome sequencing, future studies will be able to draw upon ever more extensive genetic data, offering new and unexpected insights into disease pathogenesis, and implicating critical processes both within and outside the immune system amenable to therapeutic intervention. Indeed, others have already predicted a next generation of medicine where individual patients' genomes are used to personalise treatments, based on a precise understanding of their given disease process.

Jinek M, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 2012

Alongside an increased capability to read and decode genetic data there has developed a vastly improved power to manipulate genes. Cre-recombinase systems, allowing triggered activation or inhibition of defined genes in specific cells, are already in widespread use in biomedical research. The current pinnacle of genetic manipulation is, however, currently best demonstrated by the application of CRISPR technology first outlined by Jinek and colleagues [6] in 2012. In essence, the use of guide RNAs allows an endonuclease to cut DNA at defined points, facilitating the relatively simple, and cost-effective, removal, addition or replacement of particular nucleotide sequences. The implications for many diseases, including GBS, are potentially enormous. Cell- and animal-based disease models could have individual genes modified to assess their influence on key aspects of the disease process. Ultimately, similar technology could be applied to patients, with the enticing possibility of genetically engineered cellular robots being designed to manipulate specific aspects of the immune response, enhance nerve repair, or interfere with other pathologically relevant processes.

In many ways, however, obtaining and analysing the genetic data itself is likely to be one of the simpler tasks undertaken by GBS researchers of the future. It is now appreciated that much of the complexity of the human 'biome' is not revealed by study of the genes themselves. Epigenetic factors, transcriptional control, post-translational protein modifications, lipids and carbohydrates have all already been shown to be important in health and disease. Large-scale collaborations, with ongoing advances in biochemical techniques, will ensure that the genomics revolution will be followed by similar advances in transcriptomics, proteomics and glycolipidomics. With cognition network technologies such as Definiens already operational [7], the insights offered by such increasingly complex datasets seem more likely to be successfully revealed by computer thinking, rather than by the direct application of scientists' little grey

cells.

Membranomics—Studies of the Membrane Microenvironment

Lloyd KO, et al. Cell surface accessibility of individual gangliosides in malignant melanoma cells to antibodies is influenced by the total ganglioside composition of the cells. *Cancer Research*, 1992

It was momentarily pleasing to think I had created a neologism that might be increasingly used in future years, but a quick visit to Google confirms, of course, that ‘membranomics’ is already an established term and even had its own international symposium in 2010 [8]. Away from semantics, that the physical and biochemical properties of the cell membrane as a whole have relevance to GBS was foreshadowed as long ago as 1992. Lloyd and colleagues, studying melanoma cells, realised that the ability of a ganglioside antibody to interact with its membrane target was markedly influenced by the overall ganglioside composition of the cell membrane [9]. Over a decade later, researchers working in Hugh Willison’s lab, including myself, demonstrated a similar phenomenon with anti-GM1 antibodies [10], known to be associated with the acute motor axonal neuropathy (AMAN) subtype of GBS. While all GM1-antibodies studied, including those derived from patients, were able to bind purified GM1 in solid phase assays (typically ELISA), a proportion were unable to bind when GM1 was mixed with other gangliosides, notably GD1a, a pattern we termed ‘complex attenuated’. It became apparent that these observations had direct relevance to the pathogenic potential of the antibodies. Using ex vivo preparations of neuromuscular tissue, we demonstrated that the complex attenuated antibodies were pathologically inert, whereas complex independent clones—able to bind GM1 regardless of the presence or absence of other glycolipids—caused complement dependent structural and functional injury at the neuromuscular junction. The implication is that the precise density, orientation and interactions of target antigens within the cell membrane have a critical role in modulating antibody interactions. These observations built upon the then-recent discovery by Kaida and Kusunoki of GBS-associated antibodies *only* able to bind specific heterodimeric combinations of gangliosides, which they had named ‘ganglioside complex antibodies’ [11].

In yet another exposition of the importance of the dynamism of the cell membrane, Simon Fewou and Rhona McGonigal, working on neighbouring benches in Hugh’s lab, showed that antibody internalisation substantially reduced complement activation and played a crucial role in preventing nerve injury [12,13]. Internalisation was particularly active at the nerve terminal, but much attenuated at the nodes of Ranvier, providing a potential explanation for the topographically focussed pathophysiological effects of antibodies directed against widely expressed membrane glycolipids. It is now evident that internalisation can significantly influence circulating levels of antibody, as discussed below, and this too is likely to prove an important area of future study.

Binnig G, et al. Atomic force microscope. *Physical Review Letters*, 1986

Given the obvious importance of the membrane microenvironment in GBS pathology, how will the scientists of the future study this important area? Antibodies cannot be relied upon to unambiguously indicate the presence or absence of an antigen within the cell membrane, and the *cis* interactions between neighbouring molecules in the lipid bilayer cannot be visualised by the light or even by electron microscope. Technologies already exist to address this but have so far been little utilised in this field.

One particular technique which shows promise in this area is that of atomic force microscopy. By measuring the atomic-level physical, electrical and magnetic interactions at the probe tip, the atomic force microscope, first developed by Binnig and colleagues at IBM, allows surfaces to be resolved at nanometre resolution [14]. As well as determining the precise orientation and interactions of molecules within the lipid bilayer, modifications to the methodology allow the strength and nature of ligand-receptor interactions to be assessed and manipulated in their natural environment. Such imaging has the potential to reveal why certain cells are sensitive to immunological attack whereas others are resistant, and may uncover alternative means by which this sensitivity can be modified.

GBS in a Dish and the Rise of the Robots

Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 2006

Professor Yamanaka's pioneering work in demonstrating that self-renewing, pluripotent stem cells can be generated from adult cells [15] has already begun a revolution in biomedical science which seems very likely to continue well into the second century of GBS. In the original methodology, pluripotency-associated genes are introduced into adult cells using viral vectors. By this process, patient- and disease-specific induced pluripotent stem cells (iPSCs) are produced. These cells should be immunologically inert if transplanted back into the individual they are derived from, and thus have great potential for use as disease-modifying and repair-promoting agents. Although therapeutic uses are currently limited by concerns over oncogenicity, methodological advances are likely to make this increasingly plausible in coming years.

Chambers SM, et al. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nature Biotechnology*, 2009

In neuropathy research, the ability to reliably and efficiently generate neural cells from iPSCs, made possible through the techniques pioneered by the Lorenz Studer Lab at Sloan Kettering [16], will enable stem cell-derived tissue to be used to address questions regarding disease mechanisms and to provide a biologically relevant, high-throughput screening platform for potential new therapies.

As previously discussed, the complexity of the nerve membrane microenvironment cannot be easily or accurately recapitulated by current assays. The use of in vivo or ex vivo animal neuromuscular tissue has previously been used to study antigen-antibody interactions and pathological events, but even this platform has important differences compared to its human equivalent, with rodents notably entirely lacking some types of glycolipid. Human nerve biopsy tissue is available in only limited quantities, and cannot be easily studied in a live state. Unfortunately, fixation procedures are known to disrupt the membrane antigen profile. By utilising Yamanaka and Studer's methodologies, we have been able to generate myelinating sensory nerve co-cultures with neurons derived from human iPSCs (Figure 63.1). Nodes of Ranvier, recently identified as targets for neuropathy-associated antibodies, form spontaneously in this system. The methodology is now being optimised for iPSC-derived human Schwann cells, thus generating a human-specific membrane antigen environment for use in forthcoming studies. Future developments in these techniques will also allow the generation of other components of the peripheral nerve system. Indeed, co-cultures of muscle cells and iPSC-derived motor neurons have just been used to generate motor units in microfluidic chambers. A major benefit of this methodology will be the ability to

study pathological processes in disease-relevant, live cells, in real time.

The potential ability to recreate multiple examples of human GBS across tissue culture plates will soon be exploited. The increasing availability of iPSCs generated from GBS patients and multiple controls will facilitate assessment of the importance of the polygenetic background on GBS pathology. Very high throughput studies driven by robotic management of culture conditions will enable the rapid evaluation of strategies aimed at ameliorating the disease process and/or facilitating improved recovery, whilst no doubt making the PhD student obsolete. Some of these approaches may even incorporate the previously described capacity to genetically or otherwise manipulate neural or immune cells, generating specially programmed effector cells for therapeutic use.

Immunoengineering

Betelli E, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, 2006

Betelli and colleagues' 2006 study acts as a marker of our increasing understanding of the complexities of the immune response [17]. The discovery of a whole new class of T cells was in itself a remarkable development. The observation that the precursors of these Th17-type pro-inflammatory cells could in certain circumstances instead produce regulatory anti-inflammatory cells is perhaps even more exciting [17]. It is now apparent that transdifferentiation occurs between the multiple types of mature cells, opening up the possibility that neuroimmunologists of the future will be able to specifically switch off the disease-causing components of an autoimmune response, while simultaneously enhancing the anti-inflammatory aspects of the immune system itself.

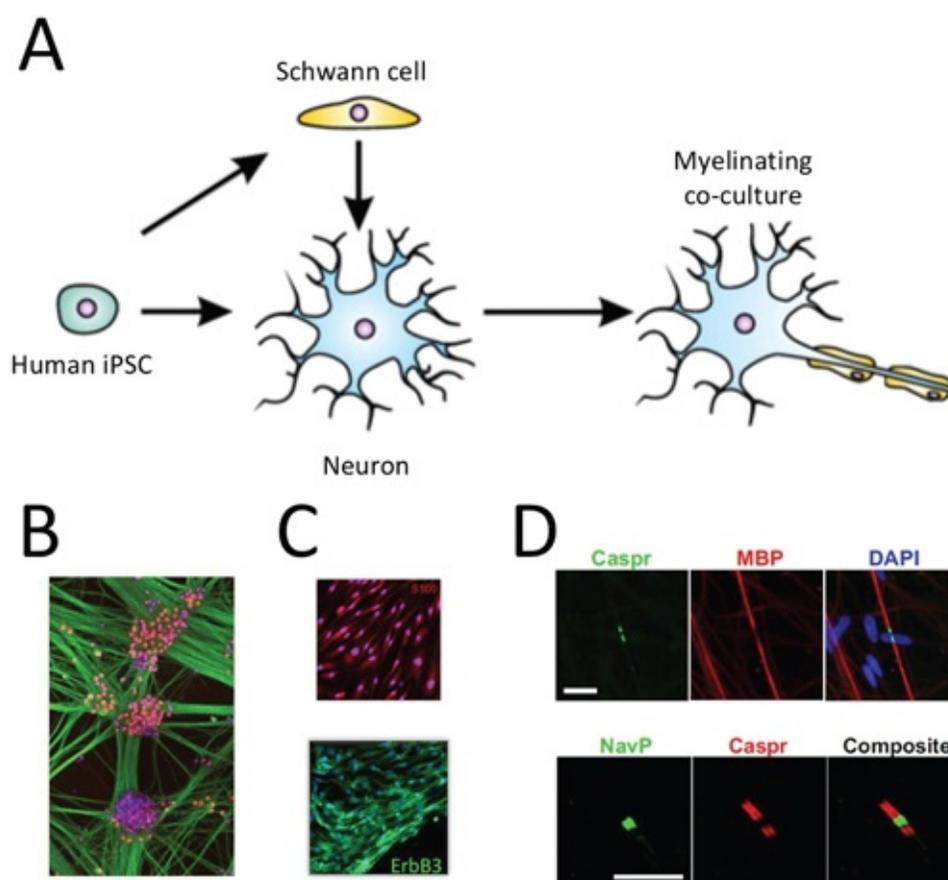


Figure 63.1 Human-induced pluripotent stem cell myelinating culture methodology

(A) Schematic of hiPSC-myelinating co-culture methodology. (B) hiPSCs differentiated to sensory neurons form ganglia-like clusters (cell bodies labelled with Brn3a, purple) and extend axons (labelled with neurofilament, green). The addition of (C) Schwann cells results in (D) myelination (myelin basic protein, MBP) and the formation of nodes of Ranvier with clustered sodium channels (NavP) and appropriately localised paranodal proteins (Caspr).

Corti D, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science*, 2011

The work of Corti and colleagues has already shown that it is possible to isolate and interrogate the function of individual antibody-producing cells [18]. A future ability to target specific subsets in vivo would clearly have therapeutic value, but even the current technology offers the ability to dissect out and study pathogenically relevant clones from within the polyclonal background. The implication, from studies of antibody internalisation on an organism-wide scale, is that the circulating levels of pathogenic antibody may be below the limits of detection, such that their identification and characterisation will depend on this ability to capture and culture the cells producing them.

Maude SL, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine*, 2014

As well as being important in autoimmune pathology, monoclonal antibodies have been at the forefront of recent advances in biological therapies which seem likely to continue into the next 100 years of GBS research. Already, stepwise improvements in the therapeutic monoclonals have produced numerous agents capable of modulating different aspects of the immune response, and a trial of the complement inhibitor eculizumab for GBS has already begun [20].

The engineered antibodies of the future could affect blocking rather than destructive processes, be conjugated to drugs to deliver directed therapies, or have their binding sites alone incorporated into other molecular scaffolds. The potential power of this latter approach has recently been demonstrated by the use of chimeric antigen receptor-T cells in the treatment of ‘incurable’ childhood leukaemia [19].

Conclusion

The future of GBS will surely involve an exponential increase in our knowledge of disease processes and our ability to precisely modulate these for therapeutic effect. New research techniques will generate enormous datasets likely to require an equivalent expansion in bioinformatic processing to fully understand. To exploit these developments in a full and timely manner, we must ensure that direct clinical research undergoes a similar revolution. A future where it is the expectation, rather than the exception, for each GBS patient to enter an informative clinical trial, is one we must strive to realise.

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Appendix

Sur Un Syndrome De Radiculo-Nevrite Avec Hyperalbuminose Du Liquide Céphalo-Rachidien Sans Réaction Cellulaire. Remarques Sur Les Caractères Cliniques Et Graphiques Des Reflexes Tendineux

Par MM. Georges Guillain, J.-A. Barre et A. Strohl.

Nous attirons l'attention, dans la présente note, sur un syndrome clinique que nous avons observé chez deux malades, syndrome caractérisé par des troubles moteurs, l'abolition des réflexes tendineux avec conservation des réflexes cutanés, des paresthésies avec troubles légers de la sensibilité objective, des douleurs à la pression des masses musculaires, des modifications peu accentuées des réactions électriques des nerfs et des muscles, de l'hyper albuminose très notable du liquide céphalo-rachidien avec absence de réaction cytologique (dissociation albumino-cytologique). Ce syndrome nous a paru dépendre d'une atteinte concomitante des racines rachidiennes, des nerfs et des muscles, vraisemblablement de nature infectieuse ou toxique. Il doit être différencié des radiculites simples, des polyneuropathies pures et des polymyosites. Des recherches expérimentales par la méthode graphique sur la vitesse des réflexes et leur temps perdu, sur les modalités, la contractilité musculaire, montrent la réalité de la participation, dans ce syndrome, de tout l'appareil moteur neuromusculaire périphérique. Nous insistons particulièrement aussi sur l'hyperalbuminose du liquide céphalo-rachidien sans réaction cytologique, fait qui, à notre connaissance, n'a pas été mentionné dans des cas semblables.

OBS. 1. – Le soldat D ..., du ... hussards, âgé de vingt-cinq ans, entre, le 20 août 1916, au Centre neurologique de la VI Armée pour des troubles moteurs des membres inférieurs et supérieurs. L'affection a débuté vers le 25 juillet par des fourmillements des pieds et de la faiblesse des membres inférieurs l'obligeant à s'arrêter au bout de 200 à 300 mètres de marche, puis des fourmillements sont apparus les jours suivants aux membres supérieurs et sur la partie inférieure de la face; la force musculaire s'est affaiblie aux membres supérieurs.

Ces différents troubles se sont développés sans cause apparente: le malade n'avait eu aucune maladie

infectieuse récente, aucune angine même légère, Il n'avait présenté aucun symptôme d'une intoxication alimentaire, il n'avait pas eu de grandes fatigues. Nous ajouterons que, dans ses antécédents pathologiques, on ne retrouvait aucun fait important, le malade n'ait toute infection syphilitique et toute habitude alcoolique.

Le premier examen du 25 Aout nous a permis de constater la symptomatologie suivante.

La force musculaire est diminuée d'une façon globale aux membres supérieurs et inférieurs sans que, toutefois, il existe une paralysie totale; cette diminution de la force musculaire est surtout accentuée aux extrémités où l'on constate une très grande faiblesse de la flexion et de l'extension des orteils, du pied sur la jambe, des doigts, de la main sur l'avant-bras.

Les muscles du tronc sont faibles, ainsi le malade, étant couché ne peut s'asseoir spontanément sans prendre de point d'appui.

La marche est possible durant quelques pas, on remarque alors une certaine instabilité et la station debout sur un pied ne peut être maintenue.

Il n'existe aucun trouble de la musculature faciale.

L'examen électrique montre qu'aux membres supérieurs l'excitabilité faradique est normale et l'excitabilité galvanique bonne pour tous les muscles avec secousses vives; il n'y a pas d'inversion polaire; on constate seulement une légère hypoexcitabilité de l'extenseur des doigts; parfois la secousse est légèrement ralentie; on constate de l'inversion polaire pour le jumeau externe, mais la réaction de dégénérescence est très incomplète.

Les réflexes rotuliens, achilléens, medio-plantaires recherchés par le marteau percuteur sont abolis, de même que les réflexes antibrachiaux, radio-et cubito-pronateurs, olécraniens.

Le réflexe cutané plantaire amène la flexion franche des orteils avec contraction à distance du tenseur du fascia lata. Les réflexes crémastériens et cutanés abdominaux sont normaux. On ne constate aucun réflexe de défense soit par pincement du cou-de-pied, soit par hyperflexion des orteils.

L'excitabilité neuromusculaire au marteau percuteur est conservée.

Le malade se plaint toujours de fourmillements dans les deux pieds jusqu'au dessus des malléoles et dans les deux mains jusqu'au-dessus du poignet. Il n'y a pas de troubles nettement appréciables de la sensibilité objective, sinon une légère hypoesthésie tactile, thermique et douloureuse aux pieds et aux mains. Les masses musculaires des membres supérieurs et inférieurs sont douloureuses à la pression.

Les pupilles, égales, réagissent à la lumière et à l'accommodation.

Il n'y a pas de troubles sphinctériens.

Aucune fièvre, aucun trouble respiratoire ou gastro-intestinal, le pouls est normal.

Les urines, examinées au Laboratoire de Bactériologie et de Chimie de l'Armée, ne contiennent ni sucre, ni albumine, ni indoxyle; les éléments chimiques sont dans leur proportion normale.

La ponction lombaire montre un liquide céphalo-rachidien clair, non hypertendu, hyperalbumineux (2 gr. 5 d'albumine par litre) sans réaction leucocytaire (2 à 4 lymphocytes par champ).

La réaction de Wassermann dans le sang est négative.

Un ensemencement du pharynx et du mucus nasal montre, l'absence de tout bacille diphtérique.

Le traitement consiste en un repos absolu au lit, des frictions sur les membres supérieurs et inférieurs, des injections de strychnine, de salicylate de soude et de salol à l'intérieur.

Le 27 aout, les fourmillements ont diminué aux membres inférieurs.

Le 2 septembre, on constate une certaine amélioration de la force musculaire, et il n'y a plus de fourmillements dans les pieds; ceux-ci persistent aux mains; les réflexes tendineux sont toujours abolis. Une nouvelle ponction lombaire montre, comme au précédent examen, une très forte hyperalbuminose sans réaction leucocytaire appréciable.

Le 19 septembre, les troubles moteurs sont très améliorés; le malade est capable de marcher durant une heure, il peut se tenir sur un seul pied; les paresthésies ont complètement disparu aux membres inférieurs, elles persistent encore, quoique atténuées, au niveau des mains; les réflexes tendineux cliniquement sont abolis, les réflexes de défense nuls, les réflexes cutanés normaux; l'excitabilité neuromusculaire au marteau percuteur paraît normale aux membres supérieurs et inférieurs et à la face.

Le malade, s'améliorant progressivement, fut envoyé en convalescence le 30 septembre.

OBS. II. – Le soldat D ..., du ... régiment d'infanterie, âgé de trente-cinq ans, entre, le 5 septembre 1916, au Centre neurologique de la VI Armée pour des troubles moteurs des membres inférieurs qui se sont montrés dans les circonstances suivantes.

Le 28 août, Après une marche de 15 kilomètres, il ressent une fatigue anormale, de la céphalée, des douleurs erratiques dans les membres supérieurs et inférieurs, il se couche, ne peut dormir et frissonne une partie de la nuit. Le lendemain matin il marche avec de grandes difficultés pour se rendre à la visite, il est exempté de service durant quatre jours consécutifs. L'état parétique a débuté par les membres inférieurs et a atteint ensuite les membres supérieurs. Le quatrième jour il veut partir vers cinq heures avec ses camarades, s'équipe mais tombe à la renverse avec sa musette et ne peut se relever. Transporté à un poste de secours, il est ensuite évacué au Centre neurologique de l'Armée. Ces différents troubles se sont développés sans cause apparente, il n'avait eu aucune maladie infectieuse récente, n'avait présenté aucun symptôme d'une intoxication alimentaire ou autre; il convient d'ajouter qu'il est très affirmatif sur ce fait qu'il n'a jamais contracté la syphilis.

Le 5 septembre, nous avons constaté la symptomatologie suivante.

Le malade esquisse avec efforts de petits mouvements de flexion et d'extension des orteils, de flexion de la jambe sur la cuisse et de la cuisse sur le bassin. La même difficulté existe pour les mouvements des membres supérieurs ou les troubles prédominent nettement à la périphérie. La tête est généralement en rotation à gauche et le malade éprouve de la difficulté pour la tourner à droite; il peut ouvrir et fermer la bouche, mais lentement et incomplètement.

L'examen des réactions électriques montre une légère hyperexcitabilité des nerfs et des muscles au courant faradique. Au courant galvanique l'excitabilité est légèrement accrue, surtout pour les nerfs du membre supérieur; il n'y a pas de réaction de dégénérescence.

Les réflexes rotuliens sont très difficiles à rechercher à cause de l'hypertonie musculaire, ils semblent exister. Les réflexes achilléens et medio-plantaires sont abolis. L'état des réflexes des membres supérieurs ne peut être déterminé à cause de l'hypertonie et de l'impossibilité d'une résolution musculaire complète. Les réflexes cutanés plantaires amènent la flexion franche des orteils; les réflexes crémastériens et cutanés abdominaux sont normaux. On ne constate aucun réflexe de défense, soit par pincement du dos du pied, soit par hyperflexion des orteils, mais le malade perçoit les sensations provoquées par ces excitations.

L'excitabilité neuromusculaire au marteau percuteur est conservée.

Le malade se plaint de fourmillements aux extrémités, il n'existe pas de trouble de la sensibilité objective sinon une légère hypoesthésie tactile, douloureuse et thermique aux pieds et aux mains.

Les masses musculaires du mollet et de l'avant-bras sont douloureuses à la pression. Les pupilles égales réagissent à la lumière et à l'accommodation. Le malade urine seul, il sent le besoin mais ne perçoit pas l'écoulement des urines.

Il n'a pas de fièvre, pas de signe de Kernig, pas de nausées, pas de vomissements.

Les urines examinées au Laboratoire de Bactériologie et de Chimie de l'Armée ne contiennent ni sucre, ni albumine, ni indoxyle; les éléments chimiques sont dans leur proportion normale.

Il convient de noter une éruption cutanée apparue depuis trois ou quatre jours, localisée principalement à la partie supérieure du thorax et à la région abdominale inférieure, éruption caractérisée par des taches érythémateuses, papuleuses. En dehors des zones que nous avons signalées, des éléments éruptifs sont disséminés sur le reste du thorax et de l'abdomen; aucun élément ne se voit sur les membres supérieurs et inférieurs.

La ponction lombaire montre un liquide céphalo-rachidien clair, non apparemment hypertendu, hyperalbumineux (plus de 0 gr. 85 d'albumine au rachi albuminimètre de Sicard), sans réaction leucocytaire notable (3 à 4 lymphocytes par champ).

Les symptômes constatés au premier examen ont eu une légère tendance à l'amélioration. Au 20 septembre, on constatait encore cependant la faiblesse des muscles de la périphérie des membres, l'abolition de tous les reflexes tendineux à l'exception du reflexe antibrachial gauche, la conservation des reflexes cutanés, la douleur des masses musculaires à la pression, les paresthésies des extrémités avec hypoesthésie légère. On observait aussi par intermittences dans les muscles du mollet et de la cuisse de petites secousses myocloniques. Une nouvelle ponction lombaire a permis de noter les mêmes particularités qu'au précédent examen: liquide clair, non hypertendu, avec une hyperalbuminose très accentuée sans réaction leucocytaire (3 ou 4 lymphocytes par champ).

Le malade a été évacué sur l'arrière le 1er octobre.

Les deux observations que nous venons de relater sont tout à fait semblables. Chez ces deux malades, sans cause apparente décelable, s'est développé un syndrome clinique caractérisé, comme nous le disions au début, par des troubles moteurs atteignant l'ensemble des muscles des membres supérieurs et inférieurs et prédominant aux extrémités de ceux-ci, l'abolition des reflexes tendineux avec conservation de tous les reflexes cutanés, des paresthésies avec troubles légers des sensibilités objectives, des douleurs à la pression des masses musculaires, des modifications minimales des réactions électriques des nerfs et des muscles, des troubles assez spéciaux du liquide céphalo-rachidien caractérisés par une forte hyperalbuminose sans réaction cytologique.

L'hyperalbuminose accentuée du liquide céphalo-rachidien sans réaction cellulaire est une particularité qui nous paraît importante à signaler. Cette dissociation albumino cytologique (Sicard et Foix) est observée le plus souvent dans certaines compressions médullaires, dans le mal de Pott, dans certains cas de syphilis du névraxe, mais on ne l'a pas décrite, nous semble-t-il, dans les radiculites pures et les polynévrites.

Chez le second de nos malades se surajoutait à l'élément paralytique une certaine hypertonie des muscles qui mérite d'être mise en relief. Le malade étant au repos, la consistance de tous les muscles est nettement supérieure à celle des muscles d'un individu sain dans la même situation. Les mouvements passifs gardent toute leur amplitude normale. Les mouvements volontaires limités, comme nous l'avons dit, se font avec une certaine raideur et lenteur. Les reflexes tendineux sont difficiles à mettre en évidence, les muscles dont la contraction est sollicitée se trouvant pour ainsi dire bridés par l'état de contraction continue des antagonistes. Malgré cet ensemble de caractères qu'on rencontre assez fréquemment dans les méningites, le malade peut être assis en gardant les membres supérieurs presque complètement étendus, et la légère flexion des genoux qui se produit alors est vaincue par une pression insignifiante. Les membres inférieurs relevés et mis presque à angle droit avec le tronc se fléchissent comme ceux d'un sujet normal. Le signe de Kernig n'existe par conséquent pas chez notre malade. Cet état d'hypertonie n'est donc nullement en rapport avec une méningite, mais avec un état spécial de la contractilité musculaire.

paraissant dépendre d'une lésion du nerf périphérique. Nous avons d'ailleurs déjà insisté sur ce fait que les états d'hypertonie peuvent se rencontrer au cours de certaines névrites périphériques et de blessures incomplètes des nerfs, et spécifié à cette occasion que les contractures fréquemment observées au cours de certaines paralysies faciales ne sont pas une exception dans les lésions périphériques des nerfs, comme on le croyait classiquement.

L'ensemble des troubles observés chez ces deux malades appartient à la pathologie simultanée des racines rachidiennes, des nerfs périphériques et des muscles. L'hyper albuminose considérable du liquide céphalo-rachidien témoigne de la participation méningée; les caractères des troubles paralytiques prédominant aux extrémités et les douleurs des masses musculaires à la pression montrent la participation névritique et musculaire. D'ailleurs, il nous semble que c'est avec une schématisation trop grande que l'on isole en neurologie les polynévrites et les polymyosites ; dans un très grand nombre de cas de polynévrites infectieuses ou toxiques, les terminaisons nerveuses intramusculaires, les fibres musculaires elles-mêmes peuvent être atteintes et en réalité il peut s'agir très souvent beaucoup plus de polynéuromyosites que de polynévrites pures.

Chez notre premier malade des recherches expérimentales par la méthode graphique nous ont permis d'apporter certains caractères nouveaux dans l'étude des reflexes et de la contractilité musculaire. La méthode graphique peut donner des éléments importants pour l'interprétation des symptômes et des lésions.

Chez ce malade, alors que les reflexes tendineux ont paru, à l'examen clinique, abolis durant tout le cours de la maladie, l'inscription graphique du gonflement des muscles quadriceps fémoral et jumeaux sous l'influence d'une percussion portant sur les tendons de ces muscles ou leurs masses musculaires ont montré des particularités intéressantes. C'est ainsi que, dès le début de la maladie, la recherche du réflexe rotulien amène une contraction que l'on voit nettement sur la figure 1 après la secousse mécanique. Cette contraction, notablement plus faible que celle obtenue chez un sujet sain, se produit après un temps perdu de 0''056 environ et n'est pas suivie d'une deuxième contraction plus ample et plus longue qui caractérise dans la courbe du reflexe normal la partie de la réponse musculaire d'origine véritablement "reflexe". C'est à peine si 0''152 après le début de l'excitation on remarque un très léger soulèvement de la courbe indiquant le vestige de la contraction reflexe. Le réflexe rotulien est ainsi resté presque entièrement réduit à une contraction idio-musculaire jusqu'à la guérison de la maladie. Durant cette période la percussion de la masse du quadriceps provoquait une belle contraction musculaire se produisant avec un retard de 0'051, suivie elle-même d'une deuxième contraction ayant tous les caractères d'une secousse d'origine reflexe (fig.1) et se produisant 0''150 après le début de l'excitation. Le muscle, qui ne répond que faiblement et partiellement à une excitation mécanique portée sur son tendon et transmise par propagation aux fibres musculaires, présente, lorsqu'il est percuté directement une double contraction à peu près normale. Il semble être le siège d'une hypoexcitabilité mécanique qui ne le rend excitable que pour des déformations brusques portées sur le corps même du muscle.

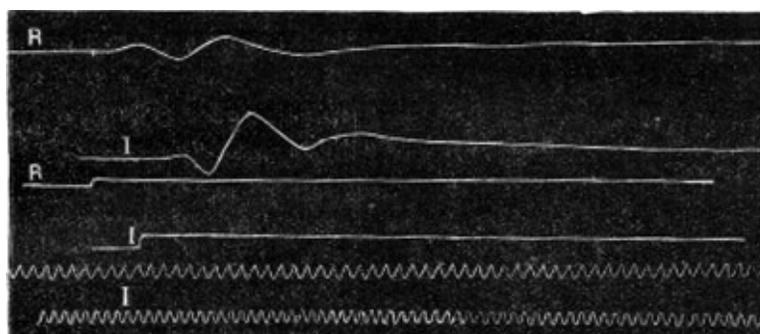


Figure 1.1 R, R, R, courbe myographique du quadriceps fémoral au cours du réflexe rotulien, avec le signal de Desprez indiquant le moment de la percussion et le temps en 1/100 de seconde.

I, I, I, les memes tracés pour la percussion directe du muscle quadriceps fémoral.

Enregistrement du 21 aout 1916.

On remarque l'absence presque totale de contraction « réflexé » qui suit la percussion du tendon rotulien, alors qu'elle existe très nettement pour la percussion directe du muscle.

Le reflexe achilléen s'est montré, au début, également très modifié et réduit presque entièrement à la secousse mécanique. Celle-ci (fig.2) d'amplitude très faible, se produit après un temps perdu extrêmement long, soit environ 0''110, et n'est suivie d'aucune contraction d'origine reflexe. Mais, à l'encontre de ce qui s'est passé pour le reflexe rotulien, ces altérations ont rétrocedé en partie, et, déjà le 5 septembre (fig.3), une nouvelle inscription du reflexe permettait de deceler une secousse musculaire plus ample, plus vive, plus rapide (0''055), suivie d'une deuxième secousse reconnaissable comme étant de nature réflexe et survenant après un retard de 0''140. La secousse neuromusculaire des jumeaux suivait une évolution parallèle et reprenait progressivement une forme se rapprochant de la normale.

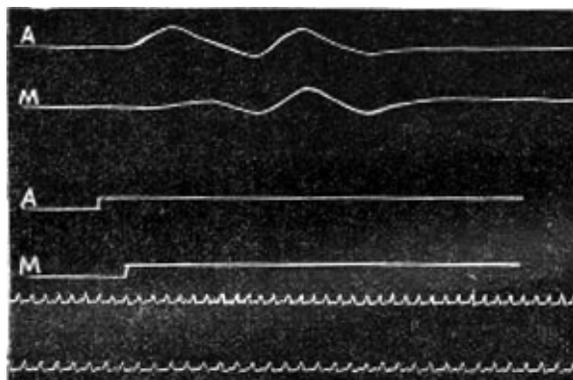


Figure 1.2 A, courbe myographique du jumeau interne au cours du réflexe achilléen.

M, la meme au cours du réflexe médio-plantaire.

Enregistrement du 21 aout 1916.

La première élévation de la courbe A est une secousse mécanique, la deuxième est une contraction « musculaire ». La partie « réflexé », qui n'existe pas dans le cas du réflexe achilleen est visible quoique très faible sur la courbe du réflexé médio-plantaire.

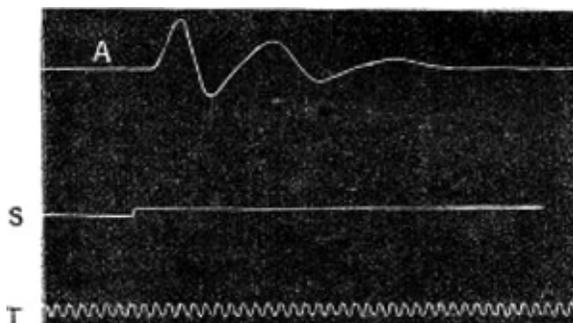


Figure 1.3 A, courbe myographique du iumeau externe au cours du réflexe achilleen.

Enregistrement du 5 septembre 1916.

Le réflexe se présente avec ses trois élévations caractéristiques. Toutefois la contraction « musculaire » et surtout Ja contraction « réflexé » sont plus faibles que chez un sujet normal.

Il est intéressant de remarquer que, tandis qu'au début de la maladie, la percussion du tendon d'Achille et celle des jumeaux ne provoquait qu'une secousse musculaire, à ce moment-la déjà, la recherche du réflexe medio-plantaire amenait une deuxième contraction ayant 0'144 de retard et que l'on doit regarder

comme une contraction reflexe (fig. 2) d'intensité faible mais très nette.

En somme, tandis que le simple examen clinique ne permet que de constater l'abolition des reflexes tendineux, l'analyse détaillée des courbes myographiques, en nous révélant sur quels éléments du reflexe portent les altérations, nous conduit à une série de remarques dignes d'intérêt. D'abord, la disparition complète de la partie *reflexe* de la courbe myographique, ou, lorsqu'elle subsiste, ses caractères morphologiques d'amplitude extrêmement réduite et de grande lenteur, enfin son temps perdu considérable, presque double de la normale, nous montrent l'altération profonde et prédominante des conducteurs nerveux ou de la partie central du reflexe. Mais, de plus, la secousse *musculaire* paraît également modifiée, diminuée de hauteur, ralentie, et retardée dans son apparition, elle nous permet de penser que l'élément musculaire a également été touché par le processus d'intoxication. Enfin, la comparaison des courbes obtenues après percussion du tendon rotulien et du tendon achilléen permet de constater une évolution différente pour ces deux reflexes. Tandis que le premier a été aboli rapidement et n'a montré jusqu'au moment où le malade a quitté l'hôpital, aucune tendance à la réapparition, le second, quoique paraissant aboli cliniquement, a pu être enregistré avec des caractères se rapprochant progressivement de la normale. *Nous insistons sur ce fait important que la méthode graphique permet beaucoup mieux que l'examen avec le marteau percuteur d'avoir des notions précises sur l'état des reflexes tendineux.*

La pathogénie du syndrome de radiculo-névrite observé chez nos malades n'a pu être précisée. Une infection ou une intoxication doivent sans doute être invoquées, mais nous n'avons pu les déceler. Le pronostic ne paraît pas être très grave, si nous en jugeons par l'évolution de l'affection chez nos deux malades, le premier était presque guéri et le second en voie d'amélioration quand ils furent évacués de l'Armée.

(1) Cette observation a été succinctement analysé par l'un de nous à une réunion médicale de la VI Armée a Villers-Cotterets au mois d'aout 1915

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comme nous l'avons observé parfois dans certains réflexes rotuliens, soit, mieux encore, qu'il s'agisse d'un réflexe pur comme dans le réflexe contra latéral des adducteurs de M. Pierre Marie, la contraction réflexe apparaît avec une période latente plus courte. Il semble que la contraction musculaire suspende l'effet de la contraction réflexe en déterminant une sorte d'état réfractaire très bref.

Quoi qu'il en soit, le réflexe médio-plantaire étant constitué par une contraction réflexe isolée et plus prolongée que celle du réflexe achilléen doit être considéré comme un réflexe plus pur que ce dernier.

Le réflexe médio-plantaire se sépare donc nettement, par ses caractères physiologiques comme par ses caractères cliniques, du réflexe achilléen avec lequel il n'a de commun que le mouvement réactionnel du pied.

SUR UN SYNDROME DE RADICULO-NÉVRITE AVEC HYPERALBUMINOSE DU LIQUIDE CÉPHALO-RACHIDIEN SANS RÉACTION CELLULAIRE. REMARQUES SUR LES CARACTÈRES CLINIQUES ET GRAPHIQUES DES RÉFLEXES TENDINEUX,

par MM. GEORGES GUILLAIN, J.-A. BARRÉ et A. STROHL.

Nous attirons l'attention, dans la présente note, sur un syndrome clinique que nous avons observé chez deux malades, syndrome caractérisé par des troubles moteurs, l'abolition des réflexes tendineux avec conservation des réflexes cutanés, des paresthésies avec troubles légers de la sensibilité objective, des douleurs à la pression des masses musculaires, des modifications peu accentuées des réactions électriques des nerfs et des muscles, de l'hyperalbuminose très notable du liquide céphalo-rachidien avec absence de réaction cytologique (dissociation albumino-cytologique). Ce syndrome nous a paru dépendre d'une atteinte concomitante des racines rachidiennes, des nerfs et des muscles, vraisemblablement de nature infectieuse ou toxique. Il doit être différencié des radiculites simples, des polynévrites pures et des polymyosites. Des recherches expérimentales par la méthode graphique sur la vitesse des réflexes et leur temps perdu, sur les modalités, la contractilité musculaire, montrent la réalité de la participation, dans ce syndrome, de tout l'appareil moteur neuro-musculaire périphérique. Nous insistons particulièrement aussi sur l'hyperalbuminose du liquide céphalo-rachidien sans réaction cytologique, fait qui, à notre connaissance, n'a pas été mentionné dans des cas semblables.

OBS. I. — Le soldat D..., du ...^e hussards, âgé de vingt-cinq ans, entre, le 20 août 1916, au Centre neurologique de la VI^e Armée, pour des troubles moteurs des membres inférieurs et supérieurs. L'affection a débuté vers le

25 juillet par des fourmillements des pieds et de la faiblesse des membres inférieurs l'obligeant à s'arrêter au bout de 200 à 300 mètres de marche, puis des fourmillements sont apparus les jours suivants aux membres supérieurs et sur la partie inférieure de la face; la force musculaire s'est affaiblie aux membres supérieurs.

Ces différents troubles se sont développés sans cause apparente : le malade n'avait eu aucune maladie infectieuse récente, aucune angine même légère, il n'avait présenté aucun symptôme d'une intoxication alimentaire, il n'avait pas eu de grandes fatigues. Nous ajouterons que, dans ses antécédents pathologiques, on ne retrouvait aucun fait important, le malade n'ait toute infection syphilitique et toute habitude alcoolique.

Le premier examen du 25 août nous a permis de constater la symptomatologie suivante.

La force musculaire est diminuée d'une façon globale aux membres supérieurs et inférieurs sans que, toutefois, il existe une paralysie totale; cette diminution de la force musculaire est surtout accentuée aux extrémités où l'on constate une très grande faiblesse de la flexion et de l'extension des orteils, du pied sur la jambe, des doigts, de la main sur l'avant-bras.

Les muscles du tronc sont faibles, ainsi le malade étant couché ne peut s'asseoir spontanément sans prendre de point d'appui.

La marche est possible durant quelques pas, on remarque alors une certaine instabilité et la station debout sur un pied ne peut être maintenue.

Il n'existe aucun trouble de la musculature faciale.

L'examen électrique montre qu'aux membres supérieurs l'excitabilité faradique est normale et l'excitabilité galvanique bonne pour tous les muscles avec secousses vives; il n'y a pas d'inversion polaire; on constate seulement une légère hypoexcitabilité de l'extenseur commun des doigts. Aux membres inférieurs l'excitabilité faradique est légèrement diminuée, l'excitabilité galvanique est diminuée aussi pour le tronc du nerf sciatique, le nerf sciatique poplité interne, le demi-tendineux, l'extenseur des doigts; parfois la secousse est légèrement ralentie; on constate de l'inversion polaire pour le jumeau externe, mais la réaction de dégénérescence est très incomplète.

Les réflexes rotuliens, achilléens, médio-plantaires recherchés par le marteau percuteur sont abolis, de même que les réflexes antibrachiaux, radio-et cubito-pronateurs, olécraniens.

Le réflexe cutané plantaire amène la flexion franche des orteils avec contraction à distance du tenseur du fascia lata. Les réflexes crémastériens et cutanés abdominaux sont normaux. On ne constate aucun réflexe de défense soit par pincement du cou-de-pied, soit par hyperflexion des orteils.

L'excitabilité neuro-musculaire au marteau percuteur est conservée.

Le malade se plaint toujours de fourmillements dans les deux pieds jusqu'au dessus des malléoles et dans les deux mains jusqu'au-dessus du poignet. Il n'y a pas de troubles nettement appréciables de la sensibilité objective, sinon une légère hypoesthésie tactile, thermique et douloureuse aux pieds et aux mains. Les masses musculaires des membres supérieurs et inférieurs sont douloureuses à la pression.

Les pupilles, égales, réagissent à la lumière et à l'accommodation.

Il n'y a pas de troubles sphinctériens.

Aucune fièvre, aucun trouble respiratoire ou gastro-intestinal, le pouls est normal.

Les urines, examinées au Laboratoire de Bactériologie et de Chimie de l'Armée, ne contiennent ni sucre, ni albumine, ni indoxyle; les éléments chimiques sont dans leur proportion normale.

La ponction lombaire montre un liquide céphalo-rachidien clair, non hypertendu, hyperalbumineux (2 gr. 5 d'albumine par litre) sans réaction leucocytaire (2 à 4 lymphocytes par champ).

La réaction de Wassermann dans le sang est négative.

Unensemencement du pharynx et du mucus nasal montre l'absence de tout bacille diphtérique.

Le traitement consiste en repos absolu au lit, frictions sur les membres supérieurs et inférieurs, injections de strychnine, salicylate de soude et salol à l'intérieur.

Le 27 août, les fourmillements ont diminué aux membres inférieurs.

Le 2 septembre, on constate une certaine amélioration de la force musculaire, et il n'y a plus de fourmillements dans les pieds; ceux-ci persistent aux mains; les réflexes tendineux sont toujours abolis. Une nouvelle ponction lombaire montre, comme au précédent examen, une très forte hyperalbuminose sans réaction leucocytaire appréciable.

Le 19 septembre, les troubles moteurs sont très améliorés: le malade est capable de marcher durant une heure, il peut se tenir sur un seul pied; les paresthésies ont complètement disparu aux membres inférieurs, elles persistent encore, quoique atténuées, au niveau des mains; les réflexes tendineux cliniquement sont abolis, les réflexes de défense nuls, les réflexes cutanés normaux; l'excitabilité neuro-musculaire au marteau percuteur paraît normale aux membres supérieurs et inférieurs et à la face.

Le malade, s'améliorant progressivement, fut envoyé en convalescence le 30 septembre.

Obs. II. — Le soldat D..., du ...^e régiment d'infanterie, âgé de trente-cinq ans, entre, le 5 septembre 1916, au Centre neurologique de la VI^e Armée pour des troubles moteurs des membres inférieurs qui se sont montrés dans les circonstances suivantes.

Le 28 août, après une marche de 15 kilomètres, il ressent une fatigue anormale, de la céphalée, des douleurs erratiques dans les membres supérieurs et inférieurs, il se couche, ne peut dormir et frissonne une partie de la nuit. Le lendemain matin il marche avec de grandes difficultés pour se rendre à la visite, il est exempté de service durant quatre jours consécutifs. L'état parétique a débuté par les membres inférieurs et a atteint ensuite les membres supérieurs. Le quatrième jour il veut partir vers cinq heures avec ses camarades, s'équipe mais tombe à la renverse avec sa musette et ne peut se relever. Transporté à un poste de secours, il est ensuite évacué au Centre neurologique de l'Armée. Ces différents troubles se sont développés sans cause apparente, il n'avait eu aucune maladie infectieuse récente, n'avait présenté aucun symptôme d'une intoxication alimentaire ou autre; il convient d'ajouter qu'il est très affirmatif sur ce fait qu'il n'a jamais contracté la syphilis.

Le 5 septembre, nous avons constaté la symptomatologie suivante.

Le malade esquisse avec efforts de petits mouvements de flexion et d'extension des orteils, de flexion de la jambe sur le cuisse et de la cuisse sur le bassin. La même difficulté existe pour les mouvements des membres supérieurs où les troubles prédominent nettement à la périphérie. La tête est généralement en rotation à gauche et le malade éprouve de la difficulté pour la tourner à droite; il peut ouvrir et fermer la bouche, mais lentement et incomplètement.

L'examen des réactions électriques montre une légère hyperexcitabilité des nerfs et des muscles au courant faradique. Au courant galvanique l'excitabilité est légèrement accrue, surtout pour les nerfs du membre supérieur; il n'y a pas de réaction de dégénérescence.

Les réflexes rotuliens sont très difficiles à rechercher à cause de l'hypertonie musculaire, ils semblent exister. Les réflexes achilléens et médio-plantaires sont abolis. L'état des réflexes des membres supérieurs ne peut être déterminé à cause de l'hypertonie et de l'impossibilité d'une résolution musculaire complète. Les réflexes cutanés plantaires amènent la flexion franche des orteils; les réflexes crémastériens et cutanés abdominaux sont normaux. On ne constate aucun réflexe de défense, soit par pincement du dos du pied, soit par hyperflexion des orteils, mais le malade perçoit les sensations provoquées par ces excitations.

L'excitabilité neuro-musculaire au marteau percuteur est conservée.

Le malade se plaint de fourmillements aux extrémités, il n'existe pas de trouble de la sensibilité objective sinon une légère hypoesthésie tactile, douloureuse et thermique aux pieds et aux mains.

Les masses musculaires du mollet et de l'avant-bras sont douloureuses à la pression.

Les pupilles égales réagissent à la lumière et à l'accommodation.

Le malade urine seul, il sent le besoin mais ne perçoit pas l'écoulement des urines.

Il n'a pas de fièvre, pas de signe de Kernig, pas de nausées, pas de vomissements.

Les urines examinées au Laboratoire de Bactériologie et de Chimie de l'Armée ne contiennent ni sucre, ni albumine, ni indoxyle; les éléments chimiques sont dans leur proportion normale.

Il convient de noter une éruption cutanée apparue depuis trois ou quatre jours, localisée principalement à la partie supérieure du thorax et à la région abdominale inférieure, éruption caractérisée par des taches érythémateuses, papuleuses. En dehors des zones que nous avons signalées, des éléments éruptifs sont disséminés sur le reste du thorax et de l'abdomen; aucun élément ne se voit sur les membres supérieurs et inférieurs.

La ponction lombaire montre un liquide céphalo-rachidien clair, non apparemment hypertendu, hyperalbumineux (plus de 0 gr. 85 d'albumine au rachialbuminimètre de Sicard), sans réaction leucocytaire notable (3 à 4 lymphocytes par champ).

Les symptômes constatés au premier examen ont eu une légère tendance à l'amélioration. Au 20 septembre, on constatait encore cependant la faiblesse des muscles de la périphérie des membres, l'abolition de tous les réflexes

tendineux à l'exception du réflexe antibrachial gauche, la conservation des réflexes cutanés, la douleur des masses musculaires à la pression, les paresthésies des extrémités avec hypoesthésie légère. On observait aussi par intermittences dans les muscles du mollet et de la cuisse de petites secousses myocloniques. Une nouvelle ponction lombaire a permis de noter les mêmes particularités qu'au précédent examen : liquide clair, non hypertendu, avec une hyperalbuminose très accentuée sans réaction leucocytaire (3 ou 4 lymphocytes par champ).

Le malade a été évacué sur l'arrière le 4^{er} octobre.

Les deux observations que nous venons de relater sont tout à fait semblables. Chez ces deux malades, sans cause apparente décelable, s'est développé un syndrome clinique caractérisé, comme nous le disions au début, par des troubles moteurs atteignant l'ensemble des muscles des membres supérieurs et inférieurs et prédominant aux extrémités de ceux-ci, l'abolition des réflexes tendineux avec conservation de tous les réflexes cutanés, des paresthésies avec troubles légers des sensibilités objectives, des douleurs à la pression des masses musculaires, des modifications minimales des réactions électriques des nerfs et des muscles, des troubles assez spéciaux du liquide céphalo-rachidien caractérisés par une forte hyperalbuminose sans réaction cytologique.

L'hyperalbuminose accentuée du liquide céphalo-rachidien sans réaction cellulaire est une particularité qui nous paraît importante à signaler. Cette dissociation albumino-cytologique (Sicard et Foix) est observée le plus souvent dans certaines compressions médullaires, dans le mal de Pott, dans certains cas de syphilis du névraxe, mais on ne l'a pas décrite, nous semble-t-il, dans les radiculites pures et les poly-névrites.

Chez le second de nos malades se surajoutait à l'élément paralytique une certaine hypertonie des muscles qui mérite d'être mise en relief. Le malade étant au repos, la consistance de tous les muscles est nettement supérieure à celle des muscles d'un individu sain dans la même situation. Les mouvements passifs gardent toute leur amplitude normale. Les mouvements volontaires limités, comme nous l'avons dit, se font avec une certaine raideur et lenteur. Les réflexes tendineux sont difficiles à mettre en évidence, les muscles dont la contraction est sollicitée se trouvant pour ainsi dire bridés par l'état de contraction continue des antagonistes. Malgré cet ensemble de caractères qu'on rencontre assez fréquemment dans les méningites, le malade peut être assis en gardant les membres supérieurs presque complètement étendus, et la légère flexion des genoux qui se produit alors est vaincue par une pression insignifiante. Les membres inférieurs relevés et mis presque à angle droit avec le tronc se fléchissent comme ceux d'un sujet normal. Le signe de Kernig n'existe par conséquent pas chez notre malade. Cet

état d'hypertonie n'est donc nullement en rapport avec une méningite, mais avec un état spécial de la contractilité musculaire paraissant dépendre d'une lésion du nerf périphérique. *Nous avons d'ailleurs déjà insisté sur ce fait que les états d'hypertonie peuvent se rencontrer au cours de certaines névrites périphériques et de blessures incomplètes des nerfs, et spécifié à cette occasion que les contractures fréquemment observées au cours de certaines paralysies faciales ne sont pas une exception dans les lésions périphériques des nerfs, comme on le croyait classiquement.*

L'ensemble des troubles observés chez ces deux malades appartient à la pathologie simultanée des racines rachidiennes, des nerfs périphériques et des muscles. L'hyperalbuminose considérable du liquide céphalo-rachidien témoigne de la participation méningée; les caractères des troubles paralytiques prédominant aux extrémités et les douleurs des masses musculaires à la pression montrent la participation névritique et musculaire. D'ailleurs, il nous semble que c'est avec une schématisation trop grande que l'on isole en neurologie les polynévrites et les polymyosites; dans un très grand nombre de cas de polynévrites infectieuses ou toxiques, les terminaisons nerveuses intramusculaires, les fibres musculaires elles-mêmes peuvent être atteintes et en réalité il peut s'agir très souvent beaucoup plus de poly-neuromyosites que de polynévrites pures.

Chez notre premier malade des recherches expérimentales par la méthode graphique nous ont permis d'apporter certains caractères nouveaux dans l'étude des réflexes et de la contractilité musculaire. La méthode graphique peut donner des éléments importants pour l'interprétation des symptômes et des lésions.

Chez ce malade, alors que les réflexes tendineux ont paru, à l'examen clinique, abolis durant tout le cours de la maladie, l'inscription graphique du gonflement des muscles quadriceps fémoral et jumeaux sous l'influence d'une percussion portant sur les tendons de ces muscles ou leurs masses musculaires ont montré des particularités intéressantes. C'est ainsi que, dès le début de la maladie, la recherche du réflexe rotulien amène une contraction que l'on voit nettement sur la figure 1 après la secousse mécanique. Cette contraction, notablement plus faible que celle obtenue chez un sujet sain, se produit après un temps perdu de 0^m036 environ et n'est pas suivie d'une deuxième contraction plus ample et plus longue qui caractérise dans la courbe du réflexe normal la partie de la réponse musculaire d'origine véritablement « réflexe ». C'est à peine si 0^m152 après le début de l'excitation on remarque un très léger soulèvement de la courbe indiquant le vestige de la contraction réflexe. Le réflexe rotulien est ainsi resté presque entièrement réduit à une contraction idio-musculaire jusqu'à la guérison de la maladie. Durant cette période la percussion de la masse du quadriceps provoquait une belle contraction musculaire se produisant avec un retard de 0^m031, suivie

elle-même d'une deuxième contraction ayant tous les caractères d'une secousse d'origine réflexe (fig. 1) et se produisant 0"150 après le début de l'excitation. Le muscle, qui ne répond que faiblement et partiellement à

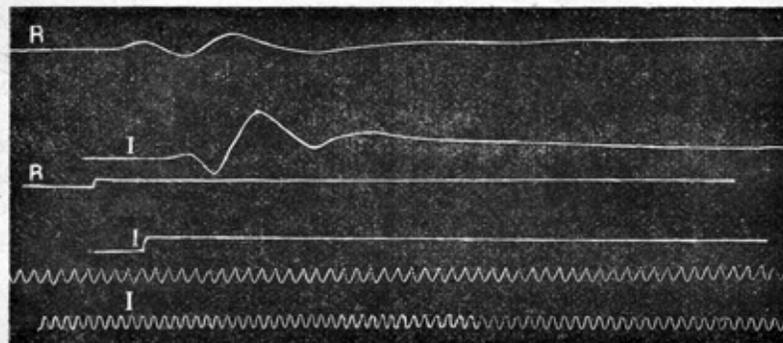


FIG. 1.

R, R, R, courbe myographique du quadriceps fémoral au cours du réflexe rotulien, avec le signal de Desprez indiquant le moment de la percussion et le temps en 1/100 de seconde.

I, I, I, les mêmes tracés pour la percussion directe du muscle quadriceps fémoral.

Enregistrement du 21 août 1916.

On remarque l'absence presque totale de contraction « réflexe » qui suit la percussion du tendon rotulien, alors qu'elle existe très nettement pour la percussion directe du muscle.

une excitation mécanique portée sur son tendon et transmise par propagation aux fibres musculaires, présente, lorsqu'il est percuté directe-

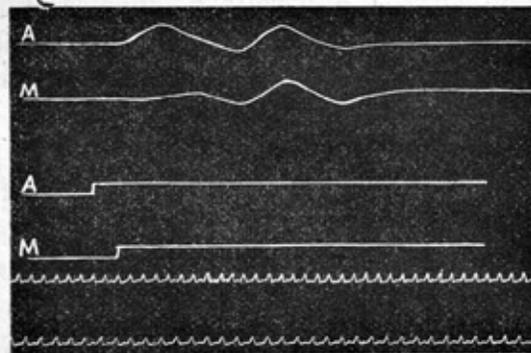


FIG. 2.

A, courbe myographique du jumeau interne au cours du réflexe achilléen.

M, la même au cours du réflexe médio-plantaire.

Enregistrement du 21 août 1916.

La première élévation de la courbe A est une secousse mécanique, la deuxième est une contraction « musculaire ». La partie « réflexe », qui n'existe pas dans le cas du réflexe achilléen est visible quoique très faible sur la courbe du réflexe médio-plantaire.

ment, une double contraction à peu près normale. Il semble être le siège d'une hypoexcitabilité mécanique qui ne le rend excitable que pour des déformations brusques portées sur le corps même du muscle.

Le réflexe achilléen s'est montré, au début, également très modifié et réduit presque entièrement à la secousse mécanique. Celle-ci (fig. 2), d'amplitude très faible, se produit après un temps perdu extrêmement

long, soit environ 0"110, et n'est suivie d'aucune contraction d'origine réflexe. Mais, à l'encontre de ce qui s'est passé pour le réflexe rotulien, ces altérations ont rétrogradé en partie, et, déjà le 5 septembre (fig. 3), une nouvelle inscription du réflexe permettait de déceler une secousse musculaire plus ample, plus vive, plus rapide (0"035), suivie d'une deuxième secousse reconnaissable comme étant de nature réflexe et survenant après un retard de 0"140. La secousse neuro-musculaire des jumeaux suivait une évolution parallèle et reprenait progressivement une forme se rapprochant de la normale.

Il est intéressant de remarquer que, tandis qu'au début de la maladie, la percussion du tendon d'Achille et celle des jumeaux ne provoquait qu'une secousse musculaire, à ce moment-là déjà, la recherche du

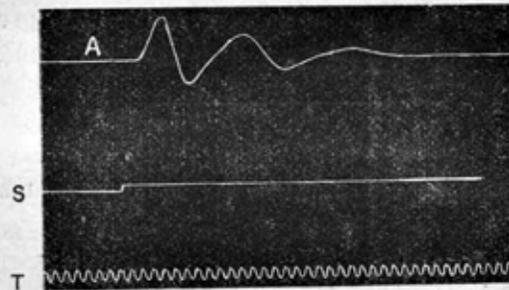


FIG. 3.

A, courbe myographique du jumeau externe au cours du réflexe achilléen. Enregistrement du 5 septembre 1916.
Le réflexe se présente avec ses trois élévations caractéristiques. Toutefois la contraction « musculaire » et surtout la contraction « réflexe » sont plus faibles que chez un sujet normal.

réflexe médio-plantaire amenait une deuxième contraction ayant 0"144 de retard et que l'on doit regarder comme une contraction réflexe (fig. 2) d'intensité faible mais très nette.

En somme, tandis que le simple examen clinique ne permet que de constater l'abolition des réflexes tendineux, l'analyse détaillée des courbes myographiques, en nous révélant sur quels éléments du réflexe portent les altérations, nous conduit à une série de remarques dignes d'intérêt. D'abord, la disparition complète de la partie *réflexe* de la courbe myographique, ou, lorsqu'elle subsiste, ses caractères morphologiques d'amplitude extrêmement réduite et de grande lenteur, enfin son temps perdu considérable, presque double de la normale, nous montrent l'altération profonde et prédominante des conducteurs nerveux ou de la partie centrale du réflexe. Mais, de plus, la secousse *musculaire* paraît également modifiée, diminuée de hauteur, ralentie, et retardée dans son apparition, elle nous permet de penser que l'élément musculaire a également été touché par le processus d'intoxication. Enfin, la comparaison des courbes obtenues après percussion du tendon

rotulien et du tendon achilléen permet de constater une évolution différente pour ces deux réflexes. Tandis que le premier a été aboli rapidement et n'a montré jusqu'au moment où le malade a quitté l'hôpital, aucune tendance à la réapparition, le second, quoique paraissant aboli cliniquement, a pu être enregistré avec des caractères se rapprochant progressivement de la normale. *Nous insistons sur ce fait important que la méthode graphique permet beaucoup mieux que l'examen avec le marteau percuteur d'avoir des notions précises sur l'état des réflexes tendineux.*

La pathogénie du syndrome de radiculo-névrite observé chez nos malades n'a pu être précisée. Une infection ou une intoxication doivent sans doute être invoquées, mais nous n'avons pu les déceler. Le pronostic ne paraît pas être très grave, si nous en jugeons par l'évolution de l'affection chez nos deux malades, le premier était presque guéri et le second en voie d'amélioration quand ils furent évacués de l'Armée.

DEUX CAS D'HÉMIPLÉGIE ORGANIQUE CONSÉCUTIVE A LA DÉFLAGRATION
DE FORTES CHARGES D'EXPLOSIFS SANS PLAIE EXTÉRIEURE,

par MM. GEORGES GUILLAIN et J.-A. BARRÉ.

Les deux observations que nous rapportons ont pour but de montrer que l'hémiplégie organique peut se constater, consécutivement à la déflagration de fortes charges d'explosifs, sans plaie extérieure. De tels troubles organiques doivent être mis en parallèle avec les troubles fonctionnels dont la réalité, dans certains cas, ne peut être mise en doute.

OBSERVATION I (1). — Le caporal L... (Etienne), du ...^e génie, âgé de trente-six ans, est envoyé, le 9 juin 1915, à l'Hôpital temporaire de Villers-Cotterets avec le diagnostic : « Hystéro-traumatisme, hémiparésie gauche de nature hystérique. »

Dans la nuit du 7 au 8 juin, il réparait, dans une tranchée de première ligne, un créneau de mitrailleuse, quand un gros obus éclata près de lui; il perdit connaissance, fut emporté par ses camarades au cantonnement. Le lendemain, il avait repris sa connaissance, se plaignait de céphalée et de rachialgie, il eut une crise convulsive et le médecin aide-major qui le vit constata une hémiplégie gauche avec léger strabisme et dysarthrie; l'intelligence était conservée, la lucidité parfaite, il n'existait pas de signe de Kernig. Dans l'après-midi, le malade accusa de violentes douleurs dans toute la partie gauche du corps, la température

(1) Cette observation a été succinctement analysée par l'un de nous à une Réunion médicale de la VI^e Armée à Villers-Cotterets au mois d'août 1915.