Review

Guillain–Barré syndrome and anti-ganglioside antibodies: a clinician-scientist's journey

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(Communicated by Kunihiko SUZUKI, M.J.A.)

Abstract: Guillain–Barré syndrome (GBS) is the most frequent cause of acute flaccid paralysis. Having seen my first GBS patient in 1989, I have since then dedicated my time in research towards understanding the pathogenesis of GBS. Along with several colleagues, we identified IgG autoantibodies against ganglioside GM1 in two patients with GBS subsequent to *Campylobacter jejuni* enteritis. We proceeded to demonstrate molecular mimicry between GM1 and bacterial lipo-oligosaccharide of *C. jejuni* isolated from a patient with GBS. Our group then established a disease model for GBS by sensitization with GM1 or GM1-like lipo-oligosaccharide. With this, a new paradigm that carbohydrate mimicry can cause autoimmune disorders was demonstrated, making GBS the first proof of molecular mimicry in autoimmune disease. Patients with Fisher syndrome, characterized by ophthalmoplegia and ataxia, can develop the disease after an infection by *C. jejuni*. We showed that the genetic polymorphism of *C. jejuni* sialyltransferase, an enzyme essential to the biosynthesis of ganglioside-like lipo-oligosaccharides determines whether patients develop GBS or Fisher syndrome. This introduces another paradigm that microbial genetic polymorphism can determine the clinical phenotype of human autoimmune diseases. Similarities between the clinical presentation of Fisher syndrome and Bickerstaff brainstem encephalitis have caused debate as to whether they are in fact the same disease. We demonstrated that IgG anti-GQ1b antibodies were common to both, suggesting that they are part of the same disease spectrum. We followed this work by clarifying the nosological relationship between the various clinical presentations within the anti-GQ1b antibody syndrome. In this review, I wanted to share my journey from being a clinician to a clinician-scientist in the hopes of inspiring younger clinicians to follow a similar path.

Keywords: autoimmune disease, *Campylobacter jejuni*, Fisher syndrome, ganglioside, Guillain–Barré syndrome, molecular mimicry

Introduction

Guillain–Barré syndrome (GBS), characterized by muscle weakness in the arms and legs (tetraplegia) as well as the loss of deep tendon reflexes (areflexia), is currently the most common cause of acute flaccid paralysis worldwide since the near-elimination of poliomyelitis. A common misconception about GBS is that it has a good prognosis, when in fact up to 20% of GBS patients remain severely disabled and approximately 5% die in the western countries.[1)] The search for more effective treatment based on a complete understanding of the molecular pathogenesis of GBS continues. In this review, I describe my first encounter with a GBS patient that prompted me to dedicate more than 20 years of all my research life to elucidate the pathogenesis of GBS and its related disorders. Hopefully, this record of the work

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Abbreviations: GBS: Guillain-Barré syndrome; ALS: amyotrophic lateral sclerosis; LOS: lipo-oligosaccharide; FS: Fisher syndrome; BBE: Bickerstaff brainstem encephalitis; AMAN: acute motor axonal neuropathy; AIDP: acute inflammatory demyelinating polyneuropathy; Nav channel: voltage-gated Na^+ channel; AMSAN: acute motor-sensory axonal neuropathy; IVIG: intravenous immunoglobulin.

doi: 10.2183/pjab.88.299

done by our group will inspire other clinicians to better understand disease pathogenesis and develop themselves to become clinician-scientists and pursue their own research, and for basic scientists to understand patient- and disease-oriented research.

Axonal Guillain–Barré syndrome

GBS following *Campylobacter jejuni* enteritis. Gangliosides constitute a large family of glycosphingolipids predominantly distributed on the cell-surface membrane and anchored in the external leaflet of the lipid bilayer by a ceramide moiety. Gangliosides, composed of a ceramide attached to one or more sugars (hexoses), contain sialic acid (*N*-acetylneuraminic acid) linked to the oligosaccharide core. Sialylated oligosaccharides are exposed extracellularly. Five gangliosides GM1, GD1a, GD1b, GT1a and GQ1b differ with regard to the number and position of their sialic acids, where M, D, T and Q stand for mono-, di-, tri- and quadri-sialosyl groups, and 'b' is used to designate gangliosides with a disialosyl group attached to the internal galactose (Fig. 1).

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 watery diarrhea 1 week before the onset. He presented with tetraplegia and areflexia without any sensory signs, although typical GBS patients describe a glove-and-stocking pattern of sensory impairment. Thomas Feasby's group had reported "an acute axonal form of Guillain–Barré polyneuropathy" in 1986,[2] but at the time most clinicians believed GBS to be a demyelinating peripheral nerve disease. Repeated nerve conduction study results in our patient supported 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 Norman 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 improved after immunotherapy,[3] 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 coated on an enzyme-linked immunoassay plate, which was confirmed by thin-layer chromatography with immunostaining.[4] The anti-GM1 antibody titers decreased with the time course of the illness. A second GBS patient was later identified who also carried IgG anti-GM1 antibodies. This patient also had antecedent diarrhea and pure motor weakness. Nerve conduction studies suggested axonal degeneration in motor nerves, but no demyelination. Unfortunately, this patient remained bed-bound even after 3 years from disease onset. Having seen the poor prognosis that can be associated with GBS, I promised the patients that I would clarify the disease mechanism and to try and develop more effective treatments based on the molecular pathogenesis. This continues to be my motivation to do my research in GBS, which has now spanned more than 20 years.

The presence of watery diarrhea prior to the illness in both patients provided the clue that the offending microbial agent might be the trigger for the development of GBS. There had been a few reports of association of GBS with diarrhea or *C. jejuni* enteritis.[5),6)] Although *C. jejuni* was not widely recognized as an antecedent infectious agent of GBS at that time, both patients were serologically confirmed as having had an antecedent *C. jejuni* infection.[4)] In contrast, we failed to identify anti-GM1 antibodies in 10 patients who had *C. jejuni* enteritis but did not develop GBS. We reported the two patients with axonal GBS following *C. jejuni* enteritis and positive IgG anti-GM1 antibodies, suggesting that they may represent a subgroup of GBS defined as "acute axonal polyneuropathy".

These cases were a learning point for me. Although my clinical experience was not extensive, careful examination of patients along with critical review of the literature allowed me to perform some basic experiments to test my hypothesis that led me to new discoveries.

I also discovered a patient with axonal GBS subsequent to *C. jejuni* enteritis, who had IgG antibodies to GD1a, but not to GM1.[7)] In collaborating with Satoshi Kuwabara's group, we demonstrated the association between axonal GBS with *C. jejuni* infection and anti-GM1- or -GD1a antibodies in a larger series.[8)] At the time the Hopkins group had also confirmed the association between anti-GD1a antibodies and axonal GBS, but not with *C. jejuni* infection that was likely due to the low specificity of their anti-*C. jejuni* antibody assay.[9)]

Experimental autoimmune neuritis, which can be induced by immunization with peripheral nerve proteins or transferred to animals by T-cells sensi-

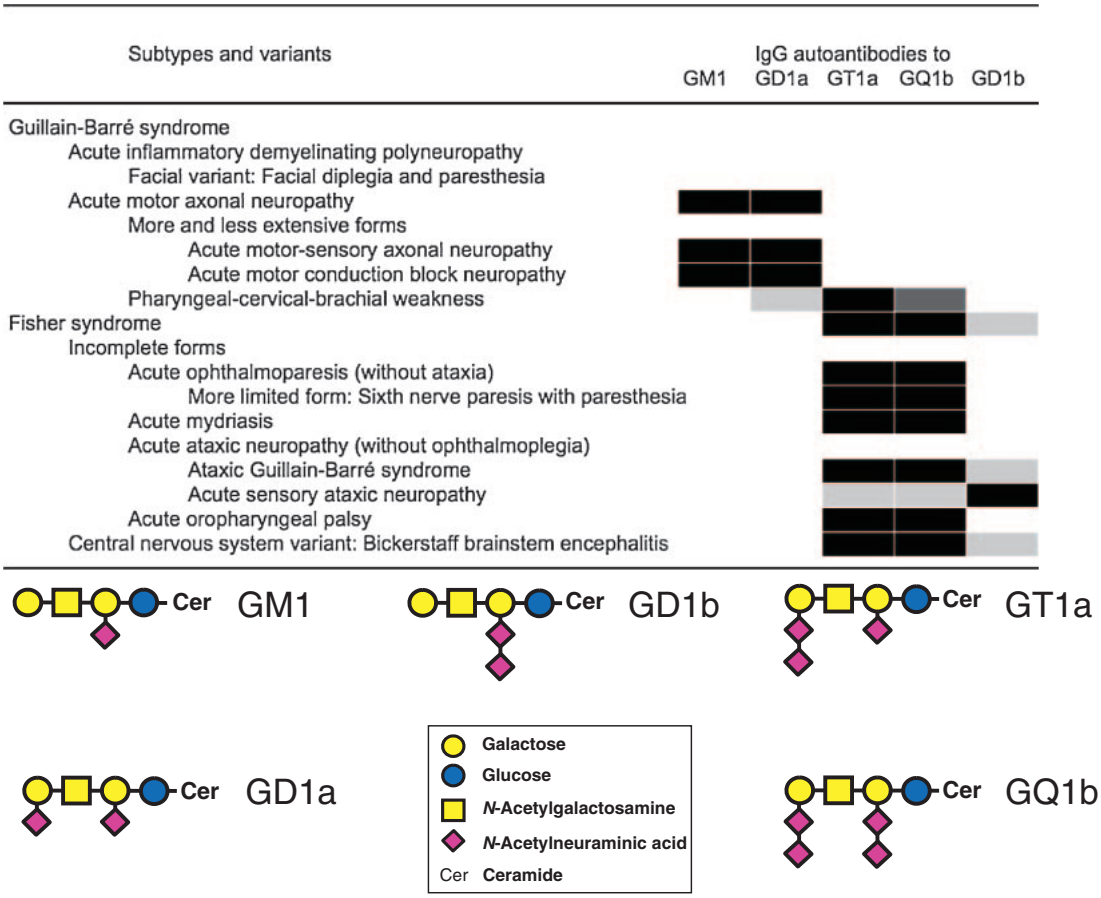

Fig. 1. Association of anti-ganglioside antibodies with Guillain–Barré and Fisher syndromes. Guillain–Barré syndrome is broadly divided into acute inflammatory demyelinating polyneuropathy and acute motor axonal neuropathy (AMAN). "Facial diplegia and paresthesia" is considered as the facial variant of acute inflammatory demyelinating polyneuropathy.[182] Acute motor-sensory axonal neuropathy and acute motor conduction block neuropathy are likely the extreme forms of the AMAN spectrum, being more and less extensive forms of AMAN. Pharyngeal-cervical-brachial weakness could be positioned as a regional form of AMAN. Fisher syndrome also includes incomplete forms, such as acute ophthalmoparesis (without ataxia), acute mydriasis, acute ataxic neuropathy (without ophthalmoplegia), acute oropharyngeal palsy, and an extensive variant Bickerstaff brainstem encephalitis. Six nerve paresis with paresthesia is a part of the "acute ophthalmoparesis" spectrum. Acute ataxic neuropahty encompasses both ataxic Guillain–Barré syndrome and acute sensory ataxic neuropathy. Pharyngeal-cervical-brachial weakness could also be considered an extensive variant of Fisher syndrome. AMAN and pharyngeal-cervical-brachial weakness can overlap with Fisher syndrome or its related conditions. IgG autoantibodies against GM1 and GD1a are associated with pure motor weakness. IgG antibodies against GQ1b and GT1a are associated with external and internal ophthalmoplegia, cerebellar-like ataxia and oropharyngeal palsy. IgG anti-GD1b antibodies which do not cross-react with GM1 are associated with sensory ataxia. "Anti-GQ1b antibody syndrome" includes Fisher syndrome, its incomplete forms, Bickerstaff brainstem encephalitis and pharyngeal-cervical-brachial weakness, although some of these patients may have anti-GD1a or -GD1b antibodies, but not anti-GQ1b antibodies.

tized to them, resembles demyelinating GBS clinically and pathologically.[10] However, there had been no conclusive evidence to support that such autoreactive T-cell response occurred in a sizable portion of GBS patients, suggesting that experimental autoimmune neuritis is not a valid model of GBS. Based on the model, however, many investigators focused on T-cells or myelin proteins such as P0. Our study published in 1990 might have provided a new insight into the understanding of the disease mechanism at least from the point of view of a post-infectious illness.[4] Richard Hughes' and the Rotterdam groups validated our findings that were published as letters to the editor of *Neurology*.[11),12] Hughes' group established an epidemiological association between *C. jejuni* infection and GBS through their prospective case-control study of 96 patients with GBS.[13] Patients and controls were systematically examined

for evidence of *C. jejuni* infection and a recent *C. jejuni* infection was noted in 26% of GBS patients, compared to 2% of the household controls (a member of the patient's household) and 1% of the age-matched hospital controls. This epidemiological study was a key criterion in proving the molecular mimicry theory and influenced our own subsequent prospective case-control study in Fisher syndrome (FS) as will be later discussed.

GBS after ganglioside administration. Gangliosides have a role in promoting nerve repair by increasing collateral sprouting. Trials of exogenous gangliosides as adjuvant treatment for various neurological disorders, however, either had gross methodological deficiencies or showed a lack in clinical improvement.[14] A clinical trial of bovine brain gangliosides (BBG) in diabetic neuropathy was performed in Japan. One patient showed limb weakness 2 months after the intramuscular administration of BBG. Upon admission to a hospital in 1991, the patient was thought to have ALS because of the gradual progression over a period of 6 months and the presence of upper motor neuron signs. Sensory and autonomic nervous dysfunctions were not as significantly affected although he did have diabetic neuropathy. Limb weakness, however, improved during the hospitalization period. When it became evident that the patient had been treated with BBG, his treating neurologist approached me, raising the possibility of an adverse reaction. My investigation detected high titers of IgM antibodies to GM2 in his serum. This led me to suggest to his primary physician to perform plasma exchange. The patient improved from not being able to stand to walking short distances after a single session of plasma exchange. We reported the case in a letter to *The Lancet* to raise awareness of the possible adverse effect of BBG to other clinicians.[15),16)]

The patient's serum contained anti-GM2 antibodies capable of killing GM2-containing neuroblastoma cells in the presence of complement.[17)] This may have been the first study to demonstrate that complement is required for anti-ganglioside antibody-mediated neuronal damage. GM2 is expressed in motor neurons and is a major ganglioside in an immortalized, murine motor neuron-like cell line.[18)] Clinical phenotypes similar to ALS have been found in some human GM2 gangliosidoses where GM2 accumulates in motor neurons.[19)] Using the patient's serum, we found lacto-ganglio-series gangliosides that bear GM2 epitope in the BBG mixture.[20)] We synthesized the minor gangliosides to find autoantibodies in some patients who were misdiagnosed as having ALS.[21),22)]

Following our case report,[15),16)] further studies on GBS and ALS in patients receiving Cronassial^{TM}, a type of BBG, led to its withdrawal from the German market in 1989.[23)] There were other reports from Italy and Spain of patients who developed GBS after ganglioside administration.[24),25)] A causal association between ganglioside injection and the precipitation of GBS was inferred, and gangliosides were eventually withdrawn from the Italian market in 1993. As described below, we developed a rabbit model of GBS by sensitization with Cronassial^{TM} and Sygen^{TM} (isolated GM1), which were previously available in the Italian market.[26)]

Molecular mimicry. The occurrence of GBS in association with ganglioside administration raised the possibility that *C. jejuni* might carry ganglioside-like structures; however, some microbiologists believed that bacteria were not likely to mimic human gangliosides. Lipo-oligosaccharides (LOSs) constitute a family of phosphorylated glycolipids, being anchored to the outer membrane of *C. jejuni*. We cultured a *C. jejuni* strain isolated from a GBS patient who was positive for IgG anti-GM1 antibodies (tested on thin-layer chromatogram plate) and also positive for IgG anti-GD1a antibodies (tested on an enzyme-linked immunoassay plate). 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 consists of silica beads.[27)] This suggested that the LOS carried GM1 epitope, and that silica bead column chromatography might be helpful in the purification of LOS with GM1 epitope. The LOS was separated by the 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. ^{1}H nuclear magnetic resonance showed that the terminal tetrasaccharide of the purified LOS was identical to those of GM1 (Fig. 2).[28)] This was the first study to demonstrate the existence of molecular mimicry between human peripheral nerve components and antecedent infectious agents in GBS. The bacterial strain also carried a GD1a-like LOS.[29)] Our study prompted further

research interest into the pathogenesis of GBS to other research groups.

Gerald Aspinall, a world-expert in chemical analysis of lipopolysaccharides, published preliminary results of the LOS structure of *C. jejuni* isolated from enteritis patients in 1992.[30] At that time, I had been informed that his group had started analyzing LOSs of *C. jejuni* from Japanese GBS patients. I was afraid of losing the competition, but I decided to analyze *C. jejuni* LOS. A year later our biochemical studies yielded the first report on the chemical structure of the LOSs,[28] followed closely by Aspinall's group the following year.[31] His group reported that the LOSs carried GD3- or GT1a-oligosaccharide structure, mimicking GQ1b, but neither GM1- nor GD1a-like structure. Going back to the clinical presentation, I found that the Japanese patients did not have typical GBS, but FS or Bickerstaff brainstem encephalitis (BBE) that overlapped with GBS.[32] This further highlights the importance of documenting an accurate clinical description. As will be later discussed, both FS and BBE are associated with IgG anti-GQ1b antibodies.[33),34]

The existence of axonal GBS. At the time that we reported acute axonal polyneuropathy, the Rotterdam group was not convinced of the existence of primary axonal form of GBS.[4),12] Triggs and Cros wrote a letter to the editor of *Neurology* by citing their paper and ours, suggesting that the entire findings in GBS patients were produced by only a primary myelinopathy.[4),35)–38] We responded to their comments by suggesting that an animal model of GBS inoculated with *C. jejuni* isolated from GBS patients might prove the existence of primary axonopathy.[39] Our correspondence generated a lot of interest leading to an editorial by Peter Dyck who was supporting our hypothesis that there might be an axonal variety.[40] Cros and Triggs went on to further dispute the presence of axonal GBS in *Muscle & Nerve*, stating that there were no neurophysiologic features characteristic of axonal GBS, although both Feasby and I responded stating the presence of axonal GBS and suggesting its possible pathogenesis.[41)–43] At the end of my rebuttal, I again explained that further studies were needed to clarify whether IgG antibodies to GM1 and GD1a produce primary axonal degeneration, which was later achieved by Hugh Willison's group.

In 1991, the Johns Hopkins group reported on the "Chinese paralytic syndrome" in *The Lancet*.[44] The distinctive epidemiological, clinical and neurophysiological characteristics of this illness suggested that the disorder was different from both GBS and poliomyelitis. In response to McKhann's personal letter, I suggested to him that the Chinese paralytic syndrome appeared very similar to the Japanese axonal GBS cases, and that it was likely that the syndrome was axonal GBS rather than being a new disease. This record was later published in *The Lancet*.[45] In 1993, McKhann and his group having accepted this possibility suggested a new term "acute motor axonal neuropathy (AMAN)" for the condition because the disease was not exclusive to the Chinese.[44] The term AMAN was certainly appropriate and more descriptive than my suggestion "acute axonal polyneuropathy".[4] His group subsequently published their autopsy studies demonstrating their impressive immunohistochemical findings. IgG and activated complement components bound to the axolemma of the motor nerves in AMAN patients, and that activated complement components bound the outer surface of Schwann cells.[46)–51] These studies provided the evidence in support of axonal GBS, which was later established by the development of the AMAN animal models.

GBS with preserved tendon reflexes. Following the increased incidence of GBS associated with the swine flu vaccination program in the United States in 1976, the *ad hoc* committee of the National Institute of Neurological and Communication Disorders and Stroke proposed a set of clinical diagnostic criteria for GBS which required the presence of universal areflexia or hyporeflexia as well as progressive motor weakness in more than one limb in 1978, which were later reaffirmed in 1981 and 1990.[52)–54] In 1990, I saw a patient with acute motor neuropathy subsequent to *C. jejuni* enteritis, who had normal tendon reflexes during the clinical course of the illness. Because of the established criteria, however, my senior colleagues disputed the diagnosis of GBS. We further saw three patients with similar presentation of acute motor neuropathy, *C. jejuni*-positive cultures and persistent preserved and even brisk tendon reflexes which did not fulfill the recognized criteria for GBS.[55] However, all four patients had IgG anti-GM1 antibodies and axonal degeneration at electrophysiology, and they were eventually diagnosed with AMAN. These findings suggested that some patients with acute paralytic syndrome subsequent to *C. jejuni* enteritis might have normal or even brisk tendon reflexes; and although they did not fulfill the stringent diagnostic criteria for GBS, their condition was not distinct from the AMAN subtype. We published our letter to

Annals of Neurology who had originally published the diagnostic criteria.[53),54)] Asbury and Cornblath responded stating that our findings were not surprising and that tendon reflexes could persist in patients whose weakness was modest.[56)] They added that the criteria had been devised to provide guidelines to non-neurologists who were investigating possible outbreaks of GBS, being purposefully restrictive.

A 24-year-old man developed acute paralysis following diarrhea and showed exaggerated tendon reflexes.[57)] He was initially diagnosed as having post-infectious myelitis at a hospital in Chiba, Japan. His friend contacted me after their internet search. Fearing that the patient might be suffering from GBS with hyperreflexia, I suggested them Kuwabara at Chiba University Hospital. Nerve conduction studies showed the AMAN pattern. He was treated with plasmapheresis, resulting in rapid recovery. This episode also suggested how important clinicians recognize the presence of hyperreflexic variant of GBS.

Recently to expand the existing diagnostic criteria for GBS with preserved tendon reflexes, we examined the clinical and laboratory findings of 213 patients in collaboration with Kuwabara's group also in Japan and Antonino Uncini's group in Italy.[58)] About 10% of patients showed normal or hyperexcitable tendon reflexes throughout the course of illness. In patients with preserved tendon reflexes, antecedent diarrhea and IgG anti-GM1 or -GD1a antibodies were frequent, although not statistically significant. Retained reflexes were also observed in some of the Chinese AMAN patients when the disease was mild.[56)] In Dutch patients with pure motor GBS, tendon reflexes were preserved in patients with weakness of Medical Research Council Grade 3.[59)] In one AMAN patient, however, all tendon reflexes were brisk although the muscle strength was graded 0 to 2 in the distal legs.[60)] In our study, 7% of the 213 GBS patients' tendon reflexes were exaggerated throughout the disease and 17% of the 23 GBS patients with tendon reflexes were bedbound at nadir. Moreover, some AMAN patients showed areflexia during the acute phase and hyperreflexia at the recovery phase.[61),62)]

The aforementioned observations suggested that preserved tendon reflexes were not limited to less severe cases and variations in tendon reflex excitability would exist in GBS. We suggested that the existing diagnostic criteria for GBS should be more inclusive of this fact so that clinicians do not miss possible patients that might benefit from immunotherapy.

Animal models. Despite our initial reports, there were some neurologists who were doubtful of the presence of axonal GBS or the pathogenic significance of anti-ganglioside antibodies. In order to provide conclusive evidence, disease models by active immunization or passive transfer were required. There were only two reports describing neurological dysfunction in GM1-immunized animals. In the first study GM1-immunized rabbits developed spastic paralysis, whereas GD1a-immunized rabbits demonstrated flaccid paralysis.[63)] Phagocytic cells containing myelin debris were observed histologically. In 1990, I administered repeated injections of 2 mg of GM1 and methylated bovine serum albumin with complete Freund's adjuvant to five rabbits according to the protocol by Nagai *et al*,[63)] but their disease model could not be reproduced.[64)] They had used male Japanese white rabbits, whereas I chose female New Zealand white rabbits. In a separate study by Latov's group, their rabbits developed subclinical neuropathy.[65)] There was mild axonal degeneration in the sciatic nerve and IgM deposits at the nodes of Ranvier. These failed to be confirmed in rodents. In contrast, Susumu Kusunoki's group 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.[66)] These findings suggested that the failure to induce neuropathy by sensitization with gangliosides might depend on species susceptibility as well as the immunization protocol used. 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^{TM}) according to the protocol by Kusunoki *et al*,[66)] at least some rabbits might develop flaccid paralysis or ataxia associated with anti-GD1a or -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 skeptical as to whether the rabbits would develop muscle weakness. Surprisingly, all 13 rabbits inoculated with the ganglioside mixture developed flaccid paralysis.[26)] Limb weakness progressed for 4 to 13 days (median, 5 days) after onset, indicating acute onset (Fig. 3). Some of the rabbits began to recover spontaneously, suggesting a monophasic course of the illness as shown in GBS patients. Unexpectedly, all the diseased rabbits developed high titers of IgG anti-GM1 antibodies, but not anti-GD1a antibodies. The antibody titers did not differ before

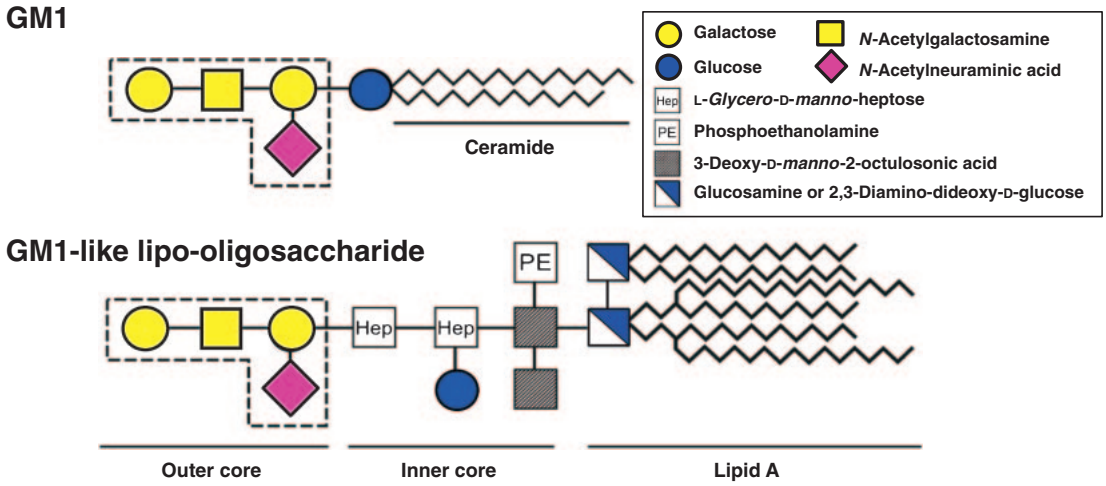

Fig. 2. Carbohydrate mimicry between gangliosides and *Campylobacter jejuni* lipo-oligosaccharide. The terminal tetrasaccharide of GM1-like lipo-oligosaccharide is identical to that of GM1 (shown by dashed lines). (Modified from ref. 179) with permission from the American Association of Immunologists.)

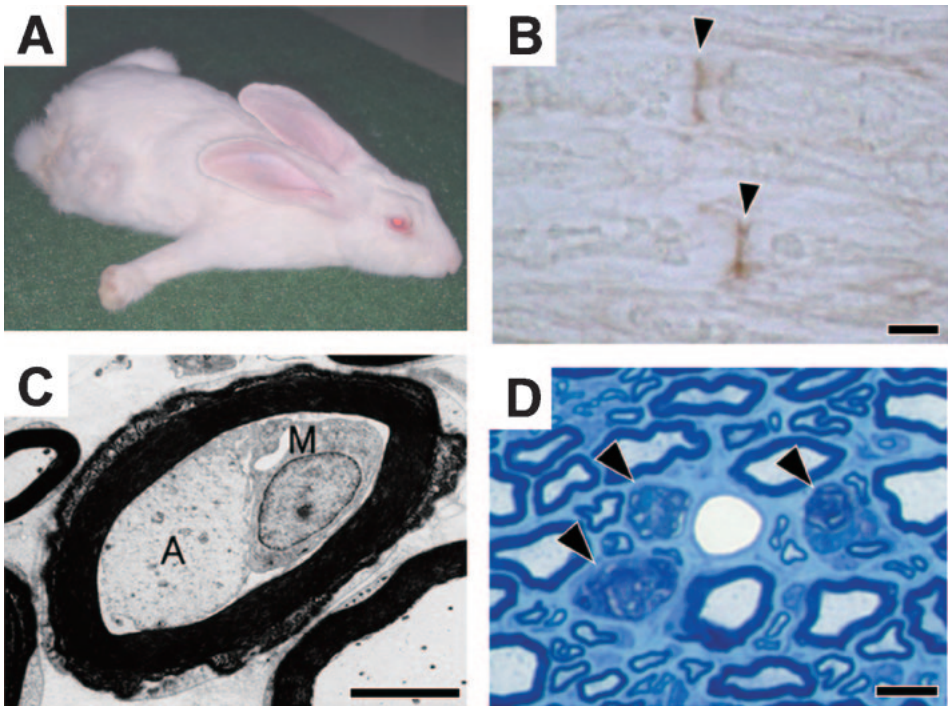

Fig. 3. Characteristic findings of the acute motor axonal neuropathy rabbit model. (A) Rabbit with flaccid limb weakness induced by sensitization with *Campylobacter jejuni* lipo-oligosaccharide. Its body is splayed, all extremities extended, head on the floor not sitting upright in the usual compact, hunched posture. (B) Longitudinal section of the cauda equina. The nodes of Ranvier are stained selectively with protein G (arrowheads). Scale bar, 10 µm. (C) Electron micrograph of a nerve fiber with macrophage infiltration. A macrophage [M] occupies the periaxonal space between the atrophic axon [A] and surrounding myelin sheath, which appears almost normal. Scale bar, 5 µm. (D) Wallerian-like degeneration of nerve fibers in a paralyzed rabbit killed 39 days after onset. Sciatic nerve cross-section with toluidine blue stain. Myelin ovoids produced by Wallerian-like degeneration of myelinated fibers are present (arrowheads). Scale bar, 10 µm. (Reproduced from ref. 183) with permission from John Wiley & Sons, Inc.)

and after the disease onset, but high affinity antibodies were detected only at the disease onset.[67] This suggested that high affinity of anti-GM1 antibodies was essential for the disease development. In contrast, none of the 10 rabbits inoculated with *keyhole limpet hemocyanin* and complete Freund's adjuvant alone developed anti-GM1 antibodies and flaccid paralysis. We started inoculating rabbits with Sygen^{TM} (isolated GM1) when a few rabbits developed IgG anti-ganglioside antibodies and acute flaccid paralysis.[26] Nine of 11 rabbits developed IgG anti-GM1 antibodies and acute flaccid paralysis. Pathological findings in the rabbit peripheral nerves were predominantly Wallerian-like degeneration with neither lymphocytic infiltration nor demyelination. GM1 is expressed in both central and peripheral nervous systems, but the pathological changes are seen only in the peripheral nerves. This may be due to the blood-brain barrier being less permeable compared to the blood-nerve barrier, thus not permitting autoantibodies to permeate the brain. IgG was deposited at the nodal or internodal axolemma in the spinal anterior roots. Anterior spinal nerves showed macrophage infiltration in the periaxonal space, but surrounding myelin sheaths remained almost intact.[68] In addition to these pathological findings, neurophysiological findings also corresponded well with those in human AMAN. A model of AMAN was established by inoculation with BBG mixture or isolated GM1. As will be discussed later, this model was instrumental in the clarification of the molecular pathogenesis of AMAN and also in developing new treatments.

The Hopkins group in their editorial commentary acknowledged that our group played a key role in proving the concept of molecular mimicry in GBS, moving us closer towards the goal of developing a robust model of AMAN.[26),69] We were also able to produce a replica of AMAN using New Zealand white rabbits and incomplete Freund's adjuvant as requested.[70),71] Toyka *et al.* in their correspondence to us enquired if passive transfer experiments or intraneural injections had been conducted to show the pathogenic role of our anti-GM1 antibody-containing sera.[72] Using Kusunoki's protocol once again,[73] we attempted to produce a passive transfer model in rabbits, but unfortunately we were unsuccessful. This was likely due to inadequate amounts of anti-GM1 antibodies. Using the protocol by Saida *et al*,[74] however, we injected rabbit anti-GM1 plasma as well as purified IgG from an AMAN patient in the presence of complement, and this produced predominantly axonal degeneration in the rat sciatic nerves.[75] Willison's group has been successful in producing murine models of axonal GBS by the passive transfer of human or mice anti-GM1 or -GD1a antibodies in the presence of human complement.[76),77] Their excellent studies provided conclusive evidence of the pathogenic roles of anti-ganglioside antibodies and complement in the development of axonal GBS.

Proof of molecular mimicry theory. The most straightforward way of verifying whether molecular mimicry between microbes and autoantigens causes GBS was to establish a disease model by immunization of animals with components of an antecedent infectious agent. We repeatedly injected Japanese white rabbits with *C. jejuni* LOS isolated from a patient with AMAN, which consisted of GM1- and GD1a-like LOSs.[78),79] 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 four of 10 rabbits developed flaccid paralysis, suggesting that sensitization with more amount of *C. jejuni* LOS should be required. Next we sensitized 10 rabbits with 10 mg of *C. jejuni* LOS, and all rabbits developed flaccid paralysis. The diseased rabbits had IgG anti-GM1 antibodies, but not anti-GD1a antibodies. In contrast, neither *Escherichia coli* nor *Salmonella minnesota* LOS induced anti-GM1 antibodies and flaccid paralysis. The pathological findings, compatible with the features of human AMAN, were evidence that rabbits inoculated with *C. jejuni* LOS constitute a valid AMAN model. The disease model studies were completed in 2001, but the work was published in 2004 to allow the inclusion of other experiments that were performed.[79] I thought there were no competitors, but it was not true. Uncini's group was doing similar experiments, and published their results 2 years later.[80]

Molecular mimicry between human tissue and microorganism has been proposed to be a pathogenic mechanism of autoimmune diseases.[79] However, no studies had convincingly demonstrated this[81] because four criteria should be satisfied to conclude that a disease is triggered by molecular mimicry:

i. Establishment of an epidemiological association between the infectious agent and immune-mediated disease;

ii. Identification of T-cells or antibodies directed against the patient's target antigens;

iii. Identification of microbial mimics of the target antigen; and

iv. Reproduction of the disease in an animal model.[82]

GBS is the first disease to fulfill all the four criteria:

i. Establishment of an epidemiological association between GBS and *C. jejuni* infection;[13]

ii. Identification of autoantibodies against GM1 or GD1a in patients with GBS subsequent to *C. jejuni* enteritis;[4),7]

iii. Identification of molecular mimicry between GM1 or GD1a and LOSs of *C. jejuni* isolated from GBS;[28),78] and

iv. A replica was produced by sensitizing rabbits with GM1 or GM1-like LOS of *C. jejuni* from GBS patients.[26),79]

Prior research on molecular mimicry and autoimmunity had focused principally on T-cell-mediated, anti-peptide responses rather than on antibody responses to carbohydrate structures. As previously mentioned, molecular mimicry between GM1 and *C. jejuni* LOS can trigger the production of pathogenic autoantibodies and development of AMAN. This new concept that carbohydrate mimicry can cause an autoimmune disease provides a clue to understand pathogenesis of other immune-mediated diseases.[83]

Ganglioside function in the nerves. GM1 and GD1a are autoantigens for AMAN, but ganglioside function of myelinated nerves was not clarified. Myelinated axons are divided into four functional regions, nodes of Ranvier, paranodes, juxtaparanodes and internodes. In myelinated nerve fibers, paranodal axo-glial junctions are important for ion channel clustering and rapid action potential propagation. Voltage-gated Na^+ (Nav) channel are highly concentrated at the nodes of Ranvier and voltage-gated K^+ channels are localized at the juxtaparanodes. Several investigators reported that anti-GM1 antibodies could block Nav channels, which are responsible for nerve conduction, whereas others negated this possibility.[84),85] Some researchers assumed that the carbohydrate portion of Nav channels carried GM1 epitope, although I noted that IgG anti-GM1 antibodies did not react with glycoproteins in the human peripheral nerves (Yuki, unpublished data). Instead, my hypothesis was that GM1 was located next to the Nav channels. Having read a paper stating that Nav channel clusters are not maintained on myelinated axons in sulfatide-deficient mice,[86] I postulated that GM1/GD1a-deficient mice might lack the functional gene for (*N*-acetylneuraminyl)-galactosylglucosylceramide *N*-acetylgalactosaminyltransferase, which would support my early hypothesis. We began these series of experiments in 2003.

In peripheral and central nervous systems, some paranodal loops failed to attach to the axolemma in the GM1/GD1a-deficient mice.[87] In the mutant mice, however, K^+ channels are aberrantly present at the paranodes (Fig. 4A). Abnormal protrusion of paranodal and nodal axolemma were seen. These findings increased with age. The defects were more prevalent in the ventral rather than dorsal roots, and less frequent in mice lacking the b-series gangliosides such as GD1b and GQ1b but with excess GM1 and GD1a. Electrophysiological studies revealed nerve conduction slowing and reduced nodal Na^+ current in the peripheral motor nerves in the GM1/GD1a-deficient mice. The amount of essential components of paranodal junctions in low density, detergent insoluble membrane fractions were reduced in the mutant brains. These results indicated that gangliosides are lipid raft components that contribute to the stability and maintenance of neuron-glia interactions at the paranodes.

Pseudo-demyelinating features in AMAN. The classification of GBS into acute inflammatory demyelinating polyneuropathy (AIDP) and AMAN are usually based on nerve conduction studies.[51),88] Several investigators reported no association between AMAN and anti-GM1 antibodies or *C. jejuni* infection.[89),90] These may have been due to less sensitive serological assays and different interpretation of nerve conduction studies. Kuwabara observed that in three GBS patients who had IgG anti-GM1 antibodies and were diagnosed as having AIDP based on conduction slowing in their first studies demonstrated resolution of this slowing within days of disease onset when the studies were repeated.[91] One patient along with three AMAN patients, moreover, showed markedly rapid increases in amplitudes of distal muscle action potentials that were not accompanied by prolonged duration of compound muscle action potentials and polyphasia. We suggested that reversible conduction failure as well as axonal degeneration were involved in the pathophysiological mechanism of IgG anti-GM1-positive GBS. We postulated that in both cases, immune-mediated attack occurs at the axolemma of motor fibers, which was later confirmed in our animal experiments described below.

C. jejuni-related GBS is usually AMAN, but previous reports described many cases of AIDP after *C. jejuni* infection. To investigate whether *C. jejuni* infection really elicits AIDP, I asked Kuwabara to

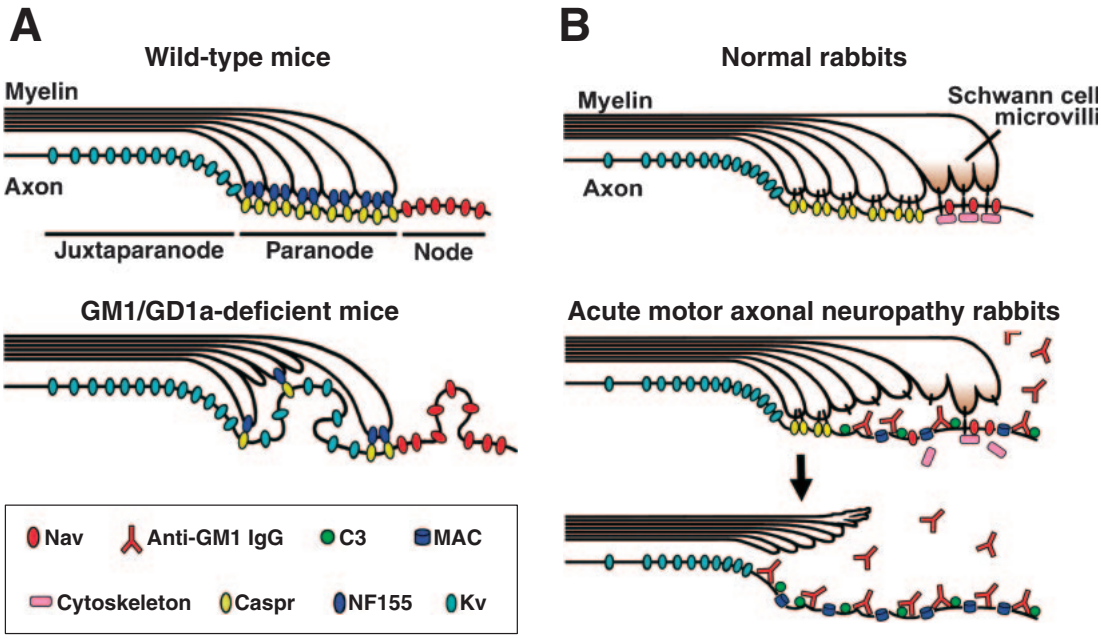

Fig. 4. Role of gangliosides in the nerves. (A) Paranodal and nodal disruption in peripheral nerves of GM1/GD1a-deficient mice. In wild-type mice, voltage-gated Na^+ (Nav) and K^+ (Kv) clusters are located at nodes and juxtaparanodes, respectively. The paranodal junction tightly attaches the myelin sheath to the axon. Neurofascin 155 (NF155) and contactin-associated protein (Caspr) are located at myelin membrane and axolemma at the paranodes, respectively. In GM1/GD1a-deficient mice, axo-glial junctions are not formed in some paranodal myelin loops. Kv channels are aberrantly localized at the paranodes. Abnormal protrusions of paranodal and nodal axolemma are frequently seen. Nodal Nav channel clusters are lengthened. (Reproduced from ref. 184) with permission from Springer.) (B) Autoimmune-mediated disruption of nodes in acute motor axonal neuropathy model. IgG anti-GM1 antibodies cause complement-mediated attack with membrane attack complex (MAC) formation at the nodal and paranodal axolemma. Nav channel clusters are altered by the destruction of structures that mediate their stabilization, including the axonal cytoskeleton at nodes, Schwann cell microvilli and paranodal junctions. As autoimmune-mediated destruction spreads, Nav channel clusters and other components at and near nodes disappear. Kv channel clusters are preserved unless immune attack extends to the juxtaparanodes. (Modified from ref. 96) with permission from the Society for Neuroscience.)

go on to review the serial nerve conduction study recordings of *C. jejuni*-related GBS patients, who met the strict criteria of positive *C. jejuni* serology and a history of a diarrheal illness within the preceding 3 weeks. According to the electrodiagnostic criteria, *C. jejuni*-related GBS patients were classified as AMAN (n = 16; 73%) or AIDP (n = 5; 23%) or unclassified (n = 1) in the first studies.[92] The five *C. jejuni*-positive patients with the initial AIDP subtype showed prolonged motor distal latencies in 2 or more nerves but these changes rapidly normalized within 2 weeks to reveal an AMAN pattern. In contrast, patients with cytomegalovirus- or Epstein–Barr virus-related AIDP showed progressive increases in distal latencies in the following 8 weeks after onset. We concluded that patients with *C. jejuni*-related GBS can show transient slowing of nerve conduction, mimicking demyelination, but *C. jejuni* infection does not appear to elicit AIDP. These observations suggested that AMAN might be underestimated when serial nerve conduction studies are not performed[93),94] and this was later also demonstrated by Uncini's group in an Italian GBS cohort.[95]

Sodium channel dysfunction in motor nerves. Following the preliminary results in the GM1/GD1a-deficent mice, I postulated that the AMAN rabbits would demonstrate similar abnormal findings, supporting the idea that anti-GM1 antibodies block Nav channels at the nodes of Ranvier.[85] We 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.[96] Nav 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 AMAN patients.[48] These pathological changes are able to produce muscle weakness. In the advanced stages of paranodal disruption, voltage-gated K^+ channel clusters at the juxtaparanodes disappeared. Complement deposition was prominent at the acute progressive phase, but decreased with clinical course. Modulation of Nav channel properties by autoantibodies has been proposed as a novel mechanism in some neuroimmunological diseases.[97] These results suggested that Nav channel alterations occurred as a consequence of complement-mediated disruption of interactions between axons and Schwann cells, providing new insights into the pathogenesis of autoimmune neuropathies.

Unexpectedly, macrophage infiltration was prominent at the recovery phase, but not at the acute progressive phase. This indicated that complement plays a crucial role in nerve injury, but that macrophages scavenge injured nerve fibers rather than acting as effector cells. Neither oral prednisolone nor intravenous methylprednisolone can significantly accelerate recovery or affect the long-term outcome in GBS.[98),99] Instead, oral corticosteroids may delay recovery.[99] The delay in recovery with steroids alone may be attributed to the fact that steroids remove macrophages, which have a role in aiding nerve regeneration through scavenging myelin debris or degenerated axons.[96]

I thereby summarize a possible pathogenesis of AMAN subsequent to *C. jejuni* enteritis (Fig. 4B):

i. Infection by *C. jejuni* bearing GM1- or GD1a-like LOS induces the production of IgG anti-GM1 or -GD1a antibodies;[78),79]

ii. These autoantibodies bind to GM1 or GD1a at the nodes of Ranvier in peripheral motor nerves;[96]

iii. Bound IgG anti-GM1 or -GD1a antibodies induce local complement activation resulting in the formation of membrane attack complex;

iv. The autoimmune attack disrupts Nav channels clusters, producing muscle weakness at the early phase of illness. Axonal degeneration subsequently occurs.

Axonal GBS subtypes. The Hopkins group further classified axonal GBS into two, AMAN and acute motor-sensory axonal neuropathy (AMSAN).[46] The pathology of AMAN and AMSAN are similar, and both conditions can be preceded by *C. jejuni* enteritis. The difference between both subtypes is the involvement of both the dorsal and ventral roots in AMSAN as opposed to the ventral roots in AMAN. IgG anti-GM1 and -GD1a antibodies are immunological markers that differentiate AMAN from AIDP, and we demonstrated that patients with AMAN and AMSAN also share these serological markers.[100] This common immunological profile supported the notion that AMAN and AMSAN are in fact the result of the same immune response against the axon rather than two separate disease entities. Uncini's group showed, through serial sensory nerve conduction studies, that sensory fibers are often subclinically involved in AMAN.[101] They also showed that reversible conduction failure occurs in sensory as well as motor fibers in AMAN and AMSAN.

Uncini's group also proposed that acute motor conduction block neuropathy was an axonal variant of GBS.[102] There is a typical history of diarrhea with evidence of *C. jejuni* infection and IgG anti-GM1 antibodies. In my editorial with Saperstein in *Neurology*, however, we recommended no further subtyping of AMAN to avoid excessive splitting in GBS.[103] Uncini agreed with the suggestion when we wrote a review together.[104] We described the full spectrum of neurophysiological changes seen in AMAN where some patients demonstrated reversible conduction block in the forearm segment, similar to that seen in acute motor conduction block neuropathy, whereas others had conduction block followed by axonal degeneration.[94] These observations suggested that acute motor conduction block neuropathy might be a less severe form of AMAN characterized by reversible conduction block in the motor nerves. At present there are a few, proposed subtypes of axonal GBS such AMAN, AMSAN and acute motor conduction block neuropathy, which are based on serial electrophysiological findings. However, these subtypes are part of the same disease spectrum and differ mainly in terms of their disease extent and severity.[105]

IgG anti-GM1 or -GD1a antibodies are associated with AMAN and AMSAN, whereas we detected monospecific IgG anti-GD1b antibodies in patients with acute sensory ataxic neuropathy.[106] These neuropathies are likely part of a continuous spectrum, although the underlying mechanisms were unclear. We hypothesized that the disruption of the nodes of Ranvier is a common mechanism whereby various anti-ganglioside antibodies, detected in these neuropathies, can lead to nervous system dysfunction. We showed that the IgG monoclonal anti-GD1a antibody injected into rat sciatic nerves caused deposition of IgG and complement products on the

nodal axolemma and disrupted clusters of nodal and paranodal molecules predominantly in motor nerves, and induced early reversible nerve conduction block.[107] Injection of IgG monoclonal anti-GD1b antibody induced nodal disruption predominantly in sensory nerves. In an acute sensory ataxic neuropathy rabbit model associated with IgG anti-GD1b antibodies, complement-mediated nodal disruption was observed predominantly in sensory nerves. In an AMAN rabbit model associated with IgG anti-GM1 antibodies, complement attack of nodes was found primarily in motor nerves, but occasionally in sensory nerves as well. Periaxonal macrophages and axonal degeneration were observed in dorsal roots from acute sensory ataxic neuropathy rabbits and AMAN rabbits. Thus, nodal disruption may be a common mechanism in immune-mediated neuropathies associated with autoantibodies to gangliosides GM1, GD1a or GD1b, providing an explanation for the similarities seen in AMAN, AMSAN, acute motor conduction block neuropathy and acute sensory ataxic neuropathy.

Mechanism of current treatments. Plasma exchange, when performed in the first 2 weeks of illness, had a significant beneficial effect on the rate of recovery.[108] The therapeutic effect is related presumably to the removal of circulating factors. Plasma concentrations of interleukin and other cytokines are elevated in GBS,[109] but given that their circulating half-lives are only a few hours, the effect of plasma exchange in reducing these levels would be short-term. Complement depletion is also brief. In contrast, the half-lives of IgM and IgA are 5 and 6 days, respectively whereas the half-life of IgG, except the IgG3 subclass, is 21 days — much longer than that of other plasma proteins. These suggested that IgG antibodies are the effector molecules responsible in the development of GBS. Results of a clinical trial suggest that at least 3 volumes of plasma exchange are required to produce an improvement.[110] As an alternative therapy, we investigated the effect of volume exchange on the reduction of IgG levels, and discovered that there was a significant decrease in IgG in the first 3 volumes of plasma exchange.[111] These findings suggested that IgG antibodies play a part in the development of GBS. Plasma exchange non-specifically removes antibodies and complement, resulting in less nerve damage and faster clinical improvement.

Intravenous immunoglobulin (IVIG) is the first line treatment of GBS in developed countries, although the action mechanism has yet to be clarified. In order to develop new therapeutic agents for GBS, the mechanism of action of IVIG must be understood. The first line of our investigation was to ascertain if IVIG had anti-idiotypic activities.[112] IVIG inhibited the cholera toxin B-subunit to GM1, and its inhibition was mediated by $F(ab')_2$ fragments of IgG. Latov's group also showed that IVIG inhibited the binding of IgM anti-GM1 antibodies from patients with multifocal motor neuropathy.[113] We then treated AMAN rabbits with IVIG and natural saline, and showed the therapeutic efficacy of IVIG in the AMAN rabbits.[114] IVIG did not affect the production or catabolism of IgG anti-GM1 antibodies, but prevented axonal degeneration of motor nerves. This may be due to the inhibition of complement activation *in situ*. Due to the difficulties in obtaining motor nerve biopsy in clinical trials, findings from animal experiments are likely surrogates to the understanding the action mechanisms of IVIG.

Alan Pestronk and his colleagues found that patients with multifocal motor neuropathy often carried IgM anti-GM1 antibodies.[115] The pathogenesis of multifocal motor neuropathy has yet to be established. We demonstrated that IgM anti-GM1 antibodies bind to GM1 and activate complement *in vitro*.[116] These results together with the findings from the AMAN rabbits experiments[96] suggested that IgM-induced, complement-mediated injury occurs at the nodes of Ranvier in peripheral motor nerves, and generates conduction block and muscle weakness. *In vitro* IVIG inhibited this type of complement activation, suggesting that *in vivo* the resulting reduction in membrane attack complex-mediated damage leads to improved muscle strength. We would need to confirm whether IVIG inhibits complement activation *in situ* to prevent axonal degeneration in the AMAN rabbits.

Development of a new treatment. Willison's group demonstrated that the neurotoxic effects of anti-ganglioside antibodies were dependent on complement rationalizing complement inhibitors as potential therapeutic agents.[117] Murine GBS model was treated successfully with complement inhibitors Mirococept and eculizumab.[118),119] The former is derived from a region of type 1 complement receptor expressed in bacteria and is given by local administration to rheumatoid arthritis patients; whereas, the latter is a humanized monoclonal antibody that binds to and blocks cleavage of C5 and is approved to treat paroxysmal nocturnal hemoglobinuria which is caused by a genetic defect that results in a deficiency

of natural membrane bound complement inhibitors. Their first study was published in 2005. The following year when I was delivering a talk on the immunopathogenesis of GBS at a seminar, Taro Kinoshita, an expert in complement, gave me an excellent suggestion that nafamostat mesilate must also be effective for GBS.

Although not widely used elsewhere, nafamostat mesilate, a synthetic serine protease inhibitor, has been clinically used in Japan for over 25 years with no serious adverse effects on patients with disseminated intravascular coagulation or acute pancreatitis. Complement system contains several serine proteases such as C1r, C1s, C3 convertase and C5 convertase. Nafamostat mesilate inhibits these serine proteases effectively to block the formation of the membrane attack complex. We began these series of experiments in 2006. Rabbits were randomly divided into two groups at disease onset, and treated using osmotic pumps with or without nafamostat mesilate.[120] Deposition of activated complement fragment and disappearance of Nav channel clusters were significantly decreased in the group treated by nafamostat mesilate.

In AMAN patients as well as in the AMAN rabbits, nodal and internodal axolemma of motor fibers are intensely immunostained for activated complement fragments and membrane attack complex.[49] In patients with AIDP, many fibers have a rim of activated complement fragments and membrane attack complex along the outer surface of Schwann cells.[50] Autopsy findings suggested that complement activation followed by membrane attack complex formation plays an important role in the development of both subtypes. In other words, complement inhibitors such as nafamostat mesilate are likely to be effective for AIDP as well as for AMAN at the progressive phase of the illness. Unfortunately, our initial plans for a clinical trial using nafamostat mesilate were hampered by the lack of support from the respective pharmaceutical company.

Post-vaccination GBS. During a 1976 mass immunization against A/NJ/1976/H1N1 swine flu in the United States, vaccinated individual had an increased risk of GBS.[121] Other seasonal influenza vaccines were not associated with the same risk. Nachamkin *et al.* reported that the 1976 swine flu vaccine induced anti-GM1 antibodies in mice.[122] Before the pandemic influenza A (H1N1) outbreak at a seminar in Genève, Switzerland in March 2009, personnel from the vaccine safety program of the World Health Organization approached me regarding producing pre-clinical data for pandemic vaccine such as influenza A (H5N1) vaccine to exclude the potential risk of GBS. At the time there were concerns that the vaccination against H1N1 might also trigger GBS, although fortunately this did not occur.[123] In our experiments, we tested serum anti-GM1 or -GD1a antibodies in human volunteers who received the pandemic 2009 influenza A/H1N1 ($n = 200$) and influenza A H5N1 ($n = 258$) as well as in mice.[124] The influenza vaccines, however, did not elicit an antibody response against GM1 or GD1a in mice and men.

Elucidating the pathogenesis of post-vaccination GBS is helpful in the development of safer vaccines. Older formulations of the rabies vaccine cultured in mammalian brain tissues are associated with a high risk of GBS; whereas, the newer formulation derived from chick embryo cells do not.[121] The World Health Organization has recommended the use of cell culture vaccines and a cessation to the production of nerve tissue vaccines. Due to its low cost, however, several countries continue to use the older form of vaccine, resulting in many patients developing GBS following vaccinations. We studied anti-rabies vaccine derived from sheep brain and chick embryo cell culture. Sheep brain-derived vaccine contained myelin basic protein, a candidate antigen that induces encephalomyelitis,[125] indicating contamination of the vaccine with nerve tissue. The brain-derived vaccine was contaminated with gangliosides, but the cell culture vaccines were not. GBS following lamb brain-derived rabies vaccination associated with anti-GM1 or -GD1a antibodies have been reported in three patients.[126] These findings along with the presence of GBS after ganglioside administration[24),25] suggested that the contamination of the brain-derived vaccine with gangliosides could induce the production of anti-ganglioside antibodies leading to the development of GBS. A GBS model by sensitization of animals with the vaccine should be reproduced to support the rare occurrence of GBS following the chick embryo cell vaccination.[121] Our preliminary biochemical results support the World Health Organization's recommendation against the use of nerve tissue vaccines to avoid the occurrence of GBS.

Fisher syndrome

Validation of molecular mimicry theory. FS is characterized by paralysis of extraocular muscles (ophthalmoplegia), loss of balance (ataxia) and areflexia.[127] Its link to GBS is strengthened by the

observation that some FS patients develop profound limb weakness typical of GBS during the clinical course of their illness.[128] Upper respiratory infectious symptoms are more common in GBS and FS than diarrhea.[129] Kusunoki's group identified IgG anti-GQ1b antibodies in patients with FS and proposed those autoantibodies as a diagnostic marker of FS.[33] Although their landmark study was published in 1992, I found the title of their presentation at a meeting in Japan and confirmed the results at the end of 1991.[130]

I have always been aware of other possible autoimmune conditions or pathogenic microorganisms that may also exhibit the molecular mimicry theory. As there were reported cases of FS subsequent to *C. jejuni* enteritis,[131] 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 enteritis patients, clinicians requested me to test anti-GQ1b antibodies in 2 FS patients from whom *C. jejuni* was isolated.[185] I therefore performed thin-layer chromatography-immunostaining to show the presence of GQ1b-like LOS of *C. jejuni* from FS 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,[132] although my efforts to purify the LOS fractions that reacted with the monoclonal anti-GQ1b antibody were unsuccessful. In 1997, Aspinall's group demonstrated that LOS of *C. jejuni* isolated from an FS patient carried GD1c-oligosaccharide (Fig. 5).[133] In collaboration with Michel Gilbert's group, we were also able to demonstrate that *C. jejuni* isolated from FS patients bore GD1c- or GT1a-like LOS mimicking GQ1b.[78),134]

About 15000 serum samples were sent to my lab at Dokkyo Medical University, Tochigi, Japan, anti-ganglioside antibody testing in patients with GBS and its related disorders between 1996 and 2007. At that time I asked clinicians to isolate microorganisms from feces, sputum or swab cultures. *Campylobacter coli*, *Campylobacter curvus* and *Campylobacter upsaliensis* are occasionally isolated from patients with GBS or FS, but we showed that these microorganisms were not pathogenic.[135),136] Instead, *C. jejuni* was the more likely candidate that led to neuropathies in this group of patients. Interestingly, *Haemophilus influenzae* was isolated from the sputum of one of Fisher's original description of patients.[127] That patient had a cough and fever prior to the neurologic onset. A chest X-ray revealed pneumonia. We also investigated the possibility of *H. influenzae* as the causative agent in GBS and FS.[137] Four of 27 patients with GBS or FS in whom *H. influenzae* was isolated were also seropositive for *C. jejuni.* Anti-ganglioside antibodies in these four patients did not cross-react with the respective *H. influenzae* LOSs, whereas anti-ganglioside antibodies in the four patients with positive serology for *H. influenzae* did. The findings indicated that the isolation of *H. influenzae* is not always suggestive that it is the causative agent in these syndromes. In collaboration with Gilbert's group, however, we were able to demonstrate that an *H. influenzae* isolate from an FS patient had GD3-like LOS which mimics GQ1b.[138] This provided good evidence that it might be pathogenic in the development of FS.

In 1996, I wanted to develop a highly specific serological testing for recent *C. jejuni* serology, which is not always sensitive, to investigate frequencies of preceding *C. jejuni* infection. Serological evidence of *C. jejuni* infection was found in 31% in 201 GBS patients and 18% of 65 FS[139] whereas serological evidence of *H. influenzae* infection was found in 2% of 110 GBS patients and 7% of 70 FS.[140] This was in contrast to Kuwabara's group who reported 13% of GBS patients with serological evidence of *H. influenzae* infection,[141] but this difference may relate to the sensitivity of the assays. These studies were retrospective, and prospective case-control studies were needed to confirm an association between microorganisms and FS. Between the period of 2000 and 2003, we received 367 serum samples from FS patients, of which 73 samples with paired hospital control were available for further analysis. During the same period, we received 1814 serum samples from patients with GBS, and 73 samples were randomly selected as disease controls. We demonstrated the serologic evidence of recent *C. jejuni* (21%) and *H. influenzae* (8%) infections was more common in FS than in the hospital controls, whereas frequencies of *Mycoplasma pneumoniae*, cytomegalovirus and Epstein-Barr virus did not differ.[78] In comparison, patients with GBS had a higher frequency of antecedent *C. jejuni* infection and lower frequency of *H. influenzae* infection but these differences were not significant.

We suggested six criteria to verify that a given microorganism is causative in the development of GBS and its related conditions:[142]

i. Epidemiological association is established between the microbial infection and the disease;

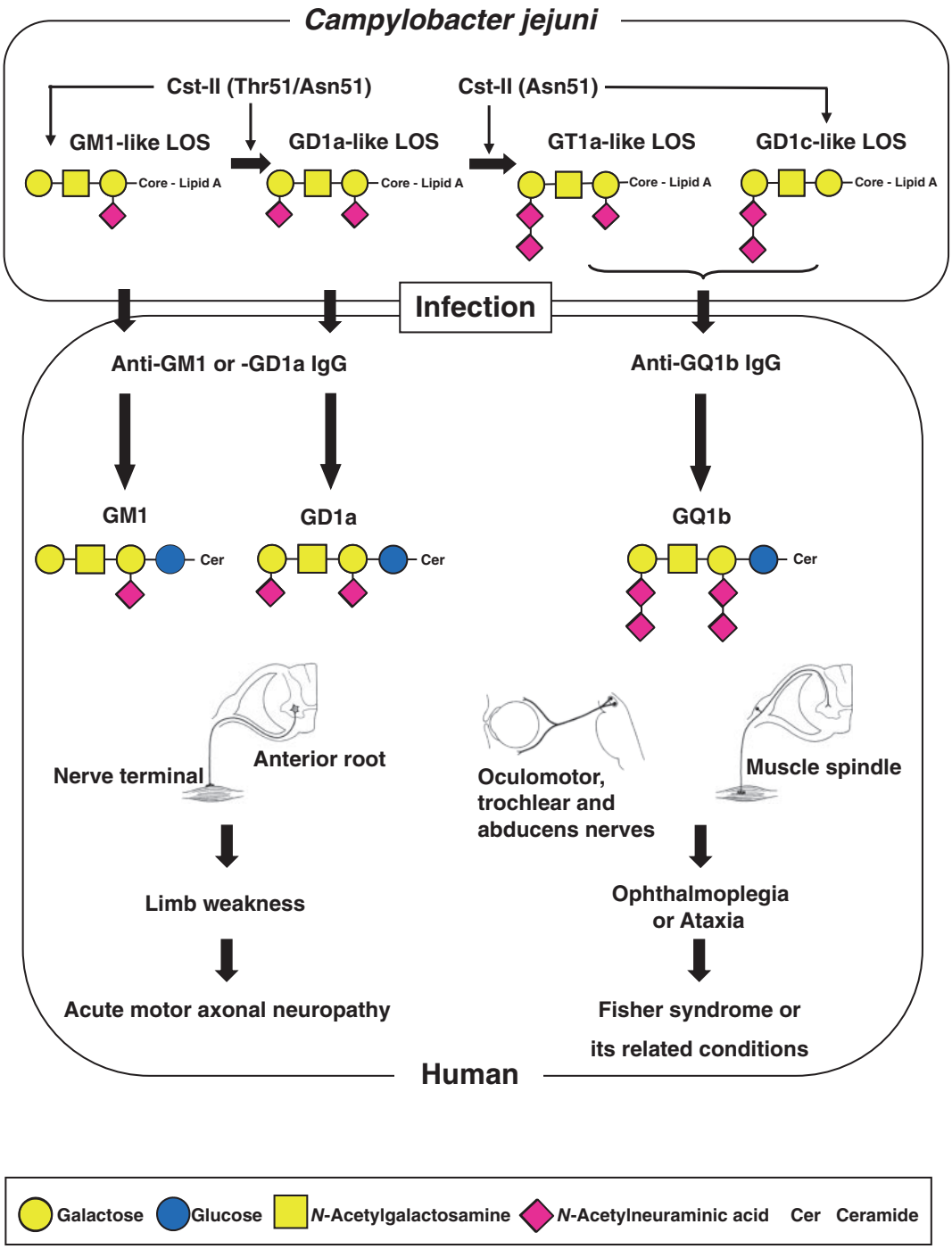

Fig. 5. *Campylobacter jejuni* gene polymorphism as a determinant of clinical phenotypes of human neuropathies. *C. jejuni* that carries *cst-II* (Thr51) can express GM1- or GD1a-like lipo-oligosaccharide (LOS) on its cell surface. Infection by such a *C. jejuni* strain may induce IgG anti-GM1 or -GD1a antibodies in some patients. The autoantibodies bind to the GM1 or GD1a expressed on motor nerves of the four limbs, inducing acute motor axonal neuropathy. By contrast, *C. jejuni* that carries *cst-II* (Asn51) expresses GT1a- or GD1c-like LOS on the cell surface. Infection by such *C. jejuni* strains may induce IgG anti-GQ1b antibodies in some patients. The autoantibodies bind to the GQ1b that is expressed on oculomotor nerves and muscle spindles, inducing Fisher syndrome or its related conditions. (Modified from ref. 183) with permission from John Wiley & Sons, Inc.)

ii. The microorganism is isolated from patients at the acute progressive phase of the illness, and not at the recovery phase;

iii. Anti-neural antibodies are detected at the acute phase, and the titers decrease at the recovery phase;

iv. Molecular mimicry is identified between the microbial and neural antigens;

v. The anti-neural antibodies are induced by sensitization with the microbe itself, or its component in animals; and

vi. Animal models are reproduced by inoculation with the microbe itself, or its component, as well as with the neural antigen.

At the very least, criteria (ii) to (v) should be fulfilled before concluding that certain microbes are probable causes of GBS, FS or related conditions. We encouraged researchers to satisfy the aforementioned definite or probable criteria to allow for more meaningful hypotheses to be formed in understanding the pathogenesis.

***C. jejuni* genes responsible for the development of autoimmune neuropathies.** Since my first encounter with a *C. jejuni*-related GBS patient in 1989, we have systematically collected *C. jejuni* strains isolated from patients with GBS and related conditions. At the time I assumed that *C. jejuni*-bearing the GM1 epitope was so rare that most patients with *C. jejuni* enteritis did not develop GBS. There are two international serotyping schemes for *Campylobacter*, the Penner method (PEN) and the Lior scheme (LIO). Kuroki *et al.* reported that four strains associated with GBS patients belonged to PEN 19 serotype.[143] We serotyped 31 isolates from patients with GBS and seven isolates from those with FS.[144] PEN 19 of *C. jejuni* was more frequently isolated from GBS patients (52%) compared to sporadic enteritis patients (5%). LIO 7 of *C. jejuni* was also more frequently isolated from GBS patients (45%) compared to enteritis patients (3%). Initially I considered the reasons why GBS was rare, despite the high incidence of *C. jejuni* enteritis, was due to the low frequencies of PEN 19 and LIO 7. The frequency of positive anti-GM1 antibody titers in the GBS patients with PEN 19 isolates was higher than that in the GBS and FS patients without PEN 19 isolates, and five of the seven isolates from the FS patients belonged to PEN 2:LIO 4. We speculated that the serotypic determinant of PEN 19 aided in the production of anti-GM1 antibodies by GM1-like LOS, and that the serotypic determinant of PEN 2 helped in the production of anti-GQ1b antibodies by LOS that bears GQ1b epitope. We later found that the serotypic determinants were not a key to the development of the disease but that the genes responsible for the biosynthesis of Penner's serotype were associated with the genes involved in the biosynthesis of LOS.

C. jejuni isolation is the standard for the diagnosis of the bacterial infection, but there were no epidemiological studies of a large number of *C. jejuni* isolates from patients with GBS and FS. When we were requested to test serum anti-ganglioside antibodies in patients with GBS and various neurological diseases, we requested each clinician to isolate *C. jejuni* at their own hospitals and concurrently send stool specimens from GBS or FS patients to the Tokyo Metropolitan Institute of Public Health. A total of 113 strains were isolated from 1049 patients between 1990 and 2003. The male/female ratios were 1.7:1 for GBS and 2.2:1 for FS.[145] The patient age range showed a peak in 10- to 30-year-old subjects who had GBS and in 10- to 20-year-old subjects who had FS. The predominance of young adults and male patients who had *C. jejuni*-associated GBS or FS may be related to the preponderance of young adults and male patients who had *C. jejuni* enteritis. The median interval from diarrhea onset to neurologic symptom onset was 10 days for GBS or FS.

Having ascertained the structure of GM1-like LOS in 1993, the next step would be to clone the genes responsible for the biosynthesis of ganglioside-like LOS but this proved to be a challenging task at the time. Ganglioside-like LOSs are synthesized by sialyltransferase Cst-II, *N*-acetylgalactosaminyltransferase CgtA and galactosyltransferase CgtB. In 2002 Gilbert's group reported that Cst-II sialyltransferase consists of 291 amino acids, the 51st determining its enzymatic activity.[146] Cst-II (Thr51) has only α-2,3-sialyltransferase activity (mono-functional) and produces GM1- and GD1a-like LOSs (Fig. 5). In contrast, Cst-II (Asn51) has both α-2,3- and α-2,8-sialyltransferase activities (bi-functional) and synthesizes GT1a- or GD1c-like LOSs mimicking GQ1b. Based on their findings, I postulated that *C. jejuni* isolates from GBS had Cst-II (Thr51) and that the isolates from FS had Cst-II (Asn51). We began the 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%).[147] 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 IgG anti-GQ1b antibodies and had ophthalmoparesis and ataxia. In contrast, patients who had *C. jejuni* (*cst-II* Thr51) were more frequently positive for IgG anti-GM1 or -GD1a antibodies and had limb weakness. Why a microbial infection lead 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 FS in others, however, was made clear by our findings. In other words, we presented another new paradigm that microbial genetic polymorphism can determine the clinical features of human autoimmune disease.

The *cst-II* gene encodes an enzyme that transfers sialic acid to LOS, and *neuA1* encodes an enzyme that synthesizes the donor (CMP-sialic acid) used by Cst-II sialyltransferase.[148] Both genes function in LOS sialylation, being essential for ganglioside-like LOS synthesis. The Rotterdam group produced and analyzed mutants of *C. jejuni* that lack these genes.[149] GM1- and GD1a-like LOSs were identified in the wild-type *C. jejuni* strains isolated from GBS patients, but neither were found in the corresponding *cst-II* and *neuA1* knockout mutants. Unlike the wild types, *cst-II* and *neuA1* knockout mutants decreased reactivity to GBS patients' sera. We took part in their study and showed GM1/GD1a-deficient mice, which were immune-naïve hosts, were used to obtain high titers of anti-ganglioside antibody responses. Immunization with the wild-type strain induced an IgG anti-GD1a antibody response in the mice, whereas mutant strain immunization did not. This meant that the genes involved in ganglioside-like LOS biosynthesis are essential for the induction of cross-reactive anti-ganglioside antibodies during *C. jejuni* infection. 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.[150] When I got the information at a conference in 2003, we began our collaborative work with Gilbert. Most isolates from GBS and FS patients belonged to classes A, B or C, and the frequency was significantly higher than isolates from uncomplicated enteritis patients.[29] In other words, *cgtA*, *cgtB* and *cst-II* are responsible genes 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 host factors are also important for the development of neuropathies after the bacterial infection.

The molecular pathogenesis of GBS or FS subsequent to *C. jejuni* enteritis is as follows (Fig. 5): *C. jejuni* strains that carry *cst-II* (Thr51) express GM1- or GD1a-like LOS on its cell surface and may induce IgG anti-GM1 or -GD1a 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- 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,[151),152] resulting in FS. GQ1b is also expressed in some large neurons in the dorsal root ganglia.[153] Rather than dorsal root ganglia, however, muscle spindles may be the lesion responsible for ataxia. This would explain the good recovery seen in FS with no sequelae.[154]

Some patients with FS overlapped by GBS carry IgG antibodies against GM1 and GD1a as well as against GQ1b, and the corresponding findings seen in such cases would be reasonable.[134] For example, a GT1a-like LOS is synthesized by Cst-II (Asn51) via GM1- and GD1a-like LOSs, and an FS isolate can carry GM1- and GD1a-like LOSs as well as a GT1a-like LOS. *C. jejuni* strains bearing *cst-II* (Asn51) can induce the synthesis of IgG anti-GM1 or -GD1a antibodies, as well as IgG anti-GQ1b antibodies, and GBS can be overlapped in some FS patients. It is the host's genetic, rather than bacterial, factors that may determine the types of autoantibodies produced as well as the clinical presentation of either FS, GBS or overlapping of FS and GBS.

Nosological relationship. Bickerstaff and Cloake published a report of three cases they called "mesencephalitis and rhombencephalitis".[155] Later Edwin Bickerstaff added five more cases to the original study and named the condition "brain stem encephalitis".[156] Seven of eight patients had ophthalmoplegia, ataxia and impaired consciousness. All the patients' conditions were severe, but seven had benign outcomes. The etiology of this condition was speculated to be similar to that of GBS because of evidence of prodromal upper respiratory infection,

areflexia and CSF albuminocytological dissociation. Bickerstaff's group subsequently reported 18 other patients who had "brainstem encephalitis and the syndrome of Miller Fisher" and argued a central origin.[157] All 18 suffered ophthalmoplegia and ataxia. Eleven experienced drowsiness, and one became comatose. Muscle stretch reflexes were absent in 11, normal in three and brisk in four. Four had positive Babinski's sign, and two long-tract sensory disturbance. Based on radiological (three patients) and pathological (one patient) changes in the brainstem, as well as abnormal electroencephalographic findings (12 patients), the authors insisted that the responsible lesion in all 18 patients were localized in the central nervous system and that the condition represents a clinical entity distinct from GBS. Their report was criticized by a fellow researcher at the time who considered six of the 18 cases typical FS and the other 12 obscure brainstem lesions without peripheral polyneuropathy.[158] One of three patients originally described by Miller Fisher also experienced drowsiness.[127] Because of the apparent similarities in the clinical presentation of FS and BBE, opinions have differed as to whether both conditions are distinct or related and whether the lesions responsible for ophthalmoplegia, ataxia and areflexia are in the peripheral or central nervous system.

While investigating sera from FS patients in an attempt to confirm the results from Chiba *et al*,[33] my attention was brought to a BBE patient my colleagues had previously treated. The patient (Patient 2 in ref. 34)) became comatose in addition to suffering acute ophthalmoplegia, ataxia and areflexia. These neurological signs disappeared 2 months after onset. At that time I thought that BBE was distinct from FS and that anti-GQ1b antibody testing could differentiate between them. Unexpectedly, the patient had IgG anti-GQ1b antibodies. I therefore investigated further two BBE patients. All three BBE patients had high anti-GQ1b antibody titers, which decreased with their clinical improvement. The finding that BBE and FS have autoantibodies in common suggested that the autoimmune mechanism is common to both, and they are not distinct conditions. At the time I was convinced that clinico-serological studies were helpful in the understanding of the nosological relationship between GBS and its related conditions. This subject has been an important part of our research.

To establish the relationship between BBE and FS, we recruited 53 with typical BBE who had impaired consciousness and 466 with typical FS who had alert consciousness and hypo- or areflexia.[159] IgG anti-GQ1b antibodies were positive in 68% of BBE patients and in 83% of FS. EEG recordings showed diffuse slow activities in the θ or δ range in 57% of 30 BBE patients and in 25% of 32 FS patients who were fully conscious. These observations indicate that central components can occasionally be affected in FS. In nerve conduction and H-reflex studies, the most frequent abnormality was the absence of soleus H-reflexes in 75% of 4 BBE patients and 74% of 28 FS patients. The coexistence of central and peripheral components refutes the idea of a simple relationship between BBE and purely central involvement, as well as between FS and a simple peripheral neuropathy. As explained above, BBE is not distinct from FS clinically, anatomically or etiologically. These two conditions therefore represent a single autoimmune disease that variably involves the peripheral and central nervous system. We proposed a new eponymic terminology "Fisher–Bickerstaff syndrome", which is more useful in the understanding of the clinical continuity between FS and BBE. Based on the historical points of view, however, FS or BBE rather than Fisher–Bickerstaff syndrome should be used at the usual clinical setting.

Whereas Bickerstaff speculated that the etiology of BBE is similar to that of GBS,[156] his group insisted that they were distinct.[157] In other words, the nosological relationship of BBE to GBS was unclear. We therefore investigated this by clarifying the clinical, electrophysiological, neuroimaging and immunological features of 62 BBE patients.[160] "Progressive, relatively symmetric external ophthalmoplegia and ataxia by four weeks" and "impaired consciousness or hyperreflexia" were clinical features in support of the diagnosis of BBE. One patient in Bickerstaff's original report had flaccid limb weakness,[156] thus our BBE cases were divided into "BBE without limb weakness" and "BBE with limb weakness". Muscle weakness was symmetric and flaccid in 37 patients who had "BBE with limb weakness".[160] IgG anti-GQ1b antibodies were present in 70%, and IgG anti-GM1 or -GD1a antibodies were present in 24% of 37 patients with BBE overlapped by GBS. These results suggested that elements of the autoimmune mechanism are common to BBE and GBS. Clinical and electrophysiological findings as well as immunological ones showed that BBE is closely related to GBS and that they form a continuous spectrum.

In 1996, I proposed a new term "anti-GQ1b antibody syndrome" for FS and related conditions because it appeared more useful in the understanding of the etiological relationships of this group of illnesses (Fig. 1).[161] This new terminology would include not only FS and BBE, but ataxic GBS,[162] acute ophthalmoparesis without ataxia,[163] isolated internal ophthalmoplegia,[164] acute oropharyngeal palsy,[165] and pharyngeal-cervical-brachial weakness.[166] In addition to the clinical similarities, the presence of common autoantibodies is evidence that these conditions are part of the same spectrum. I believe that the following clinico-serological studies are helpful for clinicians to understand the nosological relationship of this group of conditions.

Pharyngeal-cervical-brachial weakness is characterized by areflexia and weakness of the oropharyngeal, neck and shoulder muscles.[167] The electrodiagnosis of these patients supports AMAN and acute motor conduction block neuropathy, and IgG anti-GD1a antibodies are also present in some patients with pharyngeal-cervical-brachial weakness.[168),169] A significant proportion of patients with pharyngeal-cervical-brachial weakness overlap with FS as well as typical GBS.[166] Half of patients with pharyngeal-cervical-brachial weakness also carry IgG anti-GT1a antibodies, most of which cross-react with GQ1b. The most frequent antecedent infection is *C. jejuni*. The clinical, electrophysiological and serological features suggested that pharyngeal-cervical-brachial weakness can occur as a regional form of AMAN as well as an extensive form of FS. Although typical FS is associated with ataxia and ophthalmoplegia, there are incomplete FS forms with positive anti-GQ1b antibodies, where patients have only ataxia or ophthalmoplegia. The conditions can be referred to as acute ophthalmoparesis (without ataxia) or ataxic GBS.[161),162] Ataxic GBS is characterized by cerebellar-like ataxia with no ophthalmoplegia.[170] The presence of areflexia, distal paresthesia and CSF albuminocytological dissociation provides evidence that this is a GBS variant. "Sixth nerve paresis with paresthesia" can be considered a limited form of acute ophthalmoparesis.[171),172] Ataxic GBS and acute sensory ataxic neuropathy have similar features; antecedent infectious symptom (86% *versus* 83%), distal paresthesia (70% *versus* 88%), superficial sensory impairment (27% *versus* 24%), IgG antibodies against GQ1b (65% *versus* 18%) and GD1b (46% *versus* 47%) and CSF albuminocytological dissociation (30% *versus* 39%).[159] These suggested that both conditions are part of the same clinical spectrum, and could be comprehensively referred to as "acute ataxic neuropathy without ophthalmoplegia" to avoid nosological confusion because FS is not defined by the absence or presence of sensory ataxia. Mydriasis and bulbar palsy are respectively present in 42% and 26% of FS.[154] This suggests that acute mydriasis[164] and acute oropharyngeal palsy,[173] which are associated with IgG anti-GQ1b antibodies, are likely to be incomplete forms of FS, although acute oropharyngeal palsy could also be positioned as a more limited form of pharyngeal-cervical-brachial weakness.

Production mechanism of anti-ganglioside antibodies. Production of four subclasses of human IgG differs with the type of antigen. Whereas IgG1 and IgG3 subclass responses are common in viral antigens, IgG antibodies to bacterial polysaccharides are mainly restricted to the IgG2 subclass. Because IgG subclass distribution of anti-GM1 or -GQ1b antibodies is mainly restricted to IgG1 and IgG3, Willison and Veitch suggested that the antigen initiating the immune response was unidentified cross-reactive glycoprotein antigens.[174] I therefore investigated the subclasses of IgG antibodies to GM1, GQ1b and the GM1- or GQ1b-like LOS in sera from patients with GBS or FS subsequent to *C. jejuni* enteritis, and found that both belonged mainly to IgG1, but not IgG2.[175] The results suggested that the LOS that bears GM1 or GQ1b epitope participates in the production of IgG1 anti-GM1 or -GQ1b antibodies in GBS or FS following *C. jejuni* infection. We also examined the IgG subclasses of anti-GM1 antibodies in larger series of GBS patients in Japan and The Netherlands.[176),177] IgG1 antibody was associated with preceding gastroenteritis and *C. jejuni* serology, whereas IgG3 antibody was associated with preceding respiratory infection. The IgG1 subclass of anti-GM1 antibodies is a major subtype indicative of slow recovery, whereas isolated elevation of IgG3 subclass antibody titer suggests rapid recovery. This subclass pattern suggested that T-cells helped the production of IgG anti-ganglioside antibodies. We showed that oligoclonal expansion of T-cells bearing particular type T-cell receptor Vβ and Vδ genes frequently occurs in GBS, suggesting that T-cells mediate the development of these neuropathies. However, the predominant phenotypes varied even within *C. jejuni*-related GBS.[178]

Human group 1 CD1 molecules, CD1a, CD1b and CD1c, bind microbial glycolipids as well as self-glycolipids including GM1, and present the antigens to T-cells and NKT cells. Group 2 CD1 molecule,

CD1d, also binds microbial and self-glycolipids, and present the antigens to NKT cells. Because glycolipid-specific, CD1-restricted T-cells are present, we assumed that a certain group 1 CD1 molecule functioned in the production of IgG antibodies against *C. jejuni* LOS, which cross-react with self-gangliosides. We investigated *in vitro* which CD1 molecule binds *C. jejuni* LOS.[179] Neither human CD1a, CD1b nor CD1c bound GM1-like LOS, but both human and murine CD1d bound GM1-like LOS. Sensitization of GM1/GD1a-deficient mice with *C. jejuni* developed high titers of IgG antibodies against GM1 and GD1a, because they have no natural tolerance to these gangliosides. CD1d knockout mice were intercrossed to clarify whether CD1d is essential for the production of IgG anti-ganglioside antibodies, but sensitization of the mice with *C. jejuni* resulted in the development of high antibody responses against GM1 and GD1a. These results indicated that CD1d does not function in the production of anti-ganglioside IgG antibodies at least in mice.

We showed that B-cell-activating factor belonging to the tumor necrosis factor family helped murine B-cells produce anti-ganglioside antibodies against *C. jejuni* LOS.[179] In splenocyte culture, however, anti-ganglioside antibodies were produced in the presence of a soluble transmembrane activator and calcium-modulating and cyclophilin ligand interactor immunoadhesin, a receptor for B-cell-activating factor belonging to the tumor necrosis factor family. The transmembrane activator and calcium-modulating and cyclophilin ligand interactor immunoadhesin adenoviral vectors failed to decrease production of anti-ganglioside antibodies in mice sensitized with *C. jejuni* LOS and did not alter IgG subclasses, evidence that B-cell-activating factor belonging to the tumor necrosis factor family aids but is not essential for the generation of IgG anti-ganglioside antibodies in response to *C. jejuni* LOS. Further studies are required to elucidate the production mechanism of anti-ganglioside antibodies.

Conclusion

I comprehensively reviewed the infectious origins of GBS in *The Lancet Infectious Disease* in 2001,[180] the role of anti-glycolipid antibodies in peripheral neuropathies with Willison in *Brain* in 2002[181] and GBS with Hartung in *The New England Journal of Medicine*.[186] In this review, however, I wanted to share my journey from a clinician to a clinician-scientist in the hopes of inspiring younger clinicians that they too can follow a similar path. This review also allowed me to reflect on how our own work was inspired by the initial work of Latov's, Kusunoki's, Gilbert's and the Hopkins groups,[3),33),49),66),146] as well as by leading clinical and translational researches by Willison's, Hughes' and the Rotterdam groups. I have thoroughly enjoyed my journey and hope that the future brings more revelations into the pathogenesis of other autoimmune diseases.

Acknowledgements

I thank Dr. N. Shahrizaila (Department of Medicine, University of Malaya, Malaysia) for her continued help with editing; Dr. Michel Gilbert (National Research Council Canada, Canada) and Prof. Antonino Uncini (G. d'Annunzio University, Italy) for their critical reading and our fruitful collaborations; my coworkers, especially, Ms. Hiromi Fujita, Dr. Kei Funakoshi, Ms. Miyuki Funakoshi, Dr. Masafumi Ito, Ms. Saiko Koike, Dr. Norito Kokubun, Dr. Michiaki Koga, Dr. Takahide Nagashima, Dr. Yukihiro Nishimoto, Dr. Masaaki Odaka, Dr. Keiichiro Susuki, Dr. Muneto Tatsumoto and Ms. Chiaki Yanaka at my neuroimmunology lab in Dokkyo Medical University, Japan; and our many collaborators, especially Dr. Bart C. Jacobs (Erasmus MC, University Medical Center, The Netherlands), Prof. Satoshi Kuwabara (Graduate School of Medicine, Chiba University, Japan), Dr. Masaki Takahashi (Tokyo Metropolitan Institute of Public Health) and Prof. Pieter A. van Doorn (Erasmus MC, University Medical Center, The Netherlands) for their great contribution to my enjoyable research journey.

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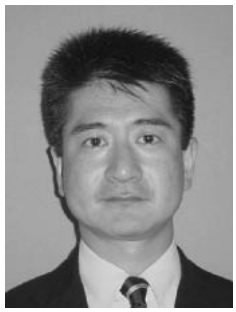
(Received Apr. 2, 2012; accepted May 2, 2012)

Profile



Prof. Nobuhiro Yuki is a neurologist whose research interest falls on neuroimmunology, particularly Guillain–Barré syndrome (GBS). He graduated from Faculty of Medicine in Niigata University and received his Ph.D in neurology at Tokyo Medical and Dental University in Japan. His life work began in 1989, when he first encountered a patient with GBS subsequent to *Campylobacter jejuni* enteritis. This later led to his research where molecular mimicry between GM1 and the lipo-oligosaccharide of *C. jejuni* has been demonstrated and their animal models established. With this work, he was able to exhibit that GBS is the first verification that molecular mimicry can cause autoimmune diseases. He continued his research endeavors in Dokkyo Medical University between 1996 and 2007. He moved from Japan to Singapore in 2010. At present, he is a Research Professor at Department of Medicine, National University of Singapore and working as a Visiting Professor at University of Malaya, Malaysia as well as an Adjunct Principal Research Scientist at the Singapore Eye Research Institute. His body of work contributed significantly in GBS and its related conditions as described in this review. His medical students branded him "Mr. GBS" but his work is not confined in a single disease but is now expanding to understand other immune-mediated diseases as well as the development of treatments for the benefit of the patients.