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An update on pathobiologic roles of anti-glycan antibodies in Guillain-Barré syndrome

Kazim A Sheikh* and Gang Zhang

Address: Department of Neurology, University of Texas Medical School at Houston, 6431 Fannin Street, Houston, TX 77030, USA

* Corresponding author: Kazim A Sheikh (kazim.sheikh@uth.tmc.edu)

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Abstract

Anti-glycan antibodies directed against gangliosides are now considered the major immune effectors that induce damage to intact nerve fibers in some variants of the monophasic neuropathic disorders that comprise Guillain-Barré syndrome. Recent experimental studies elucidating the complexity of anti-glycan antibody-mediated pathobiologic effects on intact and injured nerves undergoing repair are discussed.

Introduction and context

Guillain-Barré syndrome (GBS) is a group of acute, monophasic, and pathophysiologically heterogenous neuropathic disorders. Paralytic forms of the disorder are classified (Table 1) as demyelinating or axonal based on electrophysiology, whereas relatively rare non-paralytic variants include Fisher syndrome (FS), characterized by ophthalomplegia, ataxia, and areflexia [1,2]. With the near eradication of polio, paralytic variants of GBS are the commonest cause of acute flaccid paralysis worldwide.

GBS is considered to be an autoimmune disease triggered frequently by common infections of upper respiratory and gastrointestinal tracts [1-3] in a susceptible host [4]. What constitutes a susceptible host remains enigmatic. Autoantibodies directed against cell surface glycans carried by gangliosides (sialic acid-containing glycosphingolipids enriched in peripheral nerve fibers) have become the main focus of research in GBS. Over the last 15-20 years, several lines of evidence have linked these autoantibodies to the pathogenesis of GBS, particularly to axonal and Fisher variants of the disease. The clinical studies focusing on serological immune markers and GBS phenotype and recovery have identified associations of specific anti-glycan antibodies with different variants of GBS [3] and poor recovery [5,6]. The association studies have implied that specific anti-glycan antibodies not only can induce neuropathy (i.e., injury to intact nerve fibers) but also can adversely affect recovery by inducing more severe neuropathic disease or interfering with the nerve repair process required for recovery (or both). Identification of specific anti-glycan antibodies in GBS patients led to the development of cell culture [7], tissue culture [8-10], and animal [11-14] models that showed the pathogenetic effects of anti-glycan antibodies on intact nerves or nerve cells. Cumulatively, these studies indicate that specific anti-glycan antibodies target relevant antigens in neural cells, especially at motor nerve terminals or nodes of Ranvier (or both) to disrupt the nerve fiber function [15-17]. Human and experimental studies indicate that complement activation is involved in structural injury to the nerve fibers [18-20]. Several issues regarding the anti-glycan antibody-mediated nerve injury remain unresolved [21]. For example, unconditional passive transfer with sera containing anti-glycan antibodies obtained from patients or active immunization animal models of axonal GBS [11,13] has not been reported to induce injury to the intact nerve fibers in experimental animals. This brief review describes some salient recent developments that enhance our understanding of the complex pathobiologic mechanisms involved in anti-glycan antibody-mediated deleterious effects on intact and injured nerve fibers.

Table 1. Classification of Guillain-Barré syndrome

Paralytic forms
- Demyelinating electrophysiology
- Acute inflammatory demyelinating polyradiculoneuropathy (AIDP)
Axonal electrophysiology
- Acute motor axonal neuropathy
- Acute motor-sensory axonal neuropathy
- AIDP with secondary axonal degeneration
Regional or focal paralytic forms
- Fisher syndrome
- Oropharyngeal
Non-paralytic forms
- Sensory ataxic variant
- Acute pandysautonomia

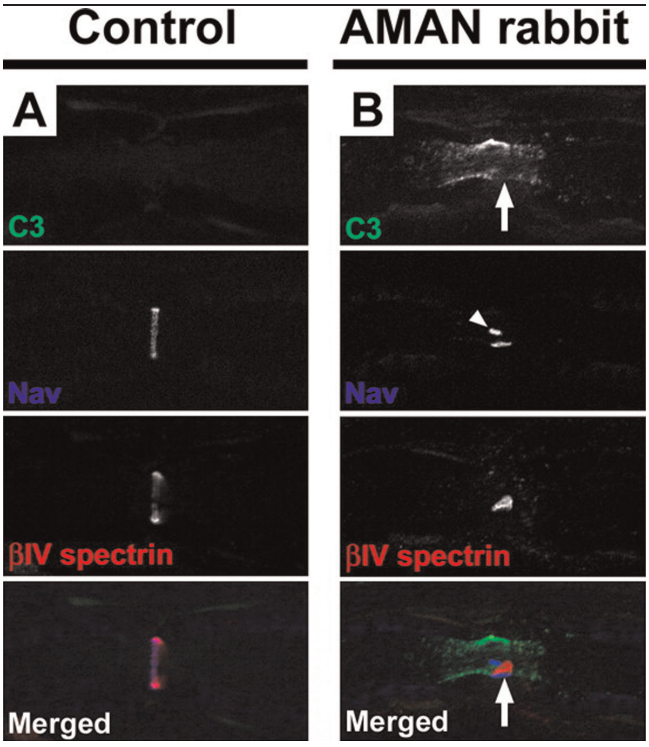
Major recent advances

Several recent observations are beginning to unravel the complexity involved in anti-glycan antibody-associated selective nerve fiber injury seen in different variants of GBS. For example, antibodies against GM1 and GD1a or related minor gangliosides are associated with acute motor axonal neuropathy (AMAN) and anti-GQ1b/GT1a with FS [1-3]. The association of specific anti-glycan antibodies with specific GBS variants had raised an important critique; that is, how do specific anti-glycan antibodies induce selective injury to different nerve fibers (e.g., motor versus sensory) or selective topographical involvement of nerves/pathways despite minor or no differences in the biochemical content of gangliosides in different nerves or nerve fibers? The group led by Kusunoki [22], in a series of studies, have presented a novel concept that some GBS sera/anti-glycan antibodies bind to ganglioside complexes (pairs of gangliosides) but not to individual components of ganglioside in solid-phase assays. The authors propose that antibodies against ganglioside complexes recognize new conformational epitope(s) formed by mixing. Whether or not gangliosides and other glycans that constitute cell surface glycocalyx form complexes or unique conformational epitopes in biological/cellular membranes remains to be determined. If this concept is validated, then this has far-reaching implications and could provide an explanation of how different neuronal/nerve fiber populations could be selectively targeted by specific anti-glycan antibodies despite similar biochemical content of individual major gangliosides. That an individual ganglioside can assume a different conformation/orientation in motor and sensory fibers was supported by data published by our group recently [23]. This study focused on anti-GD1a antibodies in the context of AMAN and selective motor fiber injury. We found that some anti-GD1a antibodies selectively bound to motor but not sensory axons and that different anti-GD1a antibodies had different binding patterns to various chemical derivatives of GD1a (fine specificity). On the basis of data derived from biochemical, immunocytochemistry, computer modeling, and enzymatic studies, we proposed a model in which critical GD1a epitopes recognized by selective motor anti-GD1a antibodies are differentially expressed in motor and sensory nerves. Yet another mechanism was suggested by a study showing that motor nerve terminal injury with anti-GM1 antibodies was enhanced with prior enzymatic treatment with sialidase [24]. Based on these findings, it was proposed that a proportion of GM1 ganglioside is cryptic due to masking by other moieties in the cell membrane, including more complex gangliosides. Overall, this set of studies suggests that selective topographical injury of nerves/nerve fibers in GBS is a complex issue and multiple complementary mechanisms are likely to contribute.

Translational progress has also been made in the area of anti-glycan antibody-mediated complement-dependent nerve fiber injury. The Willison group in Glasgow published a series of studies showing that specific anti-glycan antibodies bind to motor nerve terminals or perisynaptic Schwann cells (or both) and activate complement and this not only disrupts neuromuscular transmission but also can induce degeneration of motor nerve terminals (reviewed in [25]). Susuki *et al*. [17] extended this line of research and examined the role of complement at the nodes of Ranvier in a rabbit model of AMAN. They showed that during the acute phase of limb weakness, deposition of IgG and complement correlates with disruption of voltage-gated sodium channel clusters and paranodal axoglial junctions, the nodal cytoskeleton (Figure 1), and Schwann cell microvilli, all of which stabilize these channels at the nodes. These pathogenic studies led to translational efforts focusing on complement inhibition. Halstead *et al.*, in two separate studies [14,26], show that two inhibitors of C5 (i.e., C5-inhibiting recombinant protein rEV576 and eculizumab [Soliris®; Alexion Pharmaceuticals, Cheshire, CT, USA], a treatment approved by the US Food and Drug Administration for paroxysmal nocturnal hemoglobinuria) prevented physiological and structural damage to motor nerve fibers in preclinical models. Based on these observations, an argument has been put forth for a clinical trial with eculizumab in GBS.

In a multi-investigator collaborative study, Buchwald *et al*. [27] show that anti-glycan (GM1 and GD1a) antibodies can induce various degrees of synaptic blockade (evoked quantal release) at neuromuscular junctions independently of complement. The same antibodies significantly reduced depolarization-induced calcium influx in neurons. This study was conducted with the following clinical observations in view: (a) some patients with axonal GBS recover too rapidly to explain their recovery on the basis of axon degeneration and regeneration, and (b) some subjects (patients or animals with axonal GBS) with

Figure 1. Disruption of voltage-gated sodium (Na_v) channels and related cytoskeletal molecules at nodes of Ranvier in a rabbit model of acute motor axonal neuropathy (AMAN)



All panels show longitudinal sections of ventral roots, and nerve fibers run horizontally. Sections were immunostained with antibodies to C3 (green), Na_v channels (blue), and βIV spectrin (red). **(A)** A normal node of Ranvier in a control rabbit, showing absence of complement deposition and normal distribution of the Na_v channel and βIV spectrin. **(B)** A node of Ranvier in an AMAN rabbit at the acute progressive phase, showing that deposition of complement was associated with disruption of nodal architecture (paranodal axoglial junctions/lengthening) and Na_v channel and βIV spectrin staining. Adapted with permission from Figure 4, Susuki *et al.* [17].

severe clinical weakness were found on autopsy to lack significant pathology that would explain their weakness [28,29]. These experimental studies support the notion that anti-glycan antibody-induced deleterious effects on presynaptic transmitter release is one pathophysiological mechanism of antibody-mediated muscle weakness in AMAN.

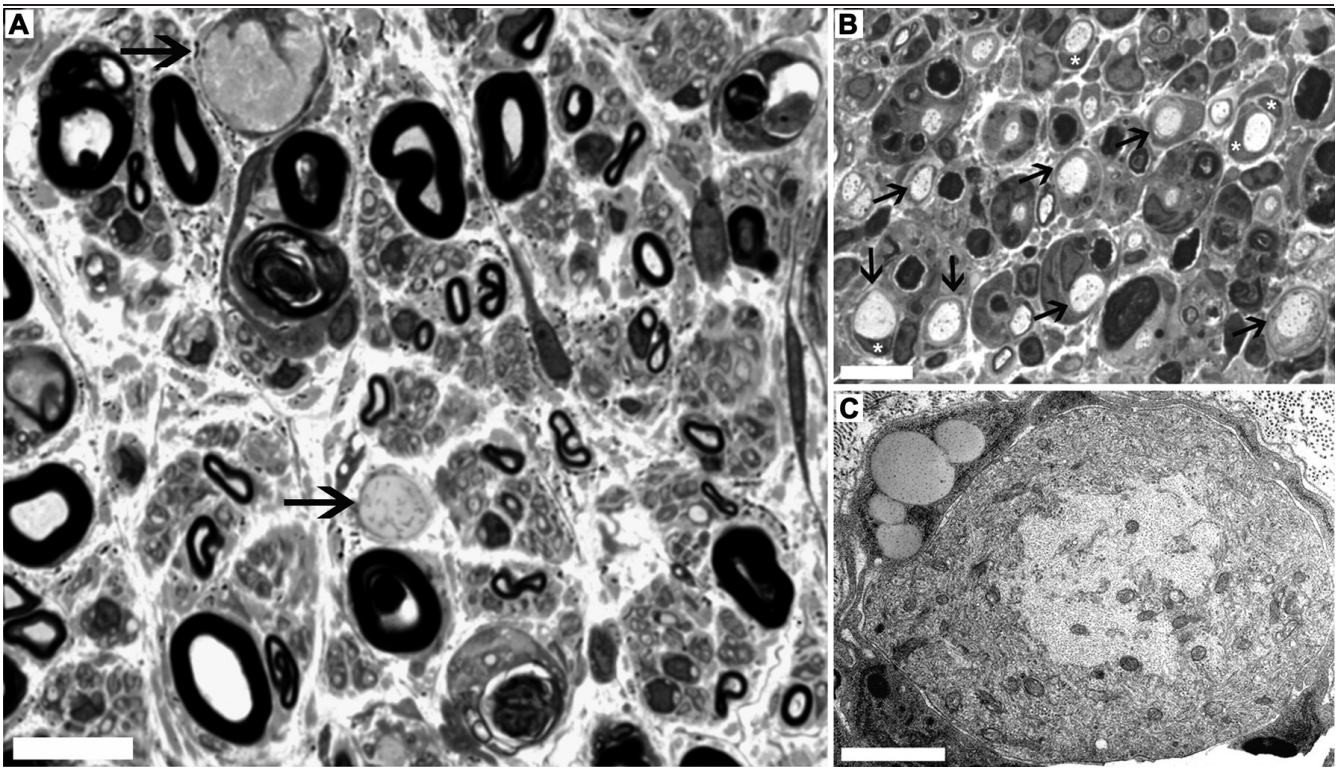
Our group examined, for the first time, the effects of anti-glycan antibodies on peripheral nerve repair/axon regeneration in an animal model [30]. We found that passive transfer of anti-GD1a reactive antibodies severely inhibited axon regeneration after peripheral nervous system injury. In mutant mice with altered ganglioside or complement expression, inhibition by antibodies was mediated directly via GD1a and was independent of complement-induced cytolytic injury. The impaired regenerative responses and ultrastructure of injured peripheral axons mimicked dystrophic and stalled growth cones (Figure 2B,C) typically seen after central nervous system injury. We have also seen dystrophic/stalled growth cones in the sural nerve biopsy of a GBS patient who had poor recovery (Figure 2A). These observations support the hypothesis that inhibition of axon regeneration is one mechanism of poor recovery in GBS patients with anti-glycan antibodies. Furthermore, these studies indicate that circulating factors such as autoimmune autoantibodies can inhibit axon regeneration and affect recovery in patients with GBS.

Future directions

The notion that anti-glycan antibodies are the primary pathogenetic effectors in GBS continues to be contested. The controversy stems mainly from the fact that experimental modeling in different studies has yielded varied results [31,32]. It is not surprising that experimental modeling is not reproducible given that traditionally only enzyme-linked immunosorbent assay has been used to define the specificity of anti-glycan antibodies for further use in experimental studies. Recent work, some of which is outlined above, indicates that anti-glycan antibody-mediated pathogenecity is quite complex, partly due to subtleties involved in the carbohydrate antigen expression on the neural cell surfaces. Furthermore, anti-glycan antibodies with specificity for a ganglioside are not created equally and these antibodies could be different in terms of fine specificity, tissue-binding patterns, affinity/avidity, Fc-based effector functions, and other as-yet-undefined properties. The development of techniques that allow the examination of different characteristics of glycan antigens and anti-glycan antibodies and increasing use of well-defined monoclonal anti-glycan antibodies is very likely to decrease discrepant experimental findings. The characterization of different properties of anti-glycan antibodies is likely to extend into the clinical arena for delineation of well-defined subgroups of GBS patients (or other immune neuropathies associated with anti-glycan antibodies) for clinical outcome research and enrolment in clinical trials as new therapies emerge.

The elucidation of mechanisms underlying anti-glycan antibody-mediated injury to intact nerve fibers has been a fruitful line of research. A direct result of this is the emergence of complement inhibitors as candidates for clinical trials in GBS and other immune neuropathies [14]. It is anticipated that detailed intracellular signaling mechanisms/pathways mediating anti-glycan antibody-induced injury to intact nerve fibers will be identified. The identification of these pathways may allow the

Figure 2. Dystrophic regenerating sprouts in a patient with Guillain-Barré syndrome (GBS) and in an animal model induced with anti-glycan antibodies.



(A) Sural nerve biopsy showing dystrophic sprouts (arrows) in a GBS patient with poor recovery. Reduced density of myelinated fibers and myelin debris are also seen. **(B)** Dystrophic sprouts (arrows) indicating abortive sprouting in a mouse model of sciatic nerve crush and treatment with anti-glycan antibodies. These sprouts are not myelinated and are surrounded by Schwann cell nuclei (*) **(C)** Electron microscopy image showing dystrophic growth cone with a core of neurofilaments and a surrounding pellet with membranous organelles. Scale bars = 10 μm (A), 20 μm (B), and 2 μm (C). Panels (B) and (C) reproduced with permission from Figure 3, Lehmann *et al.* [30]. © Copyright 2007, Society for Neuroscience.

development of rational neuroprotective therapies for use in conjunction with current immunomodulatory treatments to minimize nerve damage during the injury (acute) phase of GBS and other immune neuropathies.

Models of anti-glycan antibody-mediated inhibition of nerve repair have already been established and it is very likely that these models will allow the dissection of molecular mechanisms that prevent successful nerve repair and recovery. Novel therapeutic strategies targeting the signaling pathways that prevent successful regeneration may allow enhanced nerve repair not only in patients with GBS but in those with other neuropathic conditions.

Abbreviations

AMAN, acute motor axonal neuropathy; FS, Fisher syndrome; GBS, Guillain-Barré syndrome.

Competing interests

The authors declare that they have no competing interests.

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References

1. Hughes RA, Cornblath DR: **Guillain-Barre syndrome.** *Lancet* 2005, **366:**1653-66.
2. Van Doorn PA, Ruts L, Jacobs BC: **Clinical features, pathogenesis, and treatment of Guillain-Barre syndrome.** *Lancet Neurol* 2008, **7:**939-50.
3. Willison HJ, Yuki N: **Peripheral neuropathies and anti-glycolipid antibodies.** *Brain* 2002, **125:**2591-625.
4. Sheikh KA, Nachamkin I, Ho TW, Willison HJ, Veitch J, Ung H, Nicholson M, Li CY, Wu HS, Shen BQ, Cornblath DR, Asbury AK, McKhann GM, Griffin JW: **Campylobacter jejuni lipopolysaccharides in Guillain-Barre syndrome: molecular mimicry and host susceptibility.** *Neurology* 1998, **51:**371-8.
5. Yuki N, Yoshino H, Sato S, Shinozawa K, Miyatake T: **Severe acute axonal form of Guillain-Barre syndrome associated with IgG anti-GD_{1a} antibodies.** *Muscle Nerve* 1992, **15:**899-903.

6. Kaida K, Morita D, Kanzaki M, Kamakura K, Motoyoshi K, Hirakawa M, Kusunoki S: **Anti-ganglioside complex antibodies associated with severe disability in GBS.** *J Neuroimmunol* 2007, **182:**212-8.
7. Zhang G, Lopez PH, Li CY, Mehta NR, Griffin JW, Schnaar RL, Sheikh KA: **Anti-ganglioside antibody-mediated neuronal cytotoxicity and its protection by intravenous immunoglobulin: implications for immune neuropathies.** *Brain* 2004, **127:**1085-100.
8. Plomp JJ, Molenaar PC, O'Hanlon GM, Jacobs BC, Veitch J, Daha MR, van Doorn PA, van der Meché FG, Vincent A, Morgan BP, Willison HJ: **Miller Fisher anti-GQ1b antibodies: alpha-latrotoxin-like effects on motor end plates [published erratum appears in *Ann Neurol* 1999, 45:823].** *Ann Neurol* 1999, **45:**189-99.
9. Goodyear CS, O'Hanlon GM, Plomp JJ, Wagner ER, Morrison I, Veitch J, Cochrane L, Bullens RW, Molenaar PC, Conner J, Willison HJ: **Monoclonal antibodies raised against Guillain-Barre syndrome-associated Campylobacter jejuni lipopolysaccharides react with neuronal gangliosides and paralyze muscle-nerve preparations [published erratum appears in *J Clin Invest* 1999, 104:1771].** *J Clin Invest* 1999, **104:**697-708.
10. Buchwald B, Toyka KV, Zielasek J, Weishaupt A, Schweiger S, Dudel J: **Neuromuscular blockade by IgG antibodies from patients with Guillain-Barre syndrome: a macro-patch-clamp study.** *Ann Neurol* 1998, **44:**913-22.
11. Yuki N, Yamada M, Koga M, Odaka M, Susuki K, Tagawa Y, Ueda S, Kasama T, Ohnishi A, Hayashi S, Takahashi H, Kamijo M, Hirata K: **Animal model of axonal Guillain-Barre syndrome induced by sensitization with GM1 ganglioside.** *Ann Neurol* 2001, **49:**712-20.
12. Sheikh KA, Zhang G, Gong Y, Schnaar RL, Griffin JW: **An anti-ganglioside antibody-secreting hybridoma induces neuropathy in mice.** *Ann Neurol* 2004, **56:**228-39.
13. Yuki N, Susuki K, Koga M, Nishimoto Y, Odaka M, Hirata K, Taguchi K, Miyatake T, Furukawa K, Kobata T, Yamada M: **Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barre syndrome.** *Proc Natl Acad Sci U S A* 2004, **101:**11404-9.
14. Halstead SK, Zitman FM, Humphreys PD, Greenshields K, Verschuuren JJ, Jacobs BC, Rother RP, Plomp JJ, Willison HJ: **Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model.** *Brain* 2008, **131:**1197-208.

f1000 Factor 6.0 *Must Read*
Evaluated by Kazim Sheikh 08 May 2008

15. O'Hanlon GM, Humphreys PD, Goldman RS, Halstead SK, Bullens RW, Plomp JJ, Ushkaryov Y, Willison HJ: **Calpain inhibitors protect against axonal degeneration in a model of anti-ganglioside antibody-mediated motor nerve terminal injury.** *Brain* 2003, **126:**2497-509.
16. Halstead SK, O'Hanlon GM, Humphreys PD, Morrison DB, Morgan BP, Todd AJ, Plomp JJ, Willison HJ: **Anti-disialoside antibodies kill perisynaptic Schwann cells and damage motor nerve terminals via membrane attack complex in a murine model of neuropathy.** *Brain* 2004, **127:**2109-23.
17. Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, Hirata K, Baba H, Yuki N: **Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers.** *J Neurosci* 2007, **27:**3956-67.
18. Hafer-Macko C, Hsieh ST, Li CY, Ho TW, Sheikh K, Cornblath DR, McKhann GM, Asbury AK, Griffin JW: **Acute motor axonal neuropathy: an antibody-mediated attack on axolemma.** *Ann Neurol* 1996, **40:**635-44.
19. Hafer-Macko CE, Sheikh KA, Li CY, Ho TW, Cornblath DR, McKhann GM, Asbury AK, Griffin JW: **Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy.** *Ann Neurol* 1996, **39:**625-35.
20. Halstead SK, Humphreys PD, Goodfellow JA, Wagner ER, Smith RA, Willison HJ: **Complement inhibition abrogates nerve terminal injury in Miller Fisher syndrome.** *Ann Neurol* 2005, **58:**203-10.
21. Sheikh KA, Griffin JW: **Variants of the Guillain Barre syndrome: progress toward fulfilling "Koch's postulates".** *Ann Neurol* 2001, **49:**694-6.
22. Ogawa G, Kaida K, Kusunoki S, Ueda M, Kimura F, Kamakura K: **Antibodies to ganglioside complexes consisting of asialo-GM1 and GQ1b or GT1a in Fisher and Guillain-Barre syndromes.** *J Neuroimmunol* 2009, **214:**125-7.
23. Lopez PH, Zhang G, Bianchet MA, Schnaar RL, Sheikh KA: **Structural requirements of anti-GD1a antibodies determine their target specificity.** *Brain* 2008, **131:**1926-39.

f1000 Factor 3.0 *Recommended*
Evaluated by Hugh Willison 03 Jun 2008

24. Greenshields KN, Halstead SK, Zitman FM, Rinaldi S, Brennan KM, O'Leary C, Chamberlain LH, Easton A, Roxburgh J, Pediani J, Furukawa K, Furukawa K, Goodyear CS, Plomp JJ, Willison HJ: **The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice.** *J Clin Invest* 2009, **119:**595-610.
25. Willison HJ, Halstead SK, Beveridge E, Zitman FM, Greenshields KN, Morgan BP, Plomp JJ: **The role of complement and complement regulators in mediating motor nerve terminal injury in murine models of Guillain-Barre syndrome.** *J Neuroimmunol* 2008, **201-202:**172-82.

f1000 Factor 3.0 *Recommended*
Evaluated by Richard Lewis 16 Sep 2008

26. Halstead SK, Humphreys PD, Zitman FM, Hamer J, Plomp JJ, Willison HJ: **C5 inhibitor rEV576 protects against neural injury in an *in vitro* mouse model of Miller Fisher syndrome.** *J Peripher Nerv Syst* 2008, **13:**228-35.
27. Buchwald B, Zhang G, Vogt-Eisele AK, Zhang W, Ahangari R, Griffin JW, Hatt H, Toyka KV, Sheikh KA: **Anti-ganglioside antibodies alter presynaptic release and calcium influx.** *Neurobiol Dis* 2007, **28:**113-21.
28. Griffin JW, Li CY, Macko C, Ho TW, Hsieh ST, Xue P, Wang FA, Cornblath DR, McKhann GM, Asbury AK: **Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barre syndrome.** *J Neurocytol* 1996, **25:**33-51.
29. Susuki K, Nishimoto Y, Yamada M, Baba M, Ueda S, Hirata K, Yuki N: **Acute motor axonal neuropathy rabbit model: immune attack on nerve root axons.** *Ann Neurol* 2003, **54:**383-8.
30. Lehmann HC, Lopez PH, Zhang G, Ngyuen T, Zhang J, Kieseier BC, Mori S, Sheikh KA: **Passive immunization with anti-ganglioside antibodies directly inhibits axon regeneration in an animal model.** *J Neurosci* 2007, **27:**27-34.

f1000 Factor 6.0 *Must Read*
Evaluated by Hugh Willison 12 Feb 2007

31. Paparounas K, O'Hanlon GM, O'Leary CP, Rowan EG, Willison HJ: **Anti-ganglioside antibodies can bind peripheral nerve nodes of Ranvier and activate the complement cascade without inducing acute conduction block *in vitro*.** *Brain* 1999, **122:**807-16.
32. Ilyas AA, Chen ZW: **Lewis rats immunized with GM1 ganglioside do not develop peripheral neuropathy.** *J Neuroimmunol* 2007, **188:**34-8.