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Dysfunction of nodes of Ranvier: A mechanism for anti-ganglioside antibody-mediated neuropathies

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ABSTRACT

Autoantibodies against gangliosides GM1 or GD1a are associated with acute motor axonal neuropathy (AMAN) and acute motor-sensory axonal neuropathy (AMSAN), whereas antibodies to GD1b ganglioside are detected in acute sensory ataxic neuropathy (ASAN). These neuropathies have been proposed to be closely related and comprise a continuous spectrum, although the underlying mechanisms, especially for sensory nerve involvement, are still unclear. Antibodies to GM1 and GD1a have been proposed to disrupt the nodes of Ranvier in motor nerves via complement pathway. We hypothesized that the disruption of nodes of Ranvier is a common mechanism whereby various anti-ganglioside antibodies found in these neuropathies lead to nervous system dysfunction. Here, we show that the IgG monoclonal anti-GD1a/GT1b 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 motor nerve conduction block. Injection of IgG monoclonal anti-GD1b antibody induced nodal disruption predominantly in sensory nerves. In an ASAN 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 ASAN 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 continuous spectrum of AMAN, AMSAN, and ASAN.

Introduction

Recent discoveries of autoantibodies against gangliosides, a group of glycosphingolipids with sialic acid, have provided profound insights into the mechanisms of autoimmune neuropathies (van Doorn et al., 2008; Kaida et al., 2009). The anti-ganglioside antibody-mediated neuropathies are quite diverse in antibody profiles and clinical manifestations. For example, various antibodies can be found in one neuropathy subtype: IgG antibodies to GM1, GD1a, GalNAc-GD1a, or GM1b are detected in acute motor axonal neuropathy (AMAN). In addition, one anti-ganglioside antibody may induce different conditions: IgG anti-GM1 antibodies are associated with both AMAN (predominantly motor involvement) and acute motor-sensory axonal neuropathy (AMSAN) (both sensory and motor involvements) (Griffin et al., 1996a; Yuki et al., 1999). IgG anti-GD1b antibodies are found in the acute sensory ataxic neuropathy (ASAN) or ataxic Guillain-Barré syndrome (Ito et al., 2011; Notturno et al., 2008; Pan et al., 2001). Despite this remarkable diversity, these neuropathies may be regarded as closely related and comprise a continuous spectrum.

How can various anti-ganglioside antibodies induce distinct, but similar types of neuropathies? We hypothesized that these acute immune-mediated neuropathies are caused by the same pathophysiologic mechanism. Furthermore, we hypothesize that disruptions are more likely to occur at areas along the neuron where the axolemma is exposed, i.e. nodes of Ranvier could represent a primary target. Nodes of Ranvier are critical for action potential generation and propagation due to high densities of voltage-gated sodium (Nav) channels enriched

Abbreviations: AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensoty axonal neuropathy; ASAN, acute sensory ataxic neuropathy; Caspr, contactin-associated protein; ChAT, choline acetyltransferase; CMAP, compound muscle action potential; DRG, dorsal root ganglion; MAC, membrane attack complex; MCV, motor nerve conduction velocity; Nav, voltage-gated sodium; P/D, proximal/distal.

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at the nodal axolemma which are responsible for action potential generation (Susuki and Rasband, 2008). Gangliosides GM1, GD1a, or GD1b are highly enriched at or near nodes (for review, see Kaida et al., 2009). In human AMAN and AMSAN, an early pathological feature includes the widening of the nodes of Ranvier with deposition of complement products (Griffin et al., 1996a, 1996b; Hafer-Macko et al., 1996). The complement system plays a central role in immune response to eliminate invading pathogens, although the disruption of a fine balance of complement activation and regulation may cause injury and contribute to the pathogenesis of various diseases (Ramaglia et al., 2008). In an AMAN rabbit model induced by immunization with gangliosides, the IgG anti-GM1 antibodies bind the nodes in the ventral roots, activate the complement pathway, and disrupt the molecular organization of nodes including clustered Nav channels (Susuki et al., 2007). Consistent with these data, in *ex vivo* and *in vivo* transfer models using mutant mice overexpressing a-series gangliosides (e.g. GD1a), a monoclonal IgG antibody reactive with GD1a disrupted the nodes in distal motor nerves via the complement pathway (McGonigal et al., 2010). Thus, it is possible that the complement-mediated nodal disruption is a common mechanism in these anti-ganglioside antibody-mediated neuropathies.

In this study, we address the following questions: 1) can various anti-ganglioside antibodies cause nodal disruption, and 2) are sensory neurons affected by anti-ganglioside antibodies via the same mechanism? Here, we first provide the evidence that IgG anti-ganglioside antibodies can disrupt the nodes in sensory nerve fibers via complement pathway. Our results provide an explanation for the continuous spectrum of AMAN, AMSAN, and ASAN.

Methods

Antibodies

The following primary antibodies were used: FITC-conjugated goat IgG antibodies to C3 component of rabbit or rat complement (Nordic Immunological Laboratories); chicken polyclonal antibody to rabbit membrane attack complex (MAC), kindly provided by Dr. B.R. Lucchesi (University of Michigan Medical School, Ann Arbor, MI); mouse monoclonal antibody to rabbit macrophage (RAM11) (DAKO Cytomation); mouse monoclonal antibody against pan Nav channel (Rasband et al., 1999); guinea pig antibody to Caspr, kindly provided by Dr. J. Black (Yale University, New Haven, CT); rabbit antibody to Caspr (Schafer et al., 2004); rabbit anti-βIV spectrin SD (Berghs et al., 2000); chicken anti-βIV spectrin generated and affinity purified against the same peptide; and goat anti-choline acetyltransferase (ChAT) antibody (Millipore). For intraneural injection, the previously well-characterized mouse monoclonal anti-ganglioside antibodies were used (Lopez et al., 2008; Lunn et al., 2000; Schnaar et al., 2002, summarized in Supplementary Table 1). As control, we used mouse IgG1 and IgG2b that are not reactive to any rat antigens (abcam). AMCA-conjugated goat anti-chicken IgY were from Jackson ImmunoResearch Laboratories. Other fluorescent dye-conjugated secondary antibodies were from Invitrogen.

Intraneural injection

Adult Sprague Dawley rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (80 mg/kg body weight) and

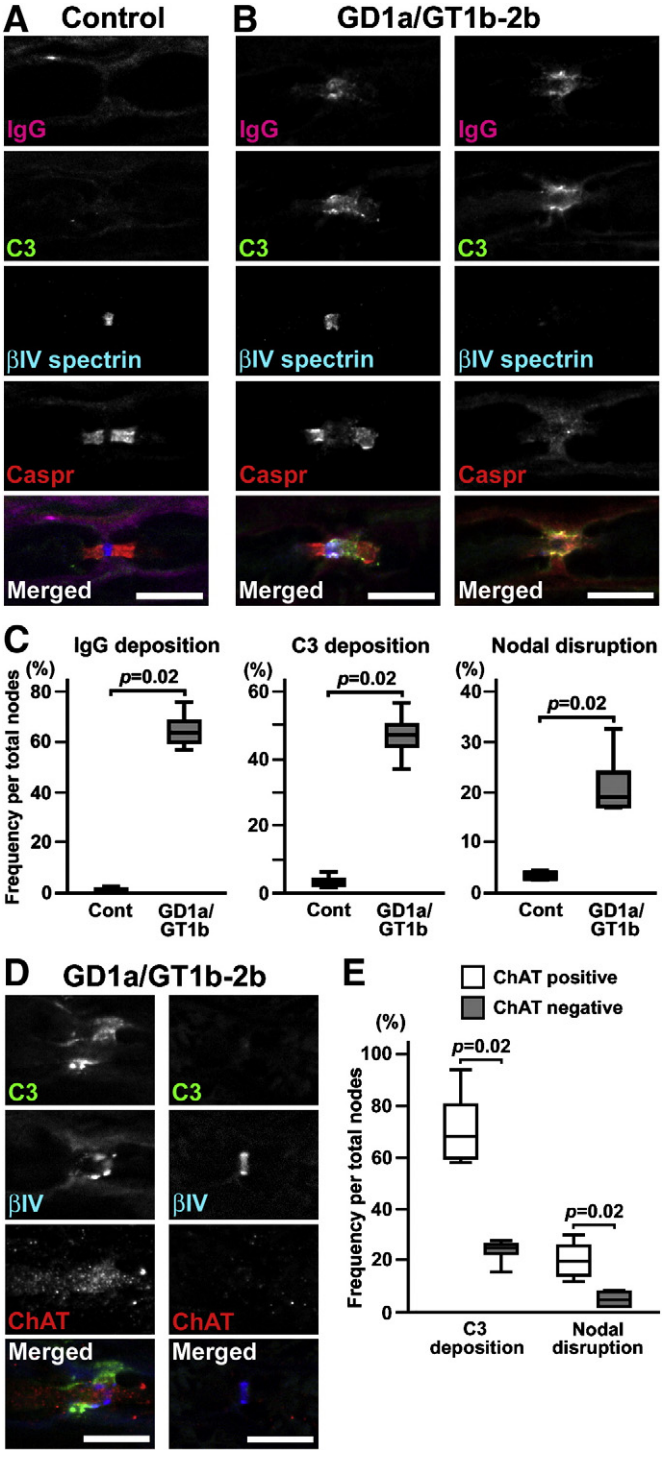

Fig. 1. GD1a/GT1b-2b disrupts predominantly motor nerve nodes via the complement pathway. (A,B) Immunofluorescence analyses in longitudinal sections of rat sciatic nerves injected with control IgG2b or GD1a/GT1b-2b. Nerve fibers run horizontally in all panels. Sections are stained by antibodies to mouse IgG (magenta), C3 component of complement (green), βIV spectrin (blue), and Caspr (red). Control IgG2b weakly binds to the outer surface of myelin, but no C3 deposition is seen (A). Deposition of GD1a/GT1b-2b and C3 is associated with the abnormally lengthened gap between paranodal Caspr clusters with preserved nodal βIV spectrin (B, left column), or with completely damaged βIV spectrin and Caspr clusters (B, right column). (C) Frequency of immune-mediated nodal disruption after repeated injection of GD1a/GT1b-2b in rat sciatic nerves at day 4. The depositions of IgG or C3, or disrupted nodes are significantly more frequent by injection of GD1a/GT1b-2b compared to control IgG2b (n = 4 in each group). For quantification, 200–300 nodes were observed in each rat sciatic nerve. (D) Representative images of nodes in sciatic nerves at day 4 after repeated injection of GD1a/GT1b-2b together with complement. Left column shows disrupted node (shown by βIV spectrin in blue) with C3 deposition (green) in ChAT positive axon (red). Right column shows preserved node in ChAT negative axon. (E) Quantitation of C3 deposition and nodal disruption in ChAT positive or negative nodes at day 4. Data were collected from four animals. Scale bars = 10 μm (A, B, D).