Anti-ganglioside antibodies can bind peripheral nerve nodes of Ranvier and activate the complement cascade without inducing acute conduction block \textit{in vitro}

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Summary
The neurophysiological effects of nine neuropathy-associated human anti-ganglioside antisera, three monoclonal antibodies to ganglioside GM1 (GM1) and of the cholera toxin B subunit (a GM1 ligand) were studied on mouse sciatic nerve \textit{in vitro}. GM1 antisera and monoclonal antibodies from patients with chronic motor neuropathies and Guillain–Barre syndrome, and GQ1b/disialosyl antisera and monoclonal antibodies from patients with chronic ataxic neuropathies and Miller Fisher syndrome were studied. \textit{In vitro} recording, for up to 6 h, of compound nerve action potentials, latencies, rise times and stimulus thresholds from isolated desheathed sciatic nerve was performed in the presence of anti-ganglioside antibodies and fresh human serum as an additional source of complement. No changes were observed over this time course, with 4–6 h values for all electrophysiological parameters being within 15\% of the starting values for both normal and antibody containing sera and for the cholera toxin B subunit. Parallel experiments on identically prepared desheathed nerves performed with 0.5 nM saxitoxin led to complete conduction block within 10 min of application. Under identical conditions to those used for electrophysiological recordings, quantitative immunohistological evaluation revealed a significant increase in IgM (immunoglobulin M) deposition at nodes of Ranvier from 5.3 ± 3.1\% to 28.7 ± 8.4\% (mean ± SEM) of desheathed nerves exposed to three normal and three antibody containing sera, respectively ($P < 0.03$). Complement activation was seen at 100\% of normal and 79\% of disease-associated IgM positive nodes of Ranvier. These data indicate that anti-ganglioside antibodies can diffuse into a desheathed nerve, bind to nodes of Ranvier and fix complement \textit{in vitro} without resulting in any overt physiological deterioration of the nerve over 4–6 h. This suggests that the node of Ranvier is relatively resistant to acute anti-ganglioside antibody mediated injury over this time scale and that anti-ganglioside antibodies and the cholera toxin B subunit are unlikely to have major direct pharmacological effects on nodal function, at least in comparison with the effect of saxitoxin. This \textit{in vitro} sciatic nerve model appears of limited use for analysing electrophysiological effects of anti-ganglioside antibodies on nerve function, possibly because its short-term viability and isolation from circulating systemic factors do not permit the evolution of an inflammatory lesion of sufficient magnitude to induce overt electrophysiological abnormalities. \textit{In vivo} models may be more suitable for identifying the effects of these antibodies on nerve conduction.

Keywords: peripheral neuropathy; conduction block; autoantibodies; gangliosides; complement

Abbreviation: CANOMAD = chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins and disialosyl antibodies
Introduction

Gangliosides are sialic acid containing glycolipids expressed in high abundance in the nervous system (Ledeen, 1985; Thomas and Brewer, 1990; Svennerholm, 1994). The extracellular display of their sialylated oligosaccharide core makes them potential target antigens for pathogenic autoantibodies in many forms of peripheral neuropathy associated with serum anti-ganglioside antibodies, including multifocal motor neuropathy, paraproteinaemic neuropathy, Guillain–Barre syndrome and the variant Miller Fisher syndrome (Freddo et al., 1986; Yuki et al., 1990; Pestronk, 1991; Ropper, 1992; Chiba et al., 1993; Willison et al., 1993a; Willison and Kennedy, 1993; Willison, 1994; Kornberg and Pestronk, 1995). Multifocal motor neuropathy and motor forms of Guillain–Barre syndrome are associated with anti-GM1 (IgM) and IgG antibodies, respectively (Pestronk et al., 1988; Yuki et al., 1990), whereas ataxic neuropathies (including the CANOMAD phenotype—chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins and disialosyl antibodies; Willison et al., 1996) and Miller Fisher syndrome are associated with anti-GQ1b (and other NeuAc(α2–8)NeuAc linked disialosyl gangliosides, including GT1a, GT1b, GD3 and GD1b, where NeuAc = N-acetylgalactosamine), IgM and IgG antibodies, respectively.

The pathogenic relationship between these peripheral neuropathies and their associated anti-ganglioside antibodies is widely debated. Debunked clinical and epidemiological observations support the notion of a pathogenic role for these antibodies (Ilyas et al., 1988; Pestronk et al., 1988; Pestronk et al., 1990; Kornberg and Pestronk, 1995). However, proving this in experimental models of disease has been technically difficult and controversial. In relation to anti-GM1 antibodies, passive transfer of antibody into animal nerves has been shown to cause electrophysiological abnormalities and demyelination in some studies (Santoro et al., 1992), but not in others (Harvey et al., 1995). Similarly, incubation of isolated nerve preparations in vitro with anti-GM1 antibodies has produced acute conduction block of myelinated nerve fibres in some studies (Arasaki et al., 1993; Takigawa et al., 1995), but not others (Hirota et al., 1997). To date, there have been no clearly positive reports of clinical neuropathy in experimental animals induced by immunization with GM1, an experiment viewed by many as representing cardinal proof of antigen specific autoimmunity (McFarlin, 1990). However, GD1b immunization has produced ataxic neuropathy in rabbits, suggesting that this condition can be fulfilled for some ganglioside antigens (Kusunoki et al., 1996).

In immunohistological studies using anti-GM1 sera (Freddo et al., 1986; Santoro et al., 1990; Gregson et al., 1991; Harvey et al., 1995; Kusunoki et al., 1997) and monoclonal antibodies (Molander et al., 1997; O’Hanlon et al., 1998), binding of antibodies to peripheral nerve structures, including nodes of Ranvier, has been demonstrated. Thus, it is theoretically plausible that, under appropriate pathophysiological circumstances, anti-GM1 antibodies should be capable of mediating nodal damage and hence electrophysiological failure of nodal conduction.

Electrophysiological effects of anti-GQ1b and anti-disialosyl antibodies associated with the CANOMAD phenotype have not been previously studied in isolated peripheral nerve segments but have been shown to induce electrophysiological abnormalities in the mouse phrenic nerve hemidiaphragm preparations (Roberts et al., 1994; Willison et al., 1996; Plomp et al., 1999). In immunofluorescence studies on mouse nerve, an anti-disialosyl antibody cloned from a CANOMAD patient was shown to bind nodes of Ranvier and other myelinated peripheral nerve structures (Willison et al., 1996). Clinical electrophysiological studies have also shown that peripheral nerve motor conduction velocities are frequently reduced in CANOMAD patients (Willison et al., 1993b) and may be abnormal in Miller Fisher syndrome (Fross and Daube, 1987). There is thus good circumstantial evidence to suggest that both Miller Fisher syndrome and CANOMAD sera might induce electrophysiological abnormalities in peripheral nerve conduction.

In an attempt to clarify the role of anti-GM1 and anti-disialosyl ganglioside antibodies in mediating acute experimental conduction block in vitro, we investigated the binding and complement fixing capacity of anti-ganglioside and control sera and monoclonal antibodies to nodes of Ranvier, in conjunction with physiological recordings from isolated mouse sciatic nerve preparations under a range of experimental conditions.

Material and methods

**Gangliosides antisera and monoclonal antibodies**

Sera were collected from patients with autoimmune neuropathies and normal control subjects and stored at −70°C until use (as approved by the Southern General Hospital NHS Trust Ethics Committee). Screening for antibodies using a panel of gangliosides comprising GM1, GM2, GM3, GD1a, GalNAc-GD1a, GD1b, GD3, GT1a, GT1b and GQ1b, was performed by ELISA (enzyme-linked immunosorbent assay) and thin layer chromatography overlay as previously described (Willison et al., 1996). We studied nine samples of human sera or plasma (see Table 1) positive for anti-ganglioside antibodies taken from the following groups of patients: (i) three patients with multifocal motor neuropathy and anti-GM1 IgM antibodies; (ii) three patients with paraproteinaemic and anti-disialosyl IgM antibodies; (iii) three patients with Guillain–Barre syndrome or Miller Fisher syndrome and anti-GM1 or GQ1b IgG antibodies. We also examined three monoclonal IgM anti-GM1 antibodies cloned from peripheral blood lymphocytes of multifocal motor neuropathy patients with elevated titres of anti-GM1 antibody (Paterson et al., 1995). In order to maintain
In vitro effects of anti-ganglioside antibodies

Table 1  Binding properties and origin of anti-ganglioside antibodies

<table>
<thead>
<tr>
<th>Code</th>
<th>Diagnosis</th>
<th>Ab class</th>
<th>GM1</th>
<th>GM2</th>
<th>GD1b</th>
<th>GQ1b</th>
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<tr>
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<tr>
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Monoclonal antibodies*

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<th>GM2</th>
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</tr>
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</tr>
<tr>
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<td>IgG</td>
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<td>250</td>
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</tbody>
</table>

neg = reactivity undetectable; MMN = multifocal motor neuropathy; PPN = paraproteinaemic neuropathy; GBS = Guillain–Barre syndrome; MFS = Miller Fisher syndrome; Ab = antibody. *Titre is expressed as the reciprocal of the antibody concentration that gave half maximal binding.

Experimental consistency throughout these studies, a single human normal control serum which did not contain anti-ganglioside antibodies was used as a source of complement and confirmed as having classical pathway (CH50 = 120 units/ml) and alternate pathway (CH50 = 71 units/ml) complement levels within the normal ranges. For the IgM and complement deposition studies at nodes of Ranvier, three additional sera from normal subjects were used as controls.

Electrophysiological recordings from mouse sciatic nerve

Male BALB/c mice (weighing 25–30 g) were killed by inhalation of carbon dioxide, according to United Kingdom Home Office guidelines. A 5–7 cm length of sciatic nerve, comprising proximal and distal regions, was dissected out and desheathed under a dissecting microscope (×50). The preparation was then mounted into a recording chamber consisting of three cylindrical compartments of 1 ml capacity each. Electrical isolation between the compartments was achieved using Vaseline. The two external compartments were filled with physiological buffer 154 mM NaCl, 5 mM KCl, 1.25 mM MgCl2, 11 mM glucose, 5.46 mM HEPES (N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]), 1.8 mM CaCl2, pH 7.4; the central compartment contained the test reagents. Before each experiment the solution was saturated with 100% O2 for 30 min. Standard extracellular recording techniques were employed to record compound nerve action potential events. Pellet type silver electrodes were dipped into each of the compartments of the recording chamber with stimulation taking place between the central and one of the external compartments. Recordings were obtained from the central pool. The ground electrode was dipped into the central pool. A Grass S48 stimulator was used to supply electrical impulses (0.4 Hz, 0.04 ms duration) supramaximally via a stimulus isolation unit, model SIU 5A (Grass Instrument Co., Quincy, Mass., USA). Signals were amplified with a CED 1902 transducer, digitized by an analogue-digital converter CED 1401 and analysed with a microcomputer program (Dempster, 1988). In each experiment the amplitude, latency, rise time and threshold of the obtained action potentials were measured.

Before the addition of test compounds, the sciatic nerve preparation was incubated in physiological buffer for 45 min under constant supramaximal stimulation to demonstrate viability in the preparation and consistency in the recordings. All test solutions were dialysed against physiological buffer at pH 7.4. Osmotic pressure was maintained at 320 ± 10 mOsm/kg. The pH and osmotic pressure were rechecked at the beginning and end of all recordings since in preliminary experiments we noted markedly deleterious effects of high and low pH and osmolarity on the physiological stability of the preparation. All test sera and plasma, including the normal serum used as a source of supplementary complement, were diluted 1/10 in physiological buffer. Monoclonal anti-GM1 antibodies and normal human IgM were diluted in physiological buffer and used at a concentration of 20 µg/ml. Cholera toxin B subunit (Sigma C9903) a high affinity ligand for GM1 (Holmgren, 1981) was used at 20 µg/ml, and the sodium channel blocker, saxitoxin at 0.5 nM concentration.

All the initial experiments were carried out at a room temperature of 20 ± 2°C. In later experiments, two anti-GM1 sera (GA and JW) were studied at 37°C, maintained using a circulating constant temperature bath. Nerves were incubated with test sera for 180–240 min, with supplementary normal serum as a fresh source of active complement being applied for a further 90–120 min. In the later experiments performed at 37°C, fresh serum was applied together with the two anti-GM1 sera from the start of the experimental recordings. Electrophysiological studies were performed with the operator blinded to the nature of the test compound.
**Immunofluorescence studies on nodal IgM and complement deposition in sciatic nerve preparations**

Male BALB/c mice (15–20 g) were killed by cervical dislocation and the sciatic nerves were dissected and desheathed as for the electrophysiology preparation described above. The nerves were incubated for 3–16 h at room temperature or at 37°C in 0.1 ml of pre-oxygenated physiological buffer containing the experimental sera (diluted 1/10) or monoclonal antibodies (20 µg/ml). Normal sera or IgM purified from normal sera were used as controls. The same fresh human serum (diluted 1/10) as was used in the electrophysiological studies was the source of complement.

In initial studies, the duration and temperature of incubation with antibody was observed to influence the extent of nodal IgM and complement deposition. We therefore followed a standardized method for quantifying the number of IgM and C3c (complement 3c) positive nodes and analysed three normal and three anti-ganglioside antibody containing heat-inactivated sera according to this method as described below.

All six samples were coded and studied blind. Sera were incubated with a 5–10 mm length of desheathed sciatic nerve for 3 h at room temperature (the time/temperature profile for the majority of electrophysiological recordings). Nerves were then washed twice for 5 min by vortexing in fresh buffer. Nerves, in a droplet of buffer, were then teased into single fibres using 25 gauge needles on 3-aminopropyltriethoxy-silane coated slides and left to air dry at room temperature. Slides were stained immediately or stored at –20°C. The fibres using 25 gauge needles on 3-aminopropyltriethoxy-silane coated slides and left to air dry at room temperature.

Slides were stained immediately or stored at –20°C. The fibres using 25 gauge needles on 3-aminopropyltriethoxy-silane coated slides and left to air dry at room temperature. Slides were stained immediately or stored at –20°C.

Slides were incubated for 1 h at 4°C in PBS (phosphate buffer solution) containing 10% lamb serum, rhodamine-labelled anti-human IgM (Southern Biotechnology Associates; diluted 1/300), and fluorescein labelled antibody to the C3c complement activation product (Dako; diluted 1/200). Some slides were also stained with fluorescein-labelled cholera toxin B subunit (0.5 µg/ml; Sigma), or the nuclear stain, DAPI (4,6-diamidino-2-phenylindole, 0.15 µg/ml; Vector Laboratories), to help orientate Schwann cell architecture around nuclei and paranodal regions. Slides were rinsed in cold PBS, mounted in Citifluor antifade (Citifluor Products, Canterbury, UK), ringed with nail-varnish to minimize drying and stored at 4°C in the dark prior to viewing. All experiments were duplicated.

**Imaging**

Slides were viewed on a monitor via a Sony colour 3CCD camera mounted on to a Zeiss Axioplan fluorescent microscope, linked to a PC driven image archiving system (Sirrius VI; Optivision). The threshold of the image acquisition equipment was set up such that the control level of staining obtained with secondary antibodies alone was zero. For the fluorescein (C3c staining) and rhodamine (IgM) channels, images were then integrated to obtain unambiguous signal intensities for counting nodes. On each slide, 25 consecutive nodes were identified at random under phase microscopy and then scored either positive or negative for IgM and C3c. Bitmap processing and annotation were conducted on PhotoMagic and Windows Draw (both by Micrographx Inc.). Images were printed directly using a photographic-quality colour printer (Kodak ColourEase).

**Statistical analysis**

The electrophysiological data are presented as mean ± standard error mean. All measurements in the sciatic nerve extracellular recordings were normalized according to the control values at time 0 min, without the presence of antibody. Statistical significance was tested with non-parametric statistics (Mann–Whitney’s test) and χ² test as appropriate. All analyses were performed using the Minitab 10.5 for Windows statistical package. Immunofluorescence data are presented as mean ± standard error mean and also analysed using a one-tailed Student’s t test.

**Results**

The effect of nerve desheathment on antibody and saxitoxin penetration

In early electrophysiological recordings from isolated ensheathed nerves during which no effects of sera were observed (data not shown) we were concerned that the antibody may be penetrating poorly. From immunohistological studies we established that the antibody failed to penetrate at detectable levels into a sheathed sciatic nerve under the incubation conditions and time course of the electrophysiological recordings (data not shown). We also compared the rate of onset of saxitoxin (a sodium channel blocking agent) action on compound nerve action potential in sheathed and desheathed nerve (Fig. 1) and noted clear differences between the two preparations, indicating that the nerve sheath acts as a significant mechanical diffusion barrier...
to small molecules delivered to the extraneural environment. Therefore, all subsequent incubations were carried out on desheathed nerve preparations.

**Electrophysiological recordings from desheathed sciatic nerve**

We first applied fresh normal human serum (as a control for the test sera) and normal human IgM (as a control for the monoclonal antibodies) to the preparation at room temperature for periods up to 4 h and observed no significant changes in any of our recorded parameters (Fig. 2 and Table 2). We also observed no effect with cholera toxin B subunit, a high affinity multimeric ligand for GM1, applied under similar conditions. When we applied test sera (human anti-ganglioside IgM and IgG containing sera GA and monoclonal antibodies), we again observed no significant changes from baseline levels of the amplitude of the compound nerve action potential, latency, rise time or stimulus. In order to ensure sufficient complement was available (if required to induce an effect) we added a fresh source at 3 or 4 h for a further 90–120 min and again did not observe any changes in recorded parameters (Table 2).

In order to assess whether any effects on the electrophysiology might become evident at normal physiological temperatures and bearing in mind that complement activation is maximal at 37°C, we made further electrophysiological recordings under these incubation conditions. We observed in preliminary experiments that nerve viability (as defined by the electrophysiological stability of our recorded parameters) began to deteriorate after 4 h at 37°C (data not shown) and we thus restricted our recording period to 3 h, plus the 45 min stabilization period prior to antibody and complement application. Under these conditions, which were considered optimal in terms of antibody binding and complement fixation for observing any potentially deleterious effects on nerve conduction in this experimental paradigm, we again observed no effects of the anti-GM1 sera from GA and JW (Fig. 3).

### Immunoglobulin and complement deposition following in vitro incubation with sera

In order to determine whether nerves incubated with sera had immunoglobulin and complement deposition at nodes of Ranvier, we performed an immunofluorescence analysis of teased nerve fibres, following incubation using the same conditions as in the physiological experiments. These studies were restricted to sera containing anti-ganglioside IgM due to the very high background signals observed when immunostaining for IgG deposits, when either normal or disease-associated sera were used at dilutions of 1/10.

In initial experiments, nodal immunoglobulin and complement deposits were observed with both normal and anti-ganglioside antibody containing sera and IgM monoclonal antibodies after 3 h and 16 h incubations at room temperature or 37°C. The frequency of IgM and complement deposition at nodes appeared greater with the disease-associated sera or monoclonal antibodies than with the control sera or control IgM, particularly after longer incubations and warmer temperatures (e.g. overnight at 37°C compared with 4 h at room temperature). Representative images are shown in Fig. 4 and show both serum IgM (from case GA) and monoclonal IgM (Wo1, cloned from case JW) and the C3c complement product deposited at nodes of Ranvier in nerves incubated overnight at 37°C. In order to confirm the differences between disease and control sera, we performed an observer blinded, quantitative immunofluorescence analysis (as described in Material and methods) of 50 nodes per serum sample under incubation conditions of equal duration and temperature to most of the electrophysiological recordings. After incubation of desheathed segments of nerve for 3 h at room temperature with the three normal control sera (heat inactivated and supplemented with the normal serum used throughout as a constant complement source), IgM deposits were observed at 0 out of 50 (0%), 2 out of 50 (4%) and 6 out of 50 (12%) nodes (mean 5.3% ± 3.1% SEM). Under the same incubation conditions, the anti-ganglioside antibody containing sera GA, JW and CH were associated with IgM deposits at 18 out of 50 (36%), 6 out of 50 (12%) and 19 out of 50 (38%) nodes (mean 28.7% ± 8.4% SEM). These data (Fig. 5) are significantly different at the 5% level ($P < 0.03$, one-tailed $t$ test). Of the IgM positive nodes, 100% of those incubated with normal sera and 79% of those incubated with disease sera were also positive for the C3c complement product.

One of the anti-GM1 sera (GA) and one of the monoclonal antibodies (Wo1) gave rise to antibody and complement deposits at over 50% of nodes of Ranvier in some teased fibre preparations after 16 h incubation at 37°C. Hence, serum from GA and JW (from whom the monoclonal antibody Wo1 was derived) were selected for the 37°C electrophysiological recordings shown in Fig. 3.

### Discussion

This study has examined the acute physiological effects of antibodies to GM1 and GQ1b (and structurally related...
Fig. 3 Electrophysiological recordings performed at 37°C on desheathed sciatic nerve exposed to anti-GM1 IgM sera from two patients (GA, JW). Points are means of eight experiments (GA: n = 4, JW: n = 6). SEM are indicated by error bars unless they were smaller than the symbols. Additional fresh human serum was added at time zero as a source of complement and was also used as the control serum.

Table 2 Electrophysiological parameters recorded from the desheathed sciatic nerve

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amplitude (mV)</th>
<th>Latency (ms)</th>
<th>Rise time (ms)</th>
<th>Threshold (V)</th>
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<tbody>
<tr>
<td></td>
<td>Time zero</td>
<td>End of incubation</td>
<td>Time zero</td>
<td>End of incubation</td>
</tr>
<tr>
<td></td>
<td>(absolute values)</td>
<td>(% time zero)</td>
<td>(absolute values)</td>
<td>(% time zero)</td>
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<tr>
<td>Control materials</td>
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<tr>
<td>NS (n = 4)</td>
<td>2.0 ± 0.1</td>
<td>99.4 ± 1.4</td>
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<td>IgM (n = 4)</td>
<td>3.8 ± 1.0</td>
<td>104.9 ± 2.0</td>
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<td>CT (n = 2)</td>
<td>3.0 ± 0.3</td>
<td>99.9 ± 2.5</td>
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<td>103.3 ± 3.3</td>
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<td>GA (n = 2)</td>
<td>3.3 ± 0.9</td>
<td>120.6 ± 0.7</td>
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<td>JW (n = 6)</td>
<td>5.7 ± 2.1</td>
<td>99.9 ± 7.0</td>
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<td>PS (n = 4)</td>
<td>2.7 ± 0.8</td>
<td>103.8 ± 3.1</td>
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<td>CH (n = 8)</td>
<td>2.8 ± 0.3</td>
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<td>VP (n = 3)</td>
<td>2.5 ± 0.3</td>
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<td>BK (n = 4)</td>
<td>3.4 ± 1.4</td>
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<td>2.2 ± 0.5</td>
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<td>AS (n = 4)</td>
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<td>92.8 ± 4.1</td>
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Test compounds were applied at room temperature for up to 4 h, followed by the addition of fresh serum as a source of complement for up to a further 2 h. NS = normal serum; IgM = normal human IgM; CT = cholera toxin B subunit.
In vitro effects of anti-ganglioside antibodies

Fig. 4 Deposition of antibody and complement in mouse sciatic nerve teased-fibre preparations. Phase-contrast images (panels A, D and G) of positively stained nodes of Ranvier (arrows). GA anti-GM1 IgM serum treated nerve (A–C) was stained with fluorescein-labelled cholera toxin B subunit (B) which binds the paranodal myelin and for human IgM deposits (C) which are deposited at the nodal gap. Wo1 anti-GM1 monoclonal antibody treated nerve (D–F) was stained with fluorescein labelled cholera toxin B subunit (E) and for human IgM deposits (F) which occupy a nodal and paranodal distribution. Wo1-treated nerve (G–I) was stained for complement C3c (H) and human IgM (I) which again occupy a nodal and paranodal distribution. Scale bar = 20 µm; ×100, under oil.

gangliosides) in the presence of complement on nerve conduction in an isolated sciatic nerve organ bath preparation over 6 h. No abnormal effects were observed despite the fact that antibody and complement products were being deposited, albeit possibly not at saturating levels, at a proportion of nodes of Ranvier during the time course of these recordings. These data infer that the node of Ranvier is relatively resistant to complement-dependent anti-GM1/GQ1b antibody mediated injury over this time frame. However, it remains possible that the node may be sensitive to more prolonged or aggressive injury mediated via these antibodies.

Previous electrophysiological studies on anti-ganglioside
Antibody mediated nerve injury have shown divergent findings. Studies of antibody injected into rat nerve showed that human GM1 antisera produced acute conduction block (Santoro et al., 1992; Uncini et al., 1993). In contrast, Harvey et al. (1995) failed to induce conduction block after intraneural injection of human anti-GM1 Ig into rat tibial nerves and histological examination of the injected nerves did not show demyelination despite the binding of the anti-GM1 antibody to the nodes of Ranvier. This latter study is entirely consistent with our findings. In in vitro studies, a minor reduction of compound nerve action potential amplitudes in desheathed rat sciatic nerve preparation has been reported with human and rabbit GM1 antisera (Arasaki et al., 1993). Takigawa et al. (1995) also found that rabbit anti-GM1 antibodies increased the potassium current elicited by step depolarization, and in the presence of active complement blocked sodium channels irreversibly. However, another recent study has shown that application of high titres of human and rabbit GM1 antisera did not cause acute conduction block or block sodium channels (Hirotta et al., 1997).

What are the possible explanations for these discrepancies? Technical factors are likely to be important: our experiments were conducted after extensive preliminary studies aimed at creating a highly stable nerve preparation for the duration of the recordings (Hubbard et al., 1968). We observed profoundly negative effects on the compound nerve action potential even with small alterations in incubation bath pH, osmolarity and ionic strength. Therefore, we controlled all these variables. In addition, tissue desheathment and appropriate placement of the desheathed nerve on the electrodes in the recording bath were critical in order to ensure a stable recording preparation; the stability of the preparation was always monitored for 45 min prior to the application of test substances. We also repeated all electrophysiological experiments on at least three occasions to accommodate any experimental variability and limited the duration of the recording to a maximum of 6 h.

Differences in the sources and fine specificities of the anti-ganglioside antibodies could also be relevant. Immunohistological studies show that different anti-GM1 antibodies are highly diverse in their peripheral nerve staining patterns, including the presence or absence of binding to nodes of Ranvier (O’Hanlon et al., 1996, 1998). Similarly there are species differences in the distribution and immunoreactivity of GM1 and other gangliosides, including differences in peripheral nerve between rat and mouse (O’Hanlon et al., 1996). For these reasons we checked for the binding of antibody and complement in the same preparations from which recordings had been made and observed nodal binding in a proportion of fibres and at a mean level above that seen with normal control sera.

The route by which the antibody penetrates the nerve is an important consideration in these experiments. Organ bath preparations have advantages over in vivo models in that direct application of a fixed antibody concentration to desheathed preparations can be studied. In studies on the in vitro phrenic nerve hemidiaphragm, the effects of anti-GQ1b ganglioside antibodies can be observed within 1–2 h of application (Robert et al., 1994; Plomp et al., 1999); hence, the in vitro approach to sciatic nerve physiology over a similar time course should also be an appropriate method for observing changes in nodal function induced by the same types of antibodies. However, this was not the case.

Despite our negative electrophysiological findings, our immunodeposition studies show the node of Ranvier is capable of binding antibodies and fixing complement. It is likely that if we were able to conduct longer term recordings under experimental circumstances in which nodes are exposed to prolonged pro-inflammatory insults, we would be able to detect electrophysiological failure of nodal conduction. The collective data from clinical studies still suggests that these antibodies are likely to be pathogenic. However, more extensive studies than the acute in vitro electrophysiological model described here will be necessary to convincingly demonstrate that anti-ganglioside antibodies play a role, on their own or in association with other undetermined factors, in causing conduction block.

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