

Research Article

High-titre IgM anti-sulfatide antibodies in individuals with IgM paraproteinaemia and associated peripheral neuropathy

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Summary The common association between monoclonal gammopathy and peripheral neuropathy was studied in seven patients with demyelinating polyneuropathy and IgM paraproteinaemia. Plasma samples from these individuals were thoroughly tested for antiperipheral nerve myelin (PNM) antibodies and then screened for glycoprotein and glycolipid reactivity by western immunoblotting and thin-layer chromatography (TLC) immunostaining. Three of the seven samples showed strong IgM anti-PNM and antisulfatide (GalS) antibody reactivity. Two of these three plasma samples showed extraordinarily high antisulfatide IgM antibody titres, ranging from 1×10^4 to 1×10^6 arbitrary units/L. These same samples also showed intense myelin staining of sciatic nerve sections (paraffin and cryostat) and teased nerve fibres. No axonal immunoreactivity was observed. These results suggest that high titre IgM antisulfatide antibodies may play a pathogenetic role in the immune demyelination associated with IgM paraproteinaemia.

Key words: antibodies, demyelination, immunoglobulin M paraproteinaemia, peripheral neuropathy, sulfatide.

Introduction

Monoclonal gammopathy is frequently associated with peripheral neuropathy. A common finding is the presence of a monoclonal antibody (M protein), which reacts with one or more myelin constituents.^{1,2} The lymphoproliferative B cell disorders associated with peripheral neuropathy include IgM, IgG, or IgA monoclonal gammopathies and multiple myeloma, Waldenström's macroglobulinaemia and plasmacytoid B cell lymphoma and chronic lymphocytic leukaemia. Among the monoclonal gammopathies, approximately 60% are of the IgG type, 10–20% of IgM and the rest of the IgA type.

Approximately 50% of patients with monoclonal gammopathy develop neuropathy. The most common type is a predominantly distal symmetrical polyneuropathy, with demyelination the most common pathology. It has been determined that approximately 50% of these patients contain M proteins that react against myelin, specifically against individual carbohydrate epitopes in peripheral myelin glycolipids and glycoproteins.^{1,2}

Raised antibody titres directed against the acidic myelin glycolipid GalS have been commonly found in patients with sensory neuropathy.³ However, investigators have also demonstrated high titre anti-GalS antibodies in sera from patients with demyelinating motor neuropathies such as Guillain-Barré syndrome (GBS).⁴

The aim of the present study was to determine whether there is a link between raised anti-GalS IgM antibody titres and the presence of IgM paraproteinaemic demyelinating neuropathy (IgMPDN). This was carried out by initially screening plasma samples from patients with IgMPDN for peripheral nerve myelin (PNM) by ELISA. To determine the specific immunoreactivity of the PNM constituents, the plasma samples were then reacted with differentiated glycolipids and glycoproteins by using TLC-immunostaining and western immunoblotting. Titres of anti-GalS IgM antibody were determined by ELISA. The peripheral nerve tissue (PNT) binding pattern of antibody was also determined by indirect immunofluorescence on cryostat and paraffin sections and teased nerve fibre preparations.

Materials and Methods

Plasma samples

Thirty-one samples were obtained from individuals with peripheral neuropathy and other neurological diseases and were subdivided as shown in Table 1.

Negative controls for the PNM and GalS ELISA comprised 48 and 24 plasma samples, respectively, from healthy blood donors without neurological symptoms.

Peripheral nerve myelin ELISA

Purification of PNM was carried out as previously described.⁵ The previously published ELISA protocol for analysing antibodies against human PNM was used.⁵ Microtitre plate wells were coated with 80 µg/mL purified myelin in 0.05 mol/L carbonate buffer (pH 9.6) overnight at 4°C. The wells were washed (three times for 5 min each) with PBS (pH 7.4) and non-specific sites were blocked

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Table 1 Plasma samples from patients with varying neurological disorders

Patient groups	PN	IgMPDN	MMN	MND	OND
Neurological disorders	Unspecified peripheral neuropathy (<i>n</i> = 4) CIDPN (<i>n</i> = 1) Inflammatory radiculopathy (<i>n</i> = 1) Neuropathy associated with diabetes (<i>n</i> = 1) AMAN (<i>n</i> = 1) Vasculitic neuropathy (<i>n</i> = 1) 'Onion bulb' neuropathy (<i>n</i> = 1)	Demyelinating neuropathy associated with IgM paraproteinaemia	Multifocal motor neuropathy	Motor neuron disease	'Stiff Man' syndrome (<i>n</i> = 2) Multiple sclerosis (<i>n</i> = 1) Transverse myelitis (<i>n</i> = 1) Paraneoplastic cerebellar syndrome (<i>n</i> = 1)
Total patient numbers (<i>n</i> = 31)	10	7	4	5	5

PN, peripheral neuropathy; IgMPDN, IgM paraproteinaemia and peripheral demyelinating neuropathy; MMN, multifocal motor neuropathy; MND, motor neuron disease; OND, other neurological diseases; CIDPN, chronic inflammatory demyelinating polyneuropathy; AMAN, acute motor-axonal neuropathy.

with 10% FCS/PBS. Test plasma diluted 1:1000 (100 μ L/well) was then added to the wells (in triplicate) and subsequently washed (three times) in PBS. Immunoreactivity was detected with peroxidase-conjugated sheep antihuman IgM antibody (Silenus Laboratories, Melbourne, Vic., Australia). After washing (three times) with PBS, the wells were developed with 20 mg *o*-phenylenediamine dihydrochloride (OPD; Sigma Chemical Company, St Louis, MO, USA). After 30 min, the peroxidase-OPD reaction was halted with 25 μ L 3 mol/L HCl and the OD of each well read at 490 nm on an ELA microplate reader (BioRad Laboratories, Hercules, CA, USA). Sample and antibody solutions were prepared using a 10% FCS/PBS diluent and all incubation steps were performed for 2 h at room temperature (RT).

Thin layer chromatography immunostaining

Peripheral nerve myelin lipids were extracted and differentiated as previously described.⁵ Standards comprised: (i) mixed bovine brain GalC; (ii) sulphatides from bovine brain; (iii) GM₁ from bovine brain; and (iv) type II gangliosides from bovine brain (including GM₄, GM₃, GM₁, GD_{1a}, GD_{1b} and GT_{1b}; all purchased from Sigma). Glycolipid bands were visualized with an α -naphthol/H₂SO₄ stain and charred at 105°C for 6 min.

Immunoglobulin M-reactive glycolipids were detected by a modification of the method of Kusunoki *et al.*⁶ (results in Petratos *et al.*⁵). The TLC plates were pretreated with 0.1% polyisobutyl methacrylate (Aldrich Chemical Co. Inc., Milwaukee, WI, USA) in *n*-hexane and then dried. Non-specific binding sites were blocked overnight at 4°C with 5% (w/v) skim milk powder (SMP; pH 7.4), washed (three times) with Tris-buffered saline (TBS) and incubated for 1 h with the test plasma (diluted 1:200) at RT. Immunoreactivity was detected with a peroxidase-conjugated sheep antihuman IgM antibody (1:1000) for 1 h at RT, then developed with a diaminobenzidine (DAB) substrate.

Absorption of patient plasma

Glycolipids were conjugated with octyl-sepharose 4B beads, mixed with 0.1 mL of test and positive control plasma at three dilutions (1:100, 1:200, 1:500) in 10% FCS/PBS and neat and left overnight at 4°C. The sepharose beads/glycolipid/antiglycolipid antibody complexes were then removed by centrifugation (5 min at 88.3 g). The supernatant was then applied to the TLC plates to determine GaIS reactivity.⁷

Octyl-sepharose beads conjugated to non-reactive glycolipid and also unconjugated glycolipids served as controls and were tested in parallel.

Western blotting and SDS-PAGE

The myelin proteins were separated by discontinuous PAGE on 15% slab gels run at constant current (40 mA/gel). Protein bands were developed by Coomassie Blue staining.

Following electrophoresis, the myelin proteins were transferred onto the protein sequencing membrane, polyvinylidene difluoride (PVDF), overnight (4°C) at a constant current of 300 mA using Towbin transfer buffer. After transfer, the membrane was washed (for 5 min three times) in distilled water and non-specific sites blocked with 5% SMP in TBS for 2 h at RT. After incubation with test plasma, diluted 1:100, the membrane was washed with TBS (for 5 min three times) and incubated with a peroxidase-conjugated sheep antihuman IgM antibody, washed with TBS (3 \times 5 min), and immunoreactivity developed using a DAB substrate. Sample and antibody solutions were prepared using a 5% SMP/TBS diluent and all incubation steps were performed for 2 h at RT.

Anti-GaIS ELISA

Anti-GaIS antibodies were measured by ELISA as previously described.⁸ This method was modified in the following ways.

Microtitre plate wells were coated with 100 μ L of a solution containing 20 μ g/mL of GalS dissolved in PBS (pH 7.25) with chenodeoxycholic acid. Non-specific sites were blocked with 10% FCS/PBS and then 100 μ L of serially diluted test plasma was added to the wells. Immunoreactivity was detected with peroxidase-conjugated sheep antihuman IgM antibody (Silenus Laboratories), developed with OPD (Sigma Chemical Company). After 30 min, the peroxidase-OPD reaction was halted with 25 μ L 3 mol/L HCl and the OD of each well read at 490 nm on an EIA microplate reader (BioRad Laboratories). Sample and antibody solutions were prepared using a 10% FCS/PBS diluent and all incubation steps were performed at RT for 2 h.

A plasma sample from a patient with IgM paraproteinaemia and high anti-GalS IgM antibodies was used as the internal laboratory control in the anti-GalS ELISA described in the present study. All test plasma samples were therefore normalized against this high titre sample. Undiluted plasma from this sample was set at 1 000 000 arbitrary units (AU)/L, corresponding to the dilution ratio still recording a positive ELISA result (i.e. $1:1 \times 10^6$). All samples were serially diluted. Readings were taken from the last part of the OD curve. The OD values in the uncoated control wells for each incubated antibody (plasma) were subtracted from the values in GalS-coated wells.

Immunofluorescence

Sciatic nerves were obtained at autopsy from individuals without neurological disease within 8 h of death. Optimum cutting temperature medium (OCT)-mounted tissue blocks were snap-frozen in isopentane, cooled in liquid nitrogen and 5 μ m sections cut on a freezing microtome. Paraffin-embedded sections were also cut at 5 μ m on a conventional microtome, mounted on silane-coated slides and passed through dewaxing and hydration steps. The sections were then incubated for 1 h at RT with blocking buffer (10% normal sheep serum (NSS) in PBS, pH 7.4). They were then incubated with human plasma diluted 1:20 in blocking buffer for 1 h. The sections were then washed (for 5 min three times) in PBS (pH 7.4). The secondary antibodies, FITC-conjugated antihuman IgM raised in sheep

(Silenus; 1:40 dilution in blocking buffer), were applied to the sections for 1 h. The slides were then washed thoroughly with PBS and coverslipped using 90% glycerine mounting medium. The sections were then examined under a fluorescence microscope (Leitz, Stuttgart, Germany).

Immunostaining of teased nerve fibres

Teased fibres were prepared by adapting the protocol of Ganser and coworkers.⁹ Briefly, a segment of sciatic nerve was obtained at autopsy from a patient who had died from non-neurological disease (within 8 h of death) and fixed in glutaraldehyde in PBS at pH 7.4 for 36 h. After treatment in glycerine over 72 h, individual nerve fibres were teased out using fine forceps under a dissecting microscope and mounted on silane-coated microscope slides.

Individual teased nerve fibres were incubated with plasma containing a high titre of anti-GalS antibodies. Identification of antibody binding sites was carried out with secondary IgM antibodies as described earlier. Antibody incubation and blocking protocols were also performed as described earlier.

Results

Immunoglobulin M ELISA

Three of the seven plasma samples from patients with demyelinating neuropathy and IgM paraproteinaemia contained IgM anti-PNM reactivity by ELISA (Fig. 1). Positive anti-PNM reactivity was determined as being 3 SD above the highest OD value observed in the negative control plasma samples. This negative control plasma sample was confirmed as being negative because it did not react with any myelin lipid or protein analysed by TLC immunostaining and western immunoblot procedures, respectively.

Plasma samples from patients with both multifocal motor neuropathy (MMN) and various peripheral neuropathies (PN) were included in the present study, due to the large volume of data demonstrating that antimyelin antibodies are

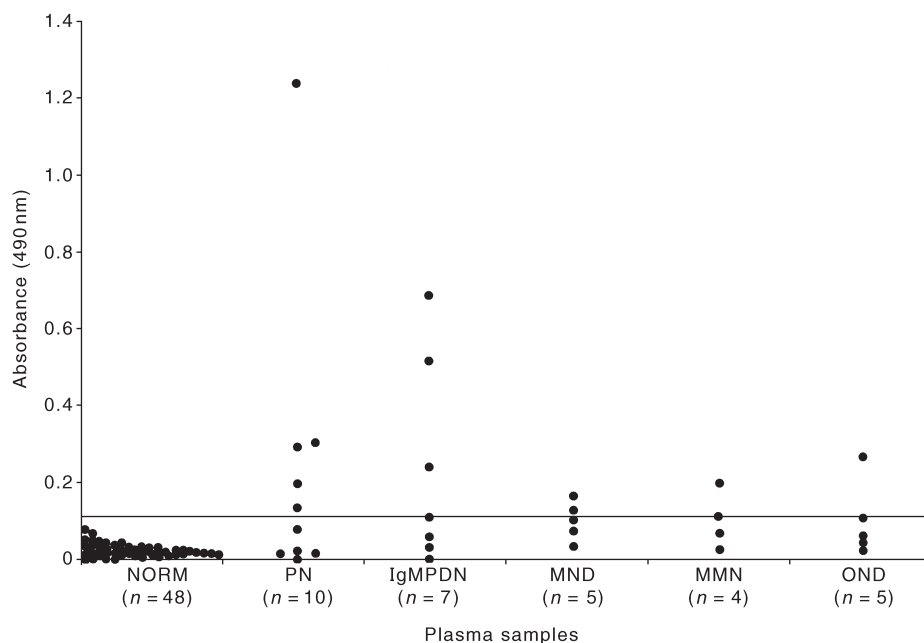


Figure 1 Immunoglobulin M reactivity determined by whole myelin ELISA at a plasma dilution of 1:1000. The horizontal line depicts the mean of the highest absorbance value obtained for a normal sample that contained no immunoreactivity with any myelin component (measured at 490 nm) plus 3 SD. Absorbance values above this line were regarded as positive. NORM, normal blood donors; PN, patients with various peripheral neuropathies; IgMPDN, IgM paraproteinaemia and demyelinating peripheral neuropathy; MND, motor neuron disease; MMN, multifocal motor neuropathy; OND, other neurological diseases.

Table 2 Clinical and pathological features of patients with neurological disease and IgM anti-PNM reactivity correlated with their IgM anti-sulfatide antibody titres

Patient	Age/Sex	Neurological assessment	Peripheral nerve biopsy studies	IgM anti-GaIS titre (AU/L)	Immunofluorescence studies using patient's plasma	Diagnosis
1	79/M	Prolonged distal latencies, absent sensory action potentials, ↓ motor amplitudes	Endoneurial oedema, loss of large diameter myelinated axons, occasional onion-bulbs, no endoneurial inflammatory cells, occasional naked axonal segments, thinly myelinated axons with shortened internodes, IgM deposits on myeline membrane	1 × 10 ⁶	Myelin stained	IgMPDN
2	58/M	↓ Sensation, absent ankle and knee jerk, prolonged F-waves, absent sensory action potentials	No biopsy taken	*	Myelin stained	IgMPDN
3	57/M	NA	NA	10 000	Myelin stained	IgMPDN
4	78/M	Distal areflexia, dysaesthesia in upper and lower limbs	No inflammatory cell infiltrates, mild loss of large and intermediate diameter myelinated fibres, occasional onion-bulbs, active axonal degeneration, several thinly myelinated segments with shortened internodes	2000	NT	CIDPN
5	64/M	NA	NA	—	NT	CIDPN
6	47/F	NA	NA	—	NT	Peripheral neuropathy
7	77/M	Lower limb weakness, dysaesthesia	Moderate number of CD68 ⁺ cells with digestion chambers containing myelin and axonal debris, strong MHC class II expression, ↓ in large and intermediate diameter myelinated axons, regenerating clusters of small axons, some thinly myelinated axons, 7% of axons undergoing active degeneration	— [†]	NT	AMAN
8	40/F	Mild to moderate dysaesthesia in feet	No biopsy taken	—*	NT	Diabetic neuropathy
9	34/M	↓ Motor amplitude and absent F-response in right median nerve	No biopsy taken	—	NT	MND
10	42/M	NA	NA	—	NT	MND
11	46/F	Progressive weakness, genetic profile showed a SOD-1 mutation	No biopsy taken	—	NT	MMN
12	52/F	NA	NA	2000	NT	'Stiff Man' syndrome

All patients contained IgM anti-myelin reactivity by ELISA at 1:1000 dilution. *Positive at a lower titre, †high titre IgG anti-GaIS antibodies. NA, not available; NT, not tested; Ig MPDN, IgM paraproteinaemia and demyelinating neuropathy; CIDPN, chronic inflammatory demyelinating polyneuropathy; AMAN, acute motor-axonal neuropathy; MND, motor neuron disease; MMN, multifocal motor neuropathy.



Figure 2 The TLC immunostaining procedure detecting IgM reactivity against differentiated glycolipids using plasma from an IgM paraproteinaemia and demyelinating peripheral neuropathy patient (this plasma sample had previously recorded an anti-peripheral nerve myelin ELISA absorbance reading of 0.7; see Fig. 1). Glycolipid (5 μ g) was loaded in each lane. All plasma samples were diluted 1:200. Lane 1, purified GalS from bovine brain; lane 2, purified type II gangliosides from bovine brain; lane 3, acidic lipid preparation from human peripheral myelin; lane 4, neutral lipid preparation from human peripheral myelin.

present in patients with these disorders. In fact, both groups showed IgM anti-PNM reactivity (five of eight in the PN group, one of four in the MMN group). By contrast, two of the five plasma samples obtained from motor neurone disease (MND) patients and one of the five plasma samples in the other neurological diseases (OND) group showed IgM antimyelin reactivity. Clinical data could be accessed from only those patients who were admitted to the Royal Melbourne Hospital, and are represented in Table 2 along with their anti-PNM antibody reactivity.

Thin layer chromatography immunostaining

Immunoreactivity of the PNM constituents was confirmed with TLC immunostaining and western immunoblotting. None of the plasma samples included in the present study contained IgM reactivity against any of the PNM proteins. This was surprising, because many investigators have associated IgM paraproteinaemic neuropathy with circulating anti-MAG IgM antibodies. The question was raised as to whether there existed a problem with antibodies binding to the glycoproteins after they were transferred onto nitrocellulose. However, this problem was resolved because

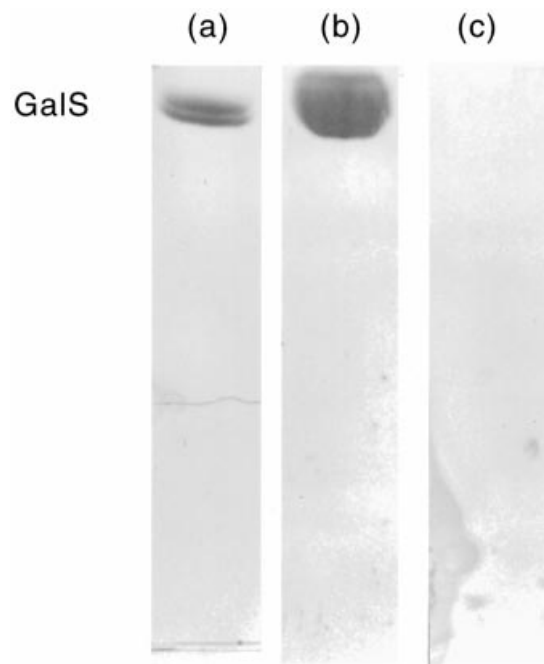


Figure 3 The TLC immunostaining of pre-incubated and post-incubated plasma samples with GalS-conjugated octyl sepharose beads. (a) Pre-incubated plasma from an IgM paraproteinaemia and demyelinating peripheral neuropathy (IgMPDN) patient showing IgM anti-GalS reactivity. (b) The same IgMPDN plasma sample post-incubation with GalC-conjugated octyl sepharose beads. (c) The same IgMPDN plasma sample post-incubation with GalS-conjugated octyl sepharose beads. Each lane was loaded with 5 μ g GalS standards from bovine brain.

anti-HNK-1 monoclonal antibodies showed perfect immunoreactivity with myelin-associated glycoprotein (MAG) and P_0 transferred onto the same nitrocellulose membrane with which the plasma samples were reacted (results not shown).

The only glycolipid that showed immunoreactivity with PNM ELISA-positive IgMPDN plasma sample group was GalS (represented by one such TLC immunostain in Fig. 2). No such reactivity was seen in the normal plasma samples. Both non-hydroxy and hydroxy bands reacted and no cross-reactivity was detected with any other glycolipid or glycoprotein containing a sulphated carbohydrate moiety (e.g. sulphoglucuronyl paragloboside (SGPG) or MAG). More importantly, reactivity was abolished by passing the positive plasma samples through a column composed of octyl-sepharose beads conjugated to GalS (Fig. 3).

Anti-GalS ELISA

Anti-GalS IgM antibody titres, analysed by ELISA, showed remarkably raised titres in two of the three IgM paraproteinaemia plasma samples. These had been positive by whole myelin ELISA and had been confirmed to react specifically against GalS by TLC immunostaining. Both of these plasma samples were well above the highest titre negative control plasma sample + 3 SD (upper limit for a positive sample;

Figure 4 Anti-GalS IgM antibody titres (represented in arbitrary units (AU)/L on a logarithmic scale) determined by ELISA. The following plasma samples were screened: 24 normal blood donors (NORM), 10 patients with various peripheral neuropathies (PN), 7 IgM paraproteinaemia and demyelinating peripheral neuropathy (IgMPDN), 5 motor neuron disease (MND), 4 multifocal motor neuropathy (MMN) and 5 other neurological diseases (OND). The horizontal line depicts the mean of the highest absorbance value obtained for a normal sample that contained no immunoreactivity with GalS (measured at 490 nm) plus 3 SD. Absorbance values above this line were regarded as positive. Numbers represented in brackets along the x axis show plasma samples that contained an antibody titre < 100 AU/L.

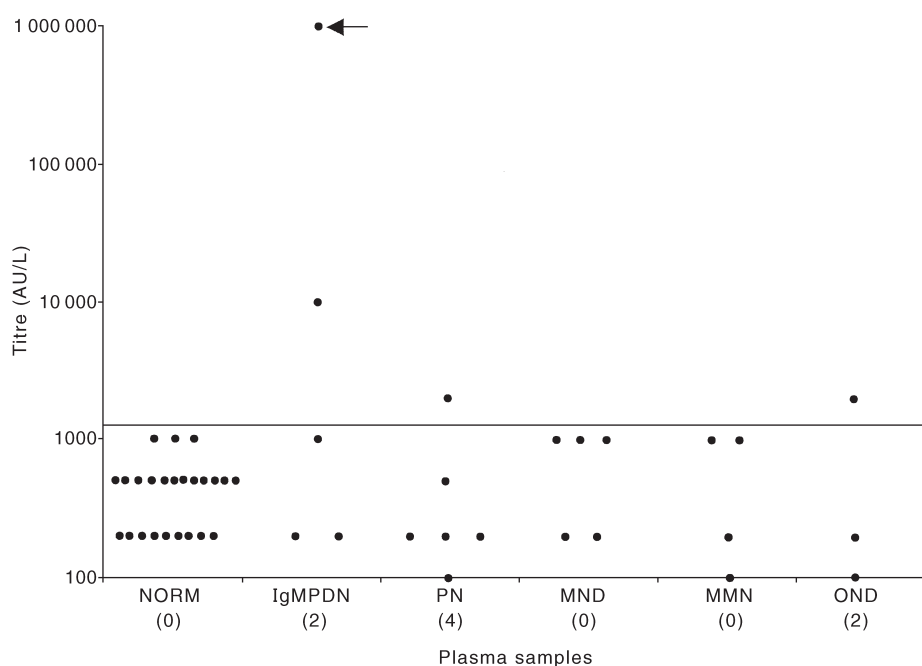


Fig. 4). One sample contained a titre of 1×10^6 AU/L and the other recorded a titre of 10 000 AU/L. The clinical and pathological information was accessible to us for only one of these two plasma samples and is outlined in Table 2. The third positive IgM paraproteinaemia plasma sample determined by whole myelin ELISA recorded a titre of 1000 AU/L, which was below the upper limit of the titre assay.

Only one of the five PNM ELISA-positive plasma samples from the PN group showed a raised IgM anti-GalS antibody titre of 2000 AU/L (Fig. 4). This anti-GalS ELISA-positive plasma sample happened to be from a patient with chronic inflammatory demyelinating polyneuropathy (CIDPN; see Tables 1,2). The plasma sample from the OND group, which showed IgM antibodies against PNM by ELISA, also showed an anti-GalS IgM antibody titre of 2000 AU/L (Fig. 4). Interestingly, this plasma sample was from a patient with clinically diagnosed 'Stiff Man' syndrome and also contained IgM antibodies against another glycoconjugate antigen, glutamic acid decarboxylase (GAD; see Table 1). The remaining plasma samples in the PN group with anti-PNM ELISA immunoreactivity did not show immunostaining against any known peripheral myelin glycolipids or glycoproteins by TLC immunostaining, western immunoblotting or by ELISA (results not shown).

Immunofluorescence

It was clearly shown, by indirect immunofluorescence, that the high titre IgM anti-GalS antibodies present in the plasma samples from both patients with IgM paraproteinaemia and demyelinating polyneuropathy bound exclusively to the external layer of the myelin sheath. No axolemmal reactivity was seen (Figs 5,6).

Discussion

Anti-GalS antibodies have rarely been described in patients with IgM paraproteinaemia and peripheral neuropathy. Antibodies reacting with MAG have been frequently reported in patients with neuropathy associated with IgM paraproteinaemia.¹⁰⁻¹³ Anti-MAG antibodies also react against the P₀ glycoprotein, neural cell adhesion molecule (NCAM) and two acidic glycolipids, SGPG and sulphoglucuronyl lactosaminyl paragloboside (SGLPG).¹⁴⁻¹⁷ Recently, cross-reactivity of some anti-MAG antibodies with GalS has also been reported.¹⁸ The sulphoglucuronyl moiety or a similar carbohydrate residue has been shown to be essential for antibody binding. This is shared by several other nervous system glycoconjugates besides MAG and P₀, including the adhesion molecules J1 and NCAM and several 20-26 kDa peripheral nerve system (PNS) glycoproteins.¹³ It is possible that the pathology in patients with these auto-antibodies may arise through their interfering with the structural integrity of PNS myelin. In addition, these antibodies may disrupt critically important interactions between axons and Schwann cells by binding to cell adhesion molecules.¹³

Our results show that anti-GalS antibodies occur in patients with IgM paraproteinaemia with associated demyelinating neuropathy, can exist at very high titres and that these antibodies preferentially bind to the outer myelin membrane without any reactivity with axons. This reactivity could also be abolished by passing plasma through a column of octyl-sepharose beads conjugated to GalS. However, our data differ from those published by previous investigators, showing no anti-GalS antibody cross-reactivity with MAG. These results suggest that, in some IgM paraproteinaemia patients, anti-GalS antibodies alone may play a significant role in demyelination.

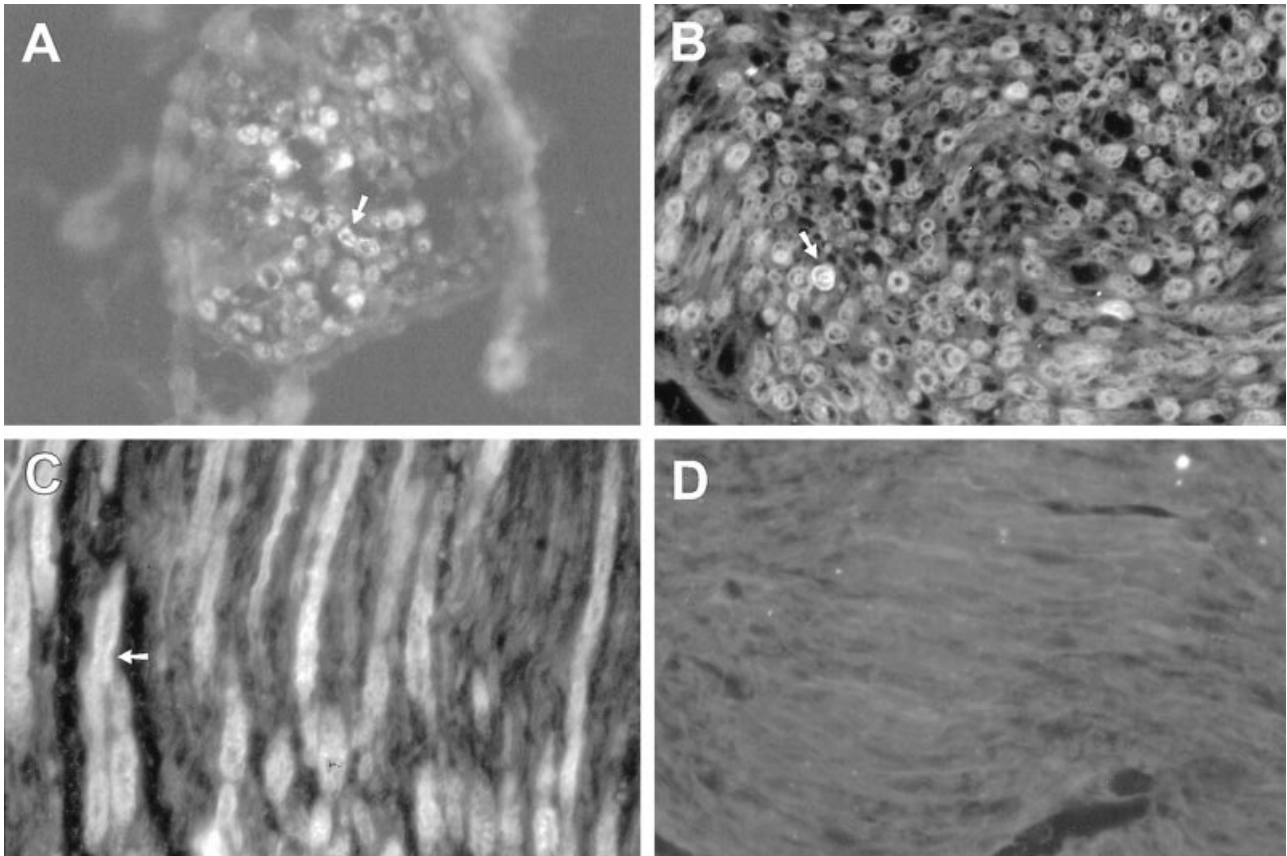


Figure 5 Immunofluorescence of fixed and unfixed sciatic nerve sections. (a,b) Anti-GalS IgM antibody reactivity (antibody titre 1×10^6 AU/L; see Fig. 4) from an IgM paraproteinaemic demyelinating neuropathy patient; (a) unfixed frozen section, (b) fixed paraffin section. (c) Fixed paraffin longitudinal section showing anti-GalS IgM antibody reactivity from another IgM paraproteinaemic demyelinating neuropathy patient (antibody titre 1×10^4 AU/L; see Fig. 4). No antibody binding to the sciatic nerve section was detected on incubation with plasma from a normal blood donor. (d) Anti-human IgM detection antibody used (frozen section). Original magnification $\times 200$. Arrows show myelin staining.

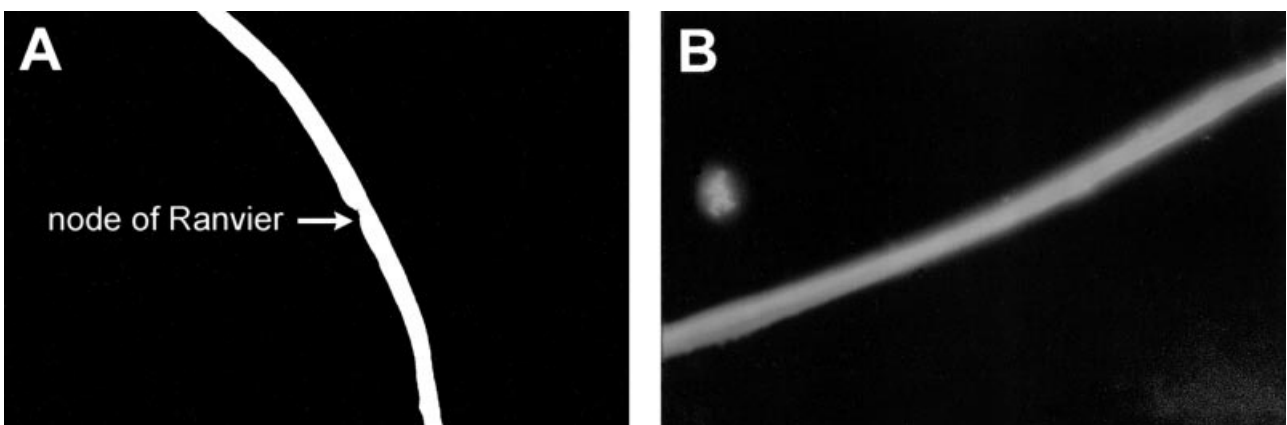


Figure 6 Immunofluorescence of fixed teased nerve fibres. (a) Anti-GalS IgM antibody reactivity from a patient with IgM paraproteinaemia and demyelinating neuropathy. No antibody binding to teased nerve fibres was detected on incubation with plasma from a normal blood donor. (b) Anti-human IgM detection antibody used. Original magnification $\times 200$.

It has been previously described that anti-GalS antibodies are prevalent in the circulation of patients with sensory deficits.^{3,19} One study, in particular, related sensory impairment to the binding of anti-GalS antibodies directly to dorsal root ganglia (DRG).³ As described in our study, these

investigators also preabsorbed the plasma in a GalS-conjugated column, which eliminated all tissue immunoreactivity. This suggested that GalS, a component of DRG neuronal cell membranes, may be a 'self' antigen. More evidence that these antibodies cause sensory symptoms arises from the

relative permeability of the blood–nerve barrier (BNB) in DRG.^{20,21} This renders DRG neurones potential targets for anti-GaIS antibodies. It is possible that these antibodies could disrupt interactions of GaIS with the extracellular adhesion molecules. This would in turn cause disruption of the close contact between axons and Schwann cells necessary for maintenance of myelination.²² These antibodies could also interfere with the function of associated β -endorphin receptors²³ or cause damage through complement fixation. The specificity of anti-GaIS antibodies is largely due to the sulphate (SO₃) and hydroxyl (OH) groups of the sugar residue.²⁴ Antibodies against GaIS have also been found to bind only to fixed sections of peripheral and central myelin.³ This tends to suggest that the GaIS epitope is normally shielded, but may be exposed in some types of nerve injury and during tissue fixation. However, it has also been shown that anti-GaIS antibodies are present in patients with demyelinating motor neuropathies.^{4,19}

Anti-GaIS antibodies are frequently found in patients with inflammatory demyelinating polyneuropathy (IDPN).^{13,25} The concentration of GaIS on the external surface of the myelin sheath has been documented.²⁶ This would suggest that anti-GaIS auto-antibodies can induce immune demyelination if these antibodies gain access to the peripheral nerve compartment and play a role in the pathogenesis of motor neuropathies such as GBS. Anti-GaIS antibodies have been detected in 65% of GBS patients and also in 87% of CIDPN patients. However, only 15% of control sera contain these antibodies.²⁵

Our results show that one CIDPN patient without IgM paraproteinaemia also had raised anti-GaIS IgM antibody titres (see Fig. 4, Table 1). Many of the other plasma samples from our PN cohort (five of 10) showed anti-PNM antibodies; however, only the CIDPN patient just described contained raised titres of IgM anti-GaIS antibodies. Because we did not find any reactivity against the protein compartment of PNM, these data may illustrate how a raised absorbance reading by ELISA using a crude substrate (such as myelin) may not necessarily translate to raised antibody titres against a single epitope within the plasma. Alternatively, this could mean that the epitope these antibodies are reacting with is a combination of two molecules in a specific conformation seen only within an intact myelin membrane (such as a glycolipid associated with a glycoprotein).

Another study has shown a direct relationship between anti-GaIS antibodies and immune demyelination in the PNS.⁴ These investigators passively transferred purified monoclonal anti-GaIS IgM antibodies from a patient with benign IgM- λ paraproteinaemic demyelinating polyneuropathy to newborn rabbits. This produced demyelinating nerve lesions similar to those seen in the donor patient. The experimental lesions showed direct binding of anti-GaIS antibodies to Schmidt–Lanterman incisures and nodes of Ranvier. The investigators also showed that these antibodies bound to satellite cells in DRG, suggesting a possible pathogenetic role for anti-GaIS antibodies in sensorimotor syndromes.

Anti-GaIS IgM antibodies are restricted to the lambda (λ) subclass, which may result from preferential utilization of particular immunoglobulin variable region genes by these antibodies,³ similar to that reported for antibodies with anti-DNA, rheumatoid factor, cold agglutinin and anti-MAG

reactivity.^{27–30} It has been suggested that low levels of anti-GaIS antibodies may be common constituents of the normal human immune repertoire and may be secreted by the CD5 positive subset of B cells, similar to other naturally occurring IgM anticarbohydrate antibodies.³¹ These B cells may be activated by GaIS incorporated into the envelope of a budding virus,³² by cross-reactive foreign antigens or by antigen complexes and may be excessively produced by expanded B cell clones in monoclonal gammopathies.

The present study has emphasized the necessity for anti-GaIS antibody screening in patients with IgM paraproteinaemia and demyelinating polyneuropathy. These antibodies can occur in isolation, unaccompanied by other possible cross-reacting antibodies such as anti-MAG or anti-SGPG antibodies. Patients presenting with high titre anti-GaIS antibodies could benefit from early treatment with plasmapheresis and/or intravenous immunoglobulin. The present study has shown a possible pathogenetic link between high titre anti-GaIS antibodies and demyelination in the setting of IgM paraproteinaemia. A prospective study is clearly required to substantiate this link, correlating clinical, electrophysiological and histopathological features with immune parameters.

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