Antiglycolipid antibodies in peripheral neuropathy: fact or fiction?

The past decade has seen the emergence of a large field of research investigating the concurrence of antiglycolipid antibodies and peripheral neuropathy (see reviews1-4). Initial scepticism about the significance of antiganglioside antibodies is being replaced by a widespread realisation that these antibodies directly contribute to the pathogenesis of neuropathy. Two incompletely resolved issues which cast doubt on this relation are that raised concentrations of antiglycolipid antibodies are very widespread in normal and disease controls and antiglycolipid antibodies may be either present or absent in clinically indistinguishable neuropathy syndromes. By contrast, some syndromes such as Miller Fisher syndrome, are very tightly linked to a specific antiglycolipid antibody in which conclusive passive transfer studies have been performed. Refining the clinicoradiological associations to aid in diagnosis and to monitor disease progress through antiglycolipid antibody measurements is becoming more relevant to patient management. From the research point of view, considerable efforts are being made to identify new antibody specificities and to elucidate the precise mechanisms of action of antiglycolipid antibodies. The purpose of this editorial is to put a large body of confusing and often conflicting data into a balanced clinical perspective with particular emphasis on currently recognised clinical syndromes and guidance about requesting and interpreting antiglycolipid antibody assays.

Glycolipid terminology, structure, and function
An early hurdle to cross concerns the complexities of glycolipid and ganglioside terminology. Glycosphingolipids are composed of the long chain aliphatic amine sphingosine (acylated ceramide) attached to one or more sugars (hexoses). The ceramide is immersed in the membrane lipid bilayer with the carbohydrate structure exposed extracellularly. A single hexose linked to ceramide (monohexosyl-ceramide) is termed a cerebroside, the most abundant of which in humans is galactocerebroside (galactosyl-ceramide). Sulphatide is galactocerebroside sulphated in the carbon-3 position and is a major component of peripheral nerve myelin and an autoantigen in predominantly sensory neuropathies.5-9

Gangliosides are complex glycosphingolipids that, by definition, must contain at least one sialic acid residue. Sialic acid is a generic term for N-acetylneuraminic acid, the acyl group generally being acetyl in the human nervous system (as opposed to glycolyl), hence N-acetylneuraminic acid, commonly abbreviated to NeuNAC or NANA. The sialic acid(s) are attached to the internal or the terminal galactose of an oligosaccharide core composed of up to four sugars with the following sequence: ceramide-glucose-galactose-N-acetylgalactosamine-galactose. Traditionally, gangliosides are named according to Svennerholm10-11 with the following formula: G refers to ganglio; M, D, T, and Q refer to the number of sialic acid residues (mono, di, tri, and quad respectively); arabic numerals and lower case letters refer to the sequence of migration as determined by thin layer chromatography. Although dependent on the solvent system used, bulky gangliosides with a longer oligosaccharide core and more sialic acid will migrate more slowly than the smaller gangliosides. For example, for three of the monosialogangliosides, GM1 (having a four sugar oligosaccharide core, Cer-Glc-Gal-GalNAC-Gal) migrates more slowly than GM2 (a three sugar oligosaccharide, Cer-Glc-Gal-GalNAC), which migrates more slowly than GM3 (a two sugar oligosaccharide, Cer-Gal-Gal). Likewise, GD1b runs ahead of GT1b, which runs ahead of GQ1b, as they contain two, three, and four sialic acids respectively. The figure shows the structure of GD1b. There are four major gangliosides in the brain (GM1, GD1a, GD1b, GT1b) and many minor gangliosides in brain and peripheral nerve tissues, which are developmentally regulated and spatially segregated in a wide range of patterns across different species. Many different gangliosides can be autoantigens in peripheral neuropathy, some of which have not been identified.1

Paragloboside is a neutral glycolipid (Cer-Glu-Gal-GlCNAC-Gal), which, when sialylated on the terminal galactose (sialosylparagloboside, SGP), is a major peripheral nerve glycolipid, also known as LM1.12 Substitution of the terminal sialic acid for sulphated glucuronic acid forms sulphated glucuronyl paragloboside (SGPG).13-14 Both LM1 and SGPG are also important peripheral nerve autoantigens in neuropathy.5-13

All antiglycolipid antibodies associated with neuropathy react with epitopes on the carbohydrate region of the glycolipid molecules. Because these carbohydrate structures are often present on several different glycolipids, glycoproteins, and other carbohydrate rich molecules such as bacterial lipopolysaccharides, there is considerable potential for shared reactivity. For example, (a) autoantibodies that react with the sulphated glucuronic acid epitope on SGPG also react with similar epitopes on many other neural glycoproteins including the myelin associated glycoprotein (MAG)15-17 and the peripheral

Editorial
Antiganglioside antibody assays: methodology and interpretation

Assaying serum samples for antiganglioside and antigangliolipid antibodies is a process with many pitfalls. There is no standardised assay method and the literature on this subject is crowded with different protocols that claim superiority. Two multicentre studies with coded samples have shown good agreement on clearly positive or negative cases but discrepancies appear with borderline samples, which may nevertheless be of important clinical relevance. 13 Most centres use a combination of enzyme linked immunosorbent assay (ELISA) with thin layer chromatography overlay as a confirmatory test. Critical factors that influence assay results include (a) the choice of ELISA plates, taking into account manufacture related batch to batch variations and subsequent storage conditions; (b) the temperature at which the assay is performed; (c) the duration of serum incubation; (d) washing and blocking buffer composition, particularly the presence or absence of detergent and the choice of blocking protein; (e) sample layout on ELISA plates, with particular attention to plate stacking artefacts and edge effects. These factors need to be built into stringent quality control practice in all laboratories performing assays, particularly when results influence decisions about diagnosis and clinical management.

Assay results are usually reported as titres calculated by end point analysis (the lowest serum dilution that still gives a raised optical density reading by ELISA). When interpreting an assay result, consideration must be given to the methodology used and the normal range established for that laboratory. Because antiganglioside antibodies form part of the normal antibody/autoantibody repertoire, 14-16 they are commonplace in normal and disease control serum samples. 17-20 For example, moderately high IgM and IgG titres (>1/10 3 < 1/10 4 ) against asialo-GM1 and sulphatide are typically found in normal serum samples and are thus not likely to be relevant at this level; by contrast, an anti-GM1 IgM titre of 1/10 4 is likely to be relevant as this is above the upper limit of the normal range (around 1/500) for this antigen in most laboratories.

Antigangliolipid antibody markers for clinical syndromes

The table lists the main clinical syndromes with a well defined antigangliolipid antibody specificity. In some situations, the antigangliolipid antibody assay result will corrob-

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<th>Clinical syndromes associated with specific antigangliolipid autoantibodies</th>
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<tr>
<td><strong>Clinical syndrome</strong></td>
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<tr>
<td>Chronic sensorimotor demyelinating neuropathy</td>
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<tr>
<td>Chronic axonal sensory neuropathy</td>
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<tr>
<td>Chronic large fibre sensory neuropathy with ataxia</td>
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<td>Multifocal motor neuropathy</td>
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<td>Miller Fisher syndrome</td>
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<td>Guillain-Barré syndrome</td>
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Glycolipids, including gangliosides, regulate many diverse physiological processes, 28, 29 including extensive modulation of neural cell function. 30 They are asymmetrically located in the plasma membrane, anchored in the bilayer by their ceramide tail, exposing their sialylated oligosaccharide core extracellularly, a site readily accessible to antibody binding. They are highly enriched in neural membranes, particularly synaptic regions. They interact with growth factor receptors, ion channels, and cell recognition/adhesion molecules and have widespread effects on signal transduction systems. At the cellular level, they influence such diverse events as cell survival, differentiation, synaptogenesis and synaptic transmission, and neurite outgrowth. In vivo effects include accelerated restoration of function in injured tissues and neuroprotection—for example, against glutamate induced neuronal injury. Considerable research is currently focused on increasing our understanding of ganglioside function.
orotate a previously suspected diagnosis, such as multifocal motor neuropathy, or indeed alert the neurophysiologist to search carefully for conduction block in an apparent case of lower motor neuron disease in whom multifocal motor neuropathy was not suspected clinically. Alternatively, a positive result will further refine an existing diagnosis, such as paraproteinaemic neuropathy with anti-MAG specificity or will help to distinguish an important differential diagnosis, such as Miller Fisher syndrome, botulism, and brain stem demyelinating disease.

The first clinical syndrome in which antigangliosid antibodies were identified was the IgM paraproteinaemic neuropathy associated with anti-MAG antibodies.41-42 As well as reacting with MAG and other glycoproteins present in peripheral nerve, it was established that the anti-MAG paraproteins also reacted with the previously unknown acidic glycolipid, SGPG, and its related homologue, sulphated glucuronyl lactosaminyl paragloboside (SGLPG).13-15 The clinical syndrome is of a relatively benign, late onset, chronic sensorimotor demyelinating neuropathy, often associated with tremor.43 A pathological feature of note in nerve biopsies is the presence of widely spaced myelins.44-46 The anti-MAG specificity accounts for 50% of patients with IgM paraproteinemic neuropathy; most of the remainder react with other glycolipids or gangliosides.1 Some anti-MAG paraproteins cross react with sulphatide7 and in addition, sulphatide can be a specific and sole antigen in patients with IgM paraproteinaemic, predominantly sensory, neuropathy.5-7 It is important to recognise, when evaluating cases of late onset demyelinating neuropathy, that low level paraproteins may be easily missed by standard electrophoretic techniques and that sensitive screening methods should be employed.

Another increasingly well defined paraproteinaemic neuropathy syndrome is a chronic large fibre sensory neuropathy with prominent ataxia, first described in a single case report almost a decade ago79 and subsequently reported by several groups.48-51 In these cases the IgM paraprotein reacts with gangliosides bearing NeuNac(a2-8)NeuNac linked disialosyl groups, including GD3, GD1b, GT1b, and GQ1b (figure). There is some variation in the fine specificity of the antibody between cases although the clinical pattern is fairly uniform. Some of these patients also have cold agglutinin disease by virtue of the presence of sialylated glycoproteins on human red blood cells. In addition, some cases have been reported with intercurrent orthomieplogia,67 53 thus producing a clinical syndrome reminiscent of Miller Fisher syndrome in which antibodies to disialylated gangliosides are also found.54-56

Interest in multifocal motor neuropathy with demyelinating conduction block77 has been intense since it was recognised that patients with this syndrome may have antibodies to GM1 ganglioside.58 Early reports indicating a significant association between anti-GM1 antibodies and motor neuron disease/amyotrophic lateral sclerosis59-60 were not confirmed by others7 57 61-63 although there do seem to be very rare, unusual cases in which this occurs.17-19 We screen all patients with predominantly lower motor neuron syndromes for anti-GM1 antibodies. Many patients with multifocal motor neuropathy and anti-GM1 antibodies have a fairly stereotyped clinical picture comprising a chronic asymmetric motor syndrome, usually with distal onset in an upper limb, as previously described.54-56 We have not found further subdivisions on the basis of antiganglioside serology useful, although they have been reported.47 These patients respond well, albeit temporarily, to intravenous immunoglobulin68 which is our routine treatment and can be regularly repeated. Our own experience with cyclophosphamide has been disappointing (lack of sufficient efficacy to warrant the high risks of toxicity) although others report success.69

Antiganglioside antibodies are also found in acute peripheral neuropathies where they appear transiently and are predominantly of the IgG class. The most clearly defined association is between anti-GQ1b ganglioside antibodies and Miller Fisher syndrome.54-56 The anti-GQ1b antibodies cross react with GT1a in the series of cases reported by Cohn et al70 and in all our cases to date (14/14) and with GD1b, GD3, or both in about half of our cases. Anti-GQ1b reactivity seems to specifically mark the presence of orthomieplogia: we have recently identified a case of acute ataxic, areflexic neuropathy without orthomieplogia whose serum samples reacted only with GD1b and GD3, thereby lending support to this view. A recent report indicates that anti-GQ1b antibodies are also present in Bickerstaff's brain stem encephalitis, suggesting that there may be a common aetiological thread to both these conditions.71

In Guillain-Barré syndrome, a wide variety of anti-ganglioside antibodies have been reported in up to 50% of cases in different series (table); although no unifying patterns have yet emerged, they may do so in the future. Considerable debate surrounds the significance of anti-GM1 antibodies in Guillain-Barré syndrome. In some studies, anti-GM1 antibodies mark a particularly severe form of the illness with prominent motor axonal involvement and poor recovery,26 27 72 although this association is refuted by others.73 74 This issue may be resolvable by further subclassification of anti-GM1 antibodies by fine specificity. In this regard, our recent studies on a panel of cloned human anti-GM1 antibodies75 have shown considerable heterogeneity in their ability to bind antigen under different conditions and in their patterns of tissue reactivity. This may account for the apparent diversity of clinical expression of anti-GM1 antibody associated neuropathies and neuronopathies.

The pathogenic relevance of antiganglioside antibodies

Despite considerable anomalies and complexities, good evidence is beginning to emerge from a variety of experimental models to support a direct role for antiganglioside antibodies in causing neuropathy. Clearly, other factors including cellular immune mechanisms may play a central part in pathogenesis. The subject is complicated by many factors common to developing an animal model for disease including qualitative and quantitative variations in glycolipid antigen composition in nerves from different species and the lack of well defined human antibodies for pathogenesis studies. The generation of disease associated human monoclonal antibodies should help to resolve this issue.79 Antibodies to SGPG, as found in the “anti-MAG” IgM paraproteinaemic neuropathy have been shown to cause demyelination and other pathological changes including widening of myelin lamellae, when injected locally into rodent nerve77 and after systemic passive transfer to the chicken.78 An intriguing case of anti-MAG IgM paraproteinaemic neuropathy occurring in conjunction with hereditary motor and sensory neuropathy has been described,79 raising the possibility that pre-existing peripheral nerve abnormalities may predispose to the development of antineur antibodies, a fact long recognised in experimental models of nerve injury.80

Experimental studies on anti-GM1 antibodies have
shown a variety of neuropathic effects including demyelina-
tion and conduction block\(^1\) and although these studies are
completely.\(^2\) Antibodies to GM1/GD1b\(^3\)
and gangliosides bearing disialosyl groups as found in
chronic sensory neuropathies,\(^4\) are toxic to cultured
motor neurons and dorsal root ganglion neurons respec-
tively. It has also recently been shown that Miller Fisher
syndrome serum containing anti-GQ1b antibody blocks
neurotransmitter release in the mouse hemidiaphragm
preparation, which we postulate accounts for the motor
manifestations of the syndrome.\(^5\) Many groups are
actively researching this field, which will undoubtedly
reveal the pathogenic relation between anti-gangliosid
antibodies and neuropathy in more detail.

Conclusion
Antiganglioside antibodies are here to stay as part of the
clinical and experimental investigation of patients with
peripheral neuropathy. The extent to which antigangli-
oside antibodies are required, however, will depend on
the investigative enthusiasm of individual neurologists
as there are few circumstances in which positive or nega-
tive results play a critical part in patient diagnosis and
management. The field is still in its infancy and a consid-
erable amount of further research is necessary to identify
putative antigens in seronegative cases, or to establish
that such cases are not antibody mediated. This informa-
tion will eventually filter into clinical practice and will
hopefully direct clinicians towards appropriate treatment
and monitoring of antibody mediated neuropathies.

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