Peripheral neuropathies and anti-glycolipid antibodies

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Summary
This review charts the progress of anti-glycolipid antibodies in neuropathy, from their original discovery 20 years ago in immunoglobulin M paraproteinaemic neuropathy through to current discoveries mapping their relationship to subtypes of Guillain–Barre syndrome. Antibodies to >20 different glycolipids have now been associated with a wide range of clinically identifiable acute and chronic neuropathy syndromes. Particular progress has been achieved in understanding the link between acute motor axonal neuropathy and antibodies to GM1, GD1a, GM1b and GalNAc-GD1a, and between the cranial, bulbar and sensory variants of GBS and antibodies to the disialylated gangliosides GQ1b, GT1a, GD1b and GD3. In addition to clinical and serological studies, the origins and measurement of anti-glycolipid antibodies and their relationships to similar carbohydrate structures on infectious organisms, particularly Campylobacter jejuni, are discussed in the context of a molecular mimicry hypothesis. The structure and nomenclature of relevant glycolipids are outlined, along with information on their localization in nerve, and the influence this has on clinical phenotypes. Major advances have been made in animal modelling of anti-glycolipid antibody-associated diseases, both in vitro and in vivo. This has advanced our understanding of the role of anti-GQ1b antibodies in Miller Fisher syndrome with particular respect to the motor nerve terminal as a potential site of injury, and led to the creation of rabbit models of anti-GD1b and anti-GM1 antibody-mediated sensory and motor neuropathy, respectively. With such information in place, it will now be possible to determine the precise mechanisms by which antibodies injure the different compartments of peripheral nerve and establish how a range of immunomodulating therapies, including current treatments, exert their therapeutic effects. Despite these very significant advances, considerable gaps in our knowledge persist, and it is likely that other pathogenic pathways operate in inflammatory neuropathy that are unrelated to glycolipid antibodies, although these are outside the scope of this review.

Keywords: autoantibody; autoimmunity; gangliosides; Guillain–Barre syndrome; peripheral neuropathy

Abbreviations: AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; AMSAN = acute motor and sensory axonal neuropathy; CANOMAD = chronic ataxic neuropathy, ophthalmoplegia, IgM paraprotein, cold agglutinins and disialosyl antibodies; CMV = cytomegalovirus; DRG = dorsal root ganglion; GBS = Guillain–Barre syndrome; Ig = immunoglobulin; LPS = lipopolysaccharide; MAG = myelin-associated glycoprotein; MFS = Miller Fisher syndrome; NMJ = neuromuscular junction; SGLPG = sulfated glucuronyl lactosaminyl paragloboside; SGPG = sulfated glucuronyl paragloboside

Introduction
The identification and characterization of neural autoantigens of pathogenic significance has been achieved successfully for a small number of disorders, including several neuromuscular junction (NMJ) diseases, but has remained elusive in major areas of clinical neuroimmunology, particularly for demyelinating diseases. Despite the ability to generate antigen-specific animal models of peripheral demyelinating diseases, such as myelin protein-induced experimental allergic neuritis,
these models in general have not led to the identification of clinically applicable disease markers, although recent progress in this area is being achieved (Hughes et al., 1999; Gabriel et al., 2000; Ritz et al., 2000; Kwa et al., 2001). One area in which considerable progress has been made is in the relationship between anti-glycolipid antibodies and neuropathy, a field of research that arose primarily from clinical-serological observations, rather than experimental studies. Thus, the identification of anti-glycolipid antibodies in large cohorts of patients with peripheral neuropathy, and their association with particular clinical phenotypes, both topographical and fibre type specific, has uncovered a wealth of clinical-serological associations. Speculation about their role in pathophysiology provides a rationale for laboratory-based research. Ironically, the reverse problem to that traditionally encountered in neuroimmunological research now exists, i.e. the difficulty in translating the human serological data into experimental models of neuropathy induced by anti-glycolipid immune responses. Despite this very significant progress in our understanding of anti-glycolipid antibody-mediated neuropathy, both gaps and inconsistencies in our knowledge persist. Some opinion holds that other pathogenic pathways operate in inflammatory neuropathy that are unrelated or even contradictory to a model based on anti-glycolipid antibodies. Identifying such pathways remains a major challenge for investigators and, where relevant, these data are discussed in outline.

An early impetus to the search for nerve autoantigens arose when it was recognized that acquired polyneuropathies occurred in association with benign monoclonal gammopathies; this now forms an important subset of predominantly late-onset neuropathy (Kyle, 1992; Quarles, 1997; Ponsford et al., 2000). It seemed hypothetically likely that the monoclonal paraprotein might have anti-neural activity, and the first such antigen to be identified was the myelin-associated glycoprotein (MAG). Stemming from this observation, it transpired that the antigen specificities of the paraproteins frequently were directed to carbohydrate determinants present on different glycolipids distributed in neural tissue, in addition to glycoproteins such as MAG. The first clinical-serological association to be studied in detail was the immunoglobulin M (IgM) paraproteinaemic neuropathy with reactivity against MAG and the cross-reactive glycolipids, sulfated glucuronyl paragloboside (SGPG) and its higher lactosaminyl homologue, sulfated glucuronyl lactosaminyl paragloboside (SGLPG) (Latov, 1994; Chassande et al., 1998; Quarles and Weiss, 1999). Chronic motor neuropathies were then identified in association with polyclonal or monoclonal IgM antibodies directed to GM1 and other Gal (β1–3) GalNAc-bearing glycolipids including GD1b and asialo-GM1: these are now known to be present in ~50% of cases of multifocal motor neuropathy with conduction block (Kornberg and Pestrkon, 1995), depending upon the clinical definition and detection methodology used (Leger et al., 2001). In 1985, the first case of IgM paraproteinaemic neuropathy in which the paraprotein reacted with NeuAc (α2–8) NeuAc (α2–3) Gal-configured disialylated gangliosides including GD1b, GD3, GD2 and GT1b was reported (Ilyas et al., 1985), and many further cases of this syndrome have now been described (Willison et al., 2001).

Although originally described in association with paraproteinaemic polyneuropathies, the main recent clinical and research impetus on the role of antibodies directed at glycolipid epitopes has been in the context of the acquired inflammatory neuropathy, Guillain–Barre syndrome (GBS). Anti-ganglioside antibodies were first found in cases of GBS in 1988 (Ilyas et al., 1988b). Early studies were performed on the basis that anti-glycolipid antibody had been identified in patients with chronic demyelinating polyneuropathies and multifocal motor neuropathies. Our knowledge of anti-glycolipid antibodies in chronic neuropathies is extensive, and it transpired that there were many interesting parallels with GBS. A wealth of new information covering different aspects of this area is now available. Thus, antibodies to a wide range of glycolipids including GM1, GM1( NeuGc), GM1b, GalNAc-GM1b, GD1a, GalNAc-GD1a, GD1b, 9-O-acetyl GD1b, GD3, GT1a, GT1b, QQ1b, QG1b, LM1, galactocerebrosides and SGPG have been reported in >200 papers on GBS and other inflammatory neuropathies, as case reports and in larger series.

Placing this literature into a clinical and pathophysiological framework is complex, and several points need emphasizing. Firstly, anti-ganglioside antibodies assays, on which much of these data are based, are technically capricious, are not served by a uniform supply of glycolipid reagents and have high inter-laboratory variation. Secondly, the epidemiological patterns of anti-ganglioside antibodies may vary substantially between geographic regions, according to the prevalent subtypes of GBS and their relationship to preceding infections, such as Campylobacter jejuni infection. Thirdly, host susceptibility factors controlling the immune response to glycolipid epitopes that are related specifically to individuals and/or populations with particular genetic or environmental backgrounds may be present. In much of the anti-ganglioside antibody literature, these factors are not controlled in a systematic fashion. Some of the most significant publications in this area have come from anti-ganglioside antibody analysis of sera collected as part of carefully controlled clinical studies and trials (Rees et al., 1995a; Jacobs et al., 1996; Hadden et al., 1998; Ang et al., 1999; Yuki et al., 2000a), rather than random ascertainment of sera from affected patients. Despite these and other caveats, very significant progress is being made, and some clear disease patterns have emerged, and others are still emerging.

**Glycosphingolipid structure and nomenclature**

Glycosphingolipids are composed of a ceramide (N-acylated sphingosine) attached to one or more sugars (hexoses) (Ledeen and Yu, 1982). A selection of some of the clinically relevant structures is shown in Fig. 1. The hydrophobic
ceramide is immersed in the lipid membrane and when the hydrophilic carbohydrate structure is exposed extracellularly, as is the case with plasma membranes, it is capable of acting as an autoantibody target. All known neuropathy-associated antibodies target this extracellular carbohydrate structure, rather than the ceramide moiety. One of the simplest glycosphingolipid structures of clinical relevance is galactocerebroside, comprising galactose linked to ceramide (monogalactosylceramide). Sulfatide is galactocerebroside sulfated on the third carbon of galactose. The term ganglioside refers to the large family of glycosphingolipids that contain sialic acid linked to the oligosaccharide core, synthesized through addition of monosaccharides in a stepwise fashion by glycosyltransferases and sialyltransferases. Gangliosides are present throughout the body but are very highly concentrated in the nervous system (Ledeen, 1985). The nomenclature proposed by Svennerholm is used most widely and accepted for gangliosides of the ganglio-series (IUPAC-IUB Commission on Biochemical Nomenclature, 1977; Svennerholm, 1994). The designations are based on the findings that brain tissue contained four major gangliosides with a ganglio-series tetraose chain of neutral sugars (i.e. asialo-GM1) that are sialylated in different positions, although it is now recognized that there are >100 structurally distinct gangliosides. Four gangliosides, GM1, GD1a, GD1b and GT1b, were designated to belong to the G1 series, where G stands for ganglio-series ganglioside. The four major gangliosides differ with regard to the number and position of their sialic acids, where M, D and T stand for mono-, di- and tri-sialosyl groups. Thus there are two disialosylgangliosides, GD1a and GD1b. Although ‘b’ normally is used to designate gangliosides with a disialosyl group attached to the internal galactose (so-called ‘b-series’ gangliosides), the term GM1b is used for the monosialosylgangliotetraosylceramide in which the sialosyl group is attached to the terminal galactose, in contrast to GM1a (normally referred to more simply as GM1) in which the sialic acid is on the internal galactose. When three sialic acids link to the internal galactose of the ganglioside, they are designated to belong to the c-series. Now that the biosynthetic pathway of gangliosides of the ganglio-series has been elucidated in large part, it is evident that this early description of the ‘a’-, ‘b’- and ‘c’-series predicted the crucial role of the sialyltransferases in ganglioside biosynthesis. Gangliosides lacking the terminal galactose, pre-terminal galactosyl-N-acetylgalactosamine or internal galactose are assigned the number 2, 3 or 4, respectively.

One major difference between the CNS and PNS is the abundance of neolacto-series gangliosides in the PNS that are localized mainly in myelin, as recently reviewed (Ogawa-Goto and Abe, 1998). There is still no general agreement about the nomenclature of the neolacto-series gangliosides. The term LM1 is used for the sialosylneolactotetraosylceramide, which is also known as sialosylparagloboside. LM1, GM1, GM3, GM2 and sialosyllactosaminylparagloboside (also known as Hex-LM1) are the major monosialosylgangliosides in human peripheral nerve, although only some of these have been identified as dominant autoantigens in peripheral nerve diseases. SGPG and its higher homologue SGLPG have structures similar to that of LM1, except for a 3-sulfated glucuronic acid instead of sialic acid on the terminal saccharide chain. SGPG and SGLPG were discovered as a direct result of studying anti-MAG IgM paraproteins from patients with chronic polynuropathy (Ariga et al., 1987). In man, most ganglioside sialic acid is in the N-acetyl form, as opposed to the N-glycolyl configuration that is common to many other species.

Detection methods for gangliosides in tissues: benefits and limitations

When considering the pathogenic relationship between the presence of an antibody and neuropathy, it is clearly important to have detailed knowledge of the glycosphingolipid composition and distribution within the PNS in both humans and experimental animals and furthermore, in species from which gangliosides are purified for experimental and
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diagnostic use. Additionally, studies using autoimmune neuropathy sera may continue to identify additional glycosphingolipids that have yet to be identified as autoantigens, as was the case for SGPG and SGLPG; these will be aided by further development of purification and analysis methods (Hirabayashi et al., 1988; Taki and Ishikawa, 1997). These issues are not straightforward since the regulation of ganglioside expression, their analysis and purification are complex. Gangliosides are developmentally regulated and spatially segregated, varying between different peripheral nerve fibre types, and between different species. A complete map of the ganglioside composition of human nerves, and a comparison between species used for experimental modelling, would be a valuable resource, although this may still have limitations. As indicated above, the pattern of anti-ganglioside activity detected in a patient’s serum correlates to some extent with the clinical pattern of neuropathy, suggestive of a differential distribution of target gangliosides throughout the PNS.

The accessibility of gangliosides to circulating antibodies, being protected in their neural environment by the blood–nerve barrier and other factors, is important (Lloyd et al., 1992). One explanation for the lack of CNS involvement in anti-glycolipid antibody-associated neuropathy, despite the wide distribution of gangliosides in the CNS, would thus be the protection from autoimmune attack afforded by the blood–brain barrier. The blood–nerve barrier also needs to be overcome in order to allow antibody access to normally cryptic sites. Blood–nerve barrier injury could be mediated by anti-ganglioside antibodies, as glycolipid antigens are expressed on intraneuronal microvascular endothelial cells, thereby potentially mediating the destruction or malfunction of the blood–nerve barrier (Kanda et al., 1994).

In addition to localization and accessibility, ganglioside function within a given structure may influence the nature and development of antibody-mediated injury (Tettamanti and Riboni, 1993; Wu and Ledeen, 1994; Yu and Ariga, 1998). The calcium-binding properties of gangliosides have been demonstrated in several model membrane systems, and it is possible that one function is to chelate extracellular calcium that may be of relevance to nerve terminal injury. Gangliosides aggregate in glycosphingolipid- and cholesterol-rich lipid membrane microdomains, termed functional rafts, where they interact with membrane proteins and modulate events such as signal transduction and receptor function (Simons and Ikonen, 1997; Stoffel and Bosio, 1997). Thus, the pathogenic role of anti-ganglioside antibodies is likely to depend not only on how they affect the number and distribution of gangliosides but also on the extent to which the target gangliosides are intimately involved in modulating neuronal function. Additionally, activated complement components may in turn affect the normal functioning of ganglioside raft-associated proteins. The relative contribution of these factors may vary from site to site, and between anti-ganglioside antibodies of differing reactivity.

The two approaches used to establish the anatomical distribution of gangliosides each have their merits and limitations. Biochemical analysis has been useful to identify significant differences in the ganglioside composition of different nerves and can reveal subtle differences in the overall lipid composition. However, this approach is limited by the pleomorphic composition of tissue and the loss of information about microanatomical distribution. In such circumstances, the second approach, that of immunohistology or other in situ ligand-binding studies (e.g. using ganglioside-binding bacterial toxins such as cholera toxin), can reveal fine structural detail about ganglioside distribution at the cellular and subcellular level. For these studies, high quality reagents such as affinity-purified antisera or monoclonal antibodies are essential. Many anti-ganglioside antibodies are not monospecific but may cross-react with structurally similar gangliosides and other glycoconjugate antigens, making extrapolation of results to ganglioside localization difficult.

Biochemical and immunohistological approaches may expose gangliosides that normally occupy cryptic sites (e.g. within compact myelin) by homogenization or tissue sectioning, and can thus misrepresent the ganglioside array that would be visible to circulating antibodies in physiological environments. Furthermore, gangliosides can be distributed heterogeneously within a membrane, as in functional rafts into which proteins such as growth factor receptors or ion channels are specifically included or excluded (Simons and Ikonen, 1997). The antigen density and the surrounding lipid environment can also markedly influence the ability of anti-ganglioside antibodies to bind; thus, failure to detect a ganglioside by immunohistology does not necessarily indicate its biochemical absence (Lloyd et al., 1992). A high local concentration of ganglioside may allow for good immunohistological detection, whereas a ganglioside which is evenly distributed throughout a membrane may have the same total tissue concentration in biochemical evaluation, yet not be detectable by immunohistology. Interpretation of both biochemical and immunohistological studies thus requires caution.

Ganglioside localization to specific nerve sites and fibre types

The simplest explanation for particular anti-ganglioside antibody-associated neuropathies being confined to a motor or sensory clinical phenotype is that the two systems are composed of different gangliosides, and this issue has been addressed in several experimental studies, including immunohistological analyses (Fig. 2). A comparison of total ganglioside composition of human spinal roots showed that GM1 (associated with antibodies in motor neuropathy) is relatively enriched in the ventral roots compared with the dorsal roots (Ogawa-Goto et al., 1992). Similarly, the cranial motor nerves supplying the extraocular muscles contain particularly high contents of GQ1b, the ganglioside antigen...
associated with ophthalmoplegia (Chiba et al., 1997). However, from these studies, it is also apparent that key gangliosides are also present at sites unaffected by the disease process. Thus, the absolute tissue distribution of gangliosides is an insufficient explanation for the regional localization of the clinical pathology.

The dorsal root ganglion (DRG) is a particularly interesting site to consider in these respects, and illustrates some of the anomalies described above. Functional subpopulations of DRG neurones can be distinguished by the expression of lacto-series and globo-series carbohydrates that correlate with their peptide and enzymatic phenotype (Dodd et al., 1984; Dodd and Jessell, 1985). It is very likely that ganglio-series antigens are also distributed selectively and that such differences may underlie the specific nature of sensory deficits. In the rodent DRG, the GM1 ligand cholera toxin B-subunit and anti-GM1 antibodies selectively identify a subset of DRG neurones. In contrast, cholera toxin and anti-GM1 antibodies bind the majority of neurones in the human DRG (O’Hanlon et al., 1996, 1998). Thus, GM1 is clearly present in the human DRG in abundance, yet the clinical phenotype of anti-GM1-associated antibody neuropathy is strikingly devoid of sensory features. Anti-GD1b antibodies are highly associated with sensory neuropathy and bind to the vast

Fig. 2 Immunolabelling of human and rodent peripheral nerve structures using anti-ganglioside monoclonal antibodies. (A and B) Mouse neuromuscular junction labelled with anti-neurofilament antibody and α-bungarotoxin (A, both green) which bind to the motor nerve axon and postsynaptic acetylcholine receptors, respectively, and a human monoclonal IgM antibody to disialylated gangliosides (B). Antibody binding is seen over the motor nerve terminal. Bar = 20 µm. (C and D) Human dorsal root ganglion labelled with anti-neurofilament antibody (C), and a human monoclonal IgM antibody to disialylated gangliosides (D). The anti-ganglioside antibody produces granular staining of the neuronal cytoplasm, and stains the plasma membrane. Bar = 20 µm. (E–G) Teased fibre from mouse sciatic nerve showing a node of Ranvier, stained with human monoclonal anti-GM1 IgM antibody (E, red) and an anti-sodium channel antibody (F, green; overlaid in G). Paranodal myelin is stained by the anti-GM1 antibody. Bar = 20 µm. (H) Three NMJs in polynervated muscle fibres within the rat extraocular muscle are labelled with a human monoclonal IgM antibody to disialylated gangliosides (red) and the motor axon is labelled with anti-neurofilament antibody (green). Bar = 20 µm. Revised from Willison and O’Hanlon (1999).
majority of DRG neurones (Kusunoki et al., 1993b; Maehara et al., 1997). Disialosylated gangliosides (including GD1b, GT1b, GQ1b, GD3 and GD2) are prominent gangliosides in cultured DRG neurones and these can be lysed by anti-disialosyl antibodies (Ohsawa et al., 1993).

The node of Ranvier is another key site of injury in autoimmune neuropathy. Immune attack directed at antigenic determinants located at the paranodal Schwann cell surface may lead to paranodal demyelination, whereas antigens targeted on the exposed axolemma may result in axonal degeneration, both of which would result in conduction failure. Ligand-binding studies have suggested that GM1, GD1b and polysialosylated gangliosides are enriched in the paranodal myelin loops of peripheral nerve (Fig. 2). In the oculomotor nerves affected in Miller Fisher syndrome (MFS), GQ1b is particularly enriched at nodes of Ranvier (Chiba et al., 1993). With respect to the neuronal components of the node of Ranvier, GM1 is present on the cytoplasmic surface of motor neurones (Corbo et al., 1992). Toxin- and antibody-binding studies have identified gangliosides on paranodal and internodal axolemma (Ganser et al., 1983; Ganser and Kirschner, 1984; Corbo et al., 1993) and the adaxonal membrane (Molander et al., 1997). Similarly, an antibody reactive with disialosylated gangliosides has been shown to bind to internodal axolemma and/or adaxonal Schwann cell cytoplasm (Willison et al., 1996). Antibodies to GD1a are associated with pure motor axonal neuropathy, and preferentially stain ventral root axons in comparison with dorsal root axons, indicating a good correlation between ganglioside localization and phenotype in this example (Gong et al., 2001).

The presynaptic NMJ recently has been considered a potential target vulnerable to autoimmune attack in GBS, with some clinical justification and also for a variety of hypothetical reasons. First, it lacks a blood–nerve barrier, thereby readily allowing access to circulating autoantibodies. Secondly, it is the site for other paralytic antibody-mediated diseases including myasthenia gravis and Lambert–Eaton myasthenic syndrome. Thirdly, it is rich in gangliosides including GQ1b, GM1 and GD1a. Fourthly, it is the binding site for a wide range of bacterial toxins that also use gangliosides as ectoacceptors (Willison and Kennedy, 1993). In particular, cholera and tetanus toxins are readily taken up into nerve terminals, loaded into synaptic vesicles and ultimately transported back to the motor neurone cell body (Wan et al., 1982; Hirakawa et al., 1992). As a result of this property, enzymatic conjugates of cholera toxin are used frequently as retrograde neuronal markers. As expected, histological analyses have demonstrated cholera toxin and anti-GM1/GD1b antibody binding to the NMJ (Lavit et al., 1988; Schlupe and Steck 1988; O’Hanlon et al., 1998). Antibodies reactive to polysialylated gangliosides also bind to the NMJ (Fig. 2). Some of the α-series gangliosides specific to cholinergic neurones (Chol-1 antigens) are also expressed at the mature NMJ which may make them potential targets for autoantibodies (Derrington and Borroni, 1990).

Measurement of anti-glycolipid antibodies in serum
In parallel with clinical developments, there has been a recent widespread increase in the use of anti-ganglioside antibody assays as both diagnostic and research tools for studying autoimmune peripheral neuropathies (Adams et al., 1991; Kornberg and Pestrkon, 1994; Willison, 1994; van Schaik et al., 1995; Taylor et al., 1996). Whilst this is highly desirable, it has also led to some inconsistencies in methodological approaches. Assays of anti-ganglioside antibodies present technical difficulties, with variables including antigen source and purity, timing of clinical sampling, details of assay method and definition of normal ranges for serum titres. The screening method used by most laboratories is an enzyme-linked immunosorbent assay, and many factors that influence this assay and contribute to the inter- and intra-laboratory variation have been identified (Ravindranath et al., 1994; Willison et al., 1999). Some attempts to recommend standard methodology have been made, although most laboratories have established local in-house immunodetection protocols based on elements of previously published assay methods (Ben Younes-Chennouf et al., 1992; Bansal et al., 1994; Bech et al., 1994). In two multicentre comparative studies, in which investigators used local methodology, there was agreement on clearly positive or negative cases but variable results with intermediate titre samples (Marcus et al., 1989; Zielasek et al., 1994). An additional technique for assaying sera for anti-glycolipid antibodies is thin-layer chromatography overlay (Figs 3 and 4); however, this is only carried out in specialized laboratories and thus not routinely available. One enzyme-linked immunosorbent assay protocol designed by a pan-European consortium that we commonly use has been described previously in detail (Willison et al., 1999). Some glycolipids, including SGPG and SGLPG, are not commercially available and thus cannot be assayed for with ease, unless diagnostic laboratories have access to glycolipid purification facilities. Antibodies to MAG, with which some anti-SGPG antibodies share reactivity, are normally sought by western blot of CNS myelin.

Acute clinical syndromes
Guillain–Barré syndrome
A large body of data indicates that anti-glycolipid antibodies are present in the acute phase sera of a proportion of patients with GBS. Whilst this falls far short of providing proof of pathogenic involvement for such antibodies, the strength of these associations warrants continued investigation. Such findings should not undermine the possible relevance of other classes of antigens; indeed, efforts are also underway to identify other pathogenic factors, including T cell and antibody responses to myelin proteins and glycosaminoglycans (Pestrkon et al., 1998; Hughes et al., 1999). A number of factors need to be highlighted at the outset of this discussion. First, only a proportion of patients with GBS have identifiable...
Neuropathy and anti-glycolipid antibodies

Anti-glycolipid antibodies. The associations of specific anti-glycolipid antibodies with definite clinical forms of GBS or variants are at best statistically significant and certainly not constant, as one would ideally expect to see if a specific antibody is indeed responsible for a specific clinical or electrophysiological presentation. The one situation where a clear and constant association exists is between anti-GQ1b/GT1a antibodies and MFS; however, other GBS variants such as acute motor axonal neuropathy (AMAN), or antibody specificities such as GM1, do not live up to this standard. Explanations for the reported differences in the clinical correlates of these antibodies are discussed in the foregoing sections.

The syndrome of symmetrical, rapidly evolving flaccid paralysis and areflexia described by Guillain, Barré and Strohl in 1916 (Guillain et al., 1916) has now been subclassified on the basis of distinct patterns of axonal and demyelinating forms of the disease. GBS formerly was considered to be a relatively homogeneous entity characterized by segmental demyelination in all cases. The most frequent pattern of GBS encountered in Europe and North America is that originally described as acute inflammatory demyelinating polyneuropathy (AIDP), characterized by demyelination and a variable degree of lymphocytic infiltration (Asbury et al., 1969). In severe cases, axonal degeneration may accompany the demyelination as a 'bystander' event.

Less frequently encountered in North America and Europe, but common in China (McKhann et al., 1993; Griffin et al., 1996a) and Japan (Kuwabara et al., 1998b), and probably other regions of the developing world, especially where C. jejuni infections are frequent, is the axonal pattern of GBS, in which primary axonal degeneration occurs with little or no demyelination. Feasby et al. (1986) first presented evidence to support the possibility that some cases of GBS might be due to primary motor and sensory axonal degeneration without preceding demyelination and that the target antigen might lie on the axon. Collaborative Chinese-African studies firmly established the presence of primary axonal GBS, and it is now recognized that the axonal patterns can be classified further into two groups, AMAN and acute motor and sensory axonal neuropathy (AMSAN) (McKhann et al., 1993; Griffin et al., 1996a). The principal clinical method for distinguishing the AMAN, AMSAN and AIDP patterns is electrodiagnostic, and clear criteria have been formulated for separating the phenotypes. One caveat that affects interpretation of anti-ganglioside antibody studies is that some patients with inexcitable nerves often cannot be classified into axonal or demyelinating groups.

Pathological findings in autopsy cases of AMAN showed axonal degeneration of the motor axons with little demyelination or lymphocytic infiltration. Early changes at the nodes of Ranvier of motor fibres are accompanied by the presence of IgG and complement deposits on the axolemma, and macrophage recruitment, the findings being strongly suggestive of highly selective antibody-mediated attack on axonal membranes, rather than T cell-mediated disease (Griffin et al., 1996b; Hafer-Macko et al., 1996a). Macrophages insert processes into the nodal gap, penetrating the overlying basal lamina of the Schwann cell, and enter the periaxonal space of the internode. The end stage of this process is interruption of motor axons, with degeneration extending as far up as the ventral root entry zone. Patients with such extensive Wallerian-like degeneration of ventral root fibres could recover only by regeneration, a process requiring very long periods of time and unlikely to be complete. Some patients with AMAN, however, recover quite rapidly, which suggests that transient loss of motor function may be related to antibody binding to nodes of Ranvier with subsequent blocking of conduction but without axonal transection, or be due to axonal injury in the very distal part of the motor nerve (Ho et al., 1997; Kuwabara et al., 1998a). In AIDP, in contrast, the immune attack is directed against components of the Schwann cell abaxonal membrane and is accompanied by the more characteristic features of vesicular demyelination (Hafer-Macko et al., 1996b). With respect to the current consideration on anti-glycolipid antibodies, it is clearly crucial to understand how this might be related to the presence or absence of particular glycolipid antigens at these sites or, alternatively, other as yet unidentified classes of antigens.

GBS is a self-limiting disease, occurring 1–3 weeks after infection, with muscle weakness usually reaching a nadir within 4 weeks, followed by partial or complete recovery taking place over weeks to months; the long-term prognosis is dependent upon the site and extent of axonal injury. This temporal pattern of evolution and decay is suggestive of...
pathophysiology centred on a primary humoral immune response, and this is also consistent with the rate of recovery being accelerated by plasma exchange or intravenous immunoglobulin. The therapeutic effect of plasma exchange presumably is related to the removal or dilution of circulating factors, and some indirect evidence suggests that the critical factors are most likely to be IgG immunoglobulins (Thornton and Griggs, 1994). Plasma concentrations of cytokines are elevated in GBS patients during the acute phase of the illness, but because their circulating half-lives are only a few hours, the effect of plasma exchange on their plasma levels is likely to be short term. Complement depletion resulting from plasma exchange is also brief, for the same reasons. The respective half-lives of IgM and IgA are 5 and 6 days. In contrast, the half-life of IgG is 21 days, except for the IgG3 subclass (7 days) that is longer than that of other plasma proteins. Plasma IgG level may be reduced for up to 5 weeks following a course of plasma exchange. Autoreactive IgG, which binds to neural components and thereby activates complement and recruits macrophages, may thus be the most important factor to be removed during plasma exchange. The mechanisms of action of intravenous immunoglobulin in ameliorating GBS are widely debated but poorly understood, although they may include antibody neutralization through a number of mechanisms (Stangel et al., 1999).

In addition to historical models of putative T cell-mediated mechanisms, antibodies directed to peripheral nerve were long believed to participate in the development of GBS, but no target molecules for the autoantibodies were found until 1988, when Ilyas et al. (1988b) first reported serum antibodies to gangliosides in five of 26 patients. IgG antibodies in one patient reacted strongly with LM1 and its hexose analogue Hex-LM1. IgG from two other patients with GBS reacted with GD1b. IgM antibodies in sera from two other patients reacted with GD1a and GT1b, which have a shared terminal carbohydrate sequence. The antibody titres in these cases decreased with clinical improvement. In the same year, Inuzuka et al. (1988) reported that a patient with GBS following Mycoplasma pneumoniae infection had IgM antibody to Hex-LM1. On the basis of these early findings, the search for anti-ganglioside antibodies in GBS accelerated rapidly throughout the 1990s.

Acute motor axonal neuropathy
The first reports of anti-GM1 antibodies in GBS appeared in the early 1990s, around the time at which concepts of GBS were emerging that led to the AMAN and AIDP subclassifications. The literature surrounding anti-GM1 antibodies that are found in both AMAN and AIDP remains confusing for a large number of reasons. First, and perhaps most unappealing in our view although maintained by others, is the possibility that anti-GM1 antibodies are irrelevant to the development of either AMAN or AIDP, but solely exist in GBS serum as bystander or secondary events. Thus they are a variable linked to the disease, either through preceding infection or as a result of a secondary immune response to nerve injury, but are independent of its pathogenesis. There are some data to support this view (Press et al., 2001). Secondly, subcategories of anti-GM1 antibodies may exist that have not been fully elucidated. For example, some anti-GM1 antibodies may be GM1 monospecific whereas others may cross-react with other gangliosides, and these subcategories of anti-GM1 antibodies may correlate with disease subgroups. Thirdly, GM1 and related epitopes may exist in both myelin and axolemmal membranes in varying concentrations or configurations that can lead to preferential binding of antibody under different circumstances in different individuals. Thus some individuals may be more susceptible to myelin injury and others to axonal injury upon exposure to a particularly subcategory or class of anti-GM1 antibody. Furthermore, this may vary during the course of the disease. Thus, at a node of Ranvier for example, axolemmal GM1 may be ‘cryptically’ disguised early in the course of the disease, but become exposed for antibody binding following paranodal demyelination induced by anti-GM1 (or other antibody) binding to paranodally sited GM1. An illness that started as AIDP could then evolve into AMAN, or AIDP with secondary axonal injury. Clearly a large number of complexities can be introduced that confound these considerations. Thus we should consider the foregoing data with an open mind and in further studies attempt to control for as many variables as possible. This applies not least to the method, definition and timing of the clinical electrophysiological analysis and the serological analyses, on which much of this discussion rests and which in some studies have only been conducted on a single occasion.

The report in 1990 of two patients with AMAN subsequent to C. jejuni enteritis in whom high titres of anti-GM1 IgG antibodies were found during the acute phase of the illness was followed by many subsequent studies (Yuki et al., 1990). Walsh et al. (1991) then reported that 14 out of 95 patients (15%) with GBS had anti-GM1 antibodies, and that the predominant immunoglobulin class was IgG rather than IgM. Kornberg et al. (1994) also reported that anti-GM1 IgG antibodies were strongly associated with AMAN. Correlations were sought between the presence of these antibodies and the prognosis in terms of long-term disability. In early studies, neither Enders et al. (1993) nor Vriesendorp et al. (1993) found a correlation between anti-GM1 antibody titres, C. jejuni infection and the severity, type (axonal versus demyelinating) or outcome of GBS. Rees et al. (1995a) showed that patients who were anti-GM1 antibody positive were more likely to have axonal degeneration and had less sensory disturbance than anti-GM1 antibody-negative patients. In a subgroup analysis of GBS cases from another large series of patients, it was also found that patients with anti-GM1 antibodies had a more severe neuropathy with predominantly distal weakness and no sensory involvement (Jacobs et al., 1996). These findings were supported further by an electrophysiological analysis showing that anti-GM1 antibodies are more common in the patient groups with axonal injury or...
inexcitable nerves (Hadden et al., 1998). Some consideration
has centred on the relative contribution of IgG and IgM
antibodies to these clinical features. Four of 24 anti-GM1-
positive patients reported by Rees et al. (1995a) had IgM
class antibody alone, but this proportion was unknown in the
study of Jacobs et al. (1997a). As outlined above, failure to
take into account anti-GM1 antibody fine specificities and
isotypes or identify the presence of other antibodies co-
occurring in the same patients (such as anti-GD1a or anti-
myelin protein antibodies) may confound the relationship
between electrodagnosis and the presence of anti-GM1
antibody. For example, in a study by Kuwabara et al.
(1998b), the relationship between electrodagnosis and anti-
GM1 IgG or IgM antibody was investigated directly, and a
significant association between axonal dysfunction and the
presence of anti-GM1 IgG antibody, but not IgM, was shown.
In this analysis of 34 GBS patients, 16 were anti-GM1 IgG-
positive, 12 of whom were classified by electrodagnostic
criteria at the first examination as having AMAN or AMSAN,
three as having AIDP, and one was unclassified. In three
patients initially diagnosed as having AIDP, conduction
slowing resolved within days, and one of the AIDP cases and
three AMAN cases showed rapid recovery of distal com-
 pound muscle action potential amplitudes without evidence
of concomitant denervation. The time courses of conduction
abnormalities were distinct from those in anti-GM1 IgG-
negative AIDP patients. Rapid resolution of conduction
slowing and block, and the absence of slow components
indicative of remyelination, suggest that the conduction
failure might be caused by impaired physiological conduction
at the nodes of Ranvier rather than segmental demyelination.
Thus it is possible that reversible conduction failure, in
addition to or in place of axonal degeneration, could account
for some of the pathophysiological events occurring in the
anti-GM1 IgG-positive GBS cases. In both cases, immune-
mediated attack may occur on the axolemma of motor fibres,
and some experimental and human pathological findings
support this, as discussed below. The extent to which these
interpretations are supported by other studies is hard to
unravel, because of methodological variations, but there are
some parallels. For example, in the study of Hadden et al.
(1998), the proportion of patients initially classified as
‘demyelinating’ who later changed to ‘axonal’ was signifi-
cantly higher in those with anti-GM1 IgG antibody (five of
27, 19%) compared with those without the antibody (one of
108, 1%; \( P = 0.0006 \)), but there was no such relationship
with anti-GM1 IgM antibody. These types of studies underlie
the rather complex considerations that need to be controlled for
if we are to unravel all the elements of anti-GM1 antibody-
associated neural injury.

In addition to the antibodies directed against GM1
described above, Kusunoki et al. (1996a) found that
antibodies to a minor monosialosylganglioside GM1b were
present in 22 out of 104 GBS cases tested and that this was
highly disease specific. Their observation was confirmed in
other studies (Yuki et al., 1997a). In a further study of 132
patients with GBS by Yuki et al. (2000a), 25 (19%) patients
had anti-GM1b antibodies; these were IgM class in 14, IgG
class in 15, and mixed isotype in four patients. The anti-
GM1b antibody-positive cases, especially those with IgG
class antibodies, had a distinct clinical pattern compared with
the 107 seronegative cases in this series, showing more
frequent serological evidence of preceding \( C. jejuni \) infection,
and a more rapidly progressive, severe and predominantly
distal weakness with slow recovery. Furthermore, cranial
nerve and sensory deficits were less common in the patients
with anti-GM1b antibodies.

Anti-GD1a IgG antibodies have also been detected in
AMAN. This was first observed in two patients with severe
axonal GBS in 1992 (Yuki et al., 1992c). In a larger series of
37 patients, a significant association was found between the
presence of anti-GD1a IgG antibody and a poor clinical
outcome, as manifested by prolonged artificial ventilation
with poor recovery at 3 months (Yuki et al., 1993d). An
autopsy in one case showed severe axonal degeneration and
degenerative demyelination of peripheral nerves, lymphocytic
infiltration and marked central chromatolysis of the lower
motor neurone cell bodies. Anti-GD1a antibodies have been
found to be highly specific for GBS in other studies,
especially when present at high titre (Carpo et al., 1996). In
a large group of Chinese patients with GBS and appropriate
controls, 24% of AMAN patients and none of the AIDP
patients or control subjects had high titre anti-GD1a IgG
antibodies. The anti-GD1a antibody was the most specific
for AMAN among other anti-glycolipid antibodies tested (GM1,
GD1b, asialo-GM1 and GQ1b), and in particular indicated that
anti-GD1a IgG antibody was better able to discriminate
between AMAN and AIDP than anti-GM1 antibody (Ho et al.,
1999). An interesting feature of this study that supports some
of the methodological considerations described above was the
definition of criteria for what constitutes a positive result for
anti-GD1a antibodies. Thus when using a cut off titre > 1 : 100,
60% of AMAN versus 4% of AIDP patients had IgG anti-
GD1a antibodies, whereas when using a cut off titre > 1 : 1000,
24% of AMAN patients and none of the AIDP patients had
IgG anti-GD1a antibodies. Thus the identification of a
relationship amongst these features depends on how one
uses the serological and electrophysiological criteria, with
stricter criteria leading to more specific, but less sensitive
results in this example.

Kusunoki et al. (1994) found that GalNAc-GD1a is yet
another target molecule for serum antibodies in the AMAN
variant of GBS, being detected in six out of 50 patients (12%),
a finding subsequently confirmed by Yuki et al. (1996b). In
a series of 147 cases, anti-GalNAc-GD1a antibodies were
found to mark a distinct clinical pattern characterized by lack
of cranial nerve involvement (87% versus 38%), distal-
dominant weakness (80% versus 25%) and no sensory
disturbance (73% versus 22%) (Hao et al., 1999).
Another recent study by Ang et al. (1999) found that anti-
GalNAc-GD1a antibodies could be detected in 19 out of 132
cases (14%) and correlated with antecedent \( C. jejuni \) infec-
tion, a rapidly progressive, more severe course with predominantly distal weakness and little sensory and cranial nerve involvement. Similar findings have been reported by Kaida et al. (2000).

As described above, anti-GM1, anti-GM1b, anti-GD1a and anti-GalNAc-GD1a IgG antibodies have been demonstrated in numerous studies to have a strong association with the AMAN pattern of GBS. In a recent extensive study of 86 consecutive Japanese GBS patients by Ogawara et al. (2000), electrodiagnostic criteria showed AIDP in 36% of the patients and AMAN in 38%. The most frequent anti-ganglioside antibodies were of the IgG class and against GM1 (40%), GD1a (30%) and GalNAc-GD1a (17%), all of which showed a strong association with AMAN. These relationships between clinical phenotype, anti-ganglioside antibody and antecedent infection are summarized schematically in Tables 1 and 2.

AIDP cases exhibit a slower recovery than AMAN, in addition to sensory fibre involvement, but the pathologies are very similar (Feasby et al., 1986; McKhann et al., 1993; Griffin et al., 1996a). Moreover, both conditions may follow C. jejuni enteritis. Griffin et al. (1996a) proposed that AMAN and AIDP are part of the spectrum of a single type of immune attack on the axon. In a study to investigate whether anti-ganglioside IgG antibodies could be used as immunological markers to differentiate AMAN from AIDP, the frequencies of anti-GM1, anti-GM1b and anti-GD1a IgG antibodies were similar (Yuki et al., 1999b). These data suggest that AMAN and AIDP share a common immunological profile and support the view that they form a spectrum, as proposed (Griffin et al., 1996a).

**Acute inflammatory demyelinating polyneuropathy**

 Clinically, patients with AIDP present with flaccid paralysis and areflexia and usually have some sensory loss either symptomatically or on physical examination. Electrophysiological testing typically reveals increased distal motor latencies and F waves accompanied by reductions in nerve conduction velocity, and temporal dispersion. AIDP was long presumed to be predominantly a T cell-mediated disorder. This presumption was based on the lymphocytic inflammation found on nerve biopsy in many cases (Asbury et al., 1969) and by analogy with the widely studied animal model, experimental allergic neuritis. Recently, the immunopathology of early and unusually well-preserved autopsies cases was evaluated and suggests that antibody-mediated injury may be more important than previously recognized, at least in some cases (Hafer-Macko et al., 1996b). In one highly informative pathological study on a patient who died 3 days after onset of symptoms, inflammation was scanty and only a few fibres had been completely demyelinated. Staining for complement activation demonstrated complement activation products on the outermost of these fibres which had early vesicular changes in the myelin sheaths, usually beginning in the outer lamellae of the sheath. The resulting pathological picture closely resembled the appearance of experimental conditions in which nerve fibres are exposed to anti-galactocerebroside antibody in the presence of complement (K. Saida et al., 1979; T. Saida et al., 1979). Thus, an attractive reconstruction based on this pathology is that an antibody directed against antigens on the outermost surface of the Schwann cell (the abaxonal Schwann cell plasmalemma) binds complement, resulting in sublytic complement activation and the development of transmembrane pore formation. Macrophages are recruited and also participate in the removal of damaged myelin. The nature of the antigen(s) on the abaxonal Schwann cell plasmalemma and within compact myelin that may be involved in directing the immune attack in AIDP to Schwann cells remains uncertain, and elusive. Some evidence suggests that anti-myelin glycolipid antibodies may be involved, along with antibodies to Schwann cell protein or carbohydrate determinants that are also expressed at the cell surface (Hughes et al., 1999).

Complement-fixing antibodies to peripheral nerve myelin have been detected in the serum of a high proportion of patients with GBS, using a sensitive assay that detects antibody binding to isolated human peripheral nerve myelin by fixation of the first component of complement C1 (Koski et al., 1985). In attempts to identify the myelin component, it was observed that some of the anti-peripheral nerve myelin antibodies in 12 GBS sera tested bound an unidentified neutral glycolipid of human peripheral nerve myelin and cross-reacted with Forssman antigen (Koski et al., 1989). However, in a follow-up study, anti-Forssman IgM and IgG antibodies could not be detected in GBS compared with controls (Ilyas et al., 1991). Whether this Forssman-like glycolipid can be verified as an autoantigen in sera from AIDP patients requires further investigation.

Two studies have suggested that galactocerebroside could be an autoantigen in GBS (Kusunoki et al., 1995; Hao et al., 1998), although in neither was the electrodiagnosis of all the anti-galactocerebroside antibody-positive cases described. In the report of Kusunoki et al. (1995), two of four patients with anti-galactocerebroside antibody were confirmed electrophysiologically as AIDP, but electrophysiological examinations were not performed in the others (S. Kusunoki, personal communication). In another study, the association of AIDP with anti-galactocerebroside antibody could not be shown (K. Susuki et al., unpublished observations). The interesting relationship between GBS and anti-galactocerebroside antibody following *M. pneumoniae* infection is described below.

Unlike CNS myelin, human peripheral nerve myelin contains LM1, Hex-LM1 and SGPG (Ogawa-Goto and Abe, 1998), and these glycolipids sensibly have been screened in a number of studies as potential antigens in AIDP. Inuzuka et al. (1988) described a single GBS patient who had IgM antibody to Hex-LM1, and Ilyas et al. (1988b) also reported one GBS patient who had IgG antibody to LM1 and Hex-LM1. A more detailed follow-up study led by Ilyas et al. (1992) showed
that 23% of GBS patients had anti-LM1 IgG, but none were found with anti-LM1 IgM antibodies. Fredman et al. (1991) detected anti-LM1 antibody in 58% of the GBS patients, but also observed such antibodies in 30% of their normal controls. In a further study from Japan, five out of 96 patients (5%) with GBS had high titres of anti-LM1 IgG, all of whom had AIDP (Yuki et al., 1996a). In another Japanese study, anti-LM1 IgG antibodies were detected in seven out of 140 patients (5%) with GBS, five of whom had AIDP but the other two were not classified (Yako et al., 1999). At odds with the view that there may be an exclusive relationship between anti-LM1 antibodies and AIDP, a study of 19 patients with AIDP and 21 patients with AMAN found two from each group with anti-LM1 IgG antibodies (Yuki et al., 1999b). In another study by Susuki et al. (2002), anti-LM1 IgG antibody was detected in only one patient with AIDP, whereas it was present in seven with AMAN and in one with AMSAN. Sera from the eight IgG anti-LM1-positive patients with AMAN/AMSAN also had IgG activity against the gangliosides GM1, GM1b, GD1a, GalNAc-GD1a, GD1b or GQ1b. Anti-LM1 IgG antibodies from the AMAN/AMSAN patients cross-reacted with other gangliosides, whereas IgG antibody from the AIDP patient was monospecific against LM1. Anti-LM1 IgG antibody, therefore, cannot be a marker of AIDP. Larger studies are needed to verify whether monospecific anti-LM1 IgG antibody could be a marker of AIDP.

With respect to anti-SGPG antibodies and AIDP, few positive data have been forthcoming despite strong hypothetical grounds for such a relationship. Ilyas et al. (1992) detected anti-SGPG IgG in 9% of GBS patients studied and anti-SGPG IgM in 15%. However, Yuki et al. (1996a) failed to detect anti-SGPG IgG in any GBS patients, with low titres of anti-SGPG IgM being found in 29% of cases. Another peripheral nerve-enriched glycolipid is GM2, and this appears to have a special relationship with preceding cytomegalovirus (CMV) infection, as discussed in detail below. The electrophysiological pattern of GBS after CMV infection is demyelinating, and anti-GM2 IgM antibody does appear in some cases of CMV-associated GBS (Visser et al., 1996; Jacobs et al., 1997c). Acute CMV infection without GBS is also associated with anti-GM2 IgM (Yuki and Tagawa, 1998). Another issue to consider is that GM2 cannot be detected in human peripheral nerves by the standard immunohistochemical techniques using anti-GM2 IgM antiserum (O’Hanlon et al., 2000). These studies raise some doubts about the pathophysiological significance of anti-GM2 IgM antibody in AIDP associated with CMV infection.

**Miller Fisher syndrome and related conditions**

**Miller Fisher syndrome**

This uncommon syndrome provides the most compelling rationale for continuing the search for anti-glycolipid antibodies in acute autoimmune neuropathy. MFS is characterized by the acute onset of ophthalmoplegia, ataxia and areflexia (Fisher, 1956), and is the most common variant of GBS with which it often overlaps clinically, accounting for 5–10% of cases. In one survey, the annual incidence has been estimated at 0.09 per 100 000 population (Emilia-Romagna Study Group on Clinical and Epidemiological Problems in GBS, 1986).

**Table 1 Clinical syndromes associated with specific anti-glycolipid antibodies**

<table>
<thead>
<tr>
<th>Clinical syndrome</th>
<th>Antibody against</th>
<th>Antibody isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic sensori-motor demyelinating neuropathy</td>
<td>SGPG, SGLPG</td>
<td>IgM (monoclonal)</td>
</tr>
<tr>
<td>Chronic ataxic neuropathy</td>
<td>GD1b, GD2, GD3</td>
<td>IgM (monoclonal)</td>
</tr>
<tr>
<td></td>
<td>GT1b, GQ1b</td>
<td></td>
</tr>
<tr>
<td>Multifocal motor neuropathy</td>
<td>GM1, GD1b, asialo-GM1</td>
<td>IgM (monoclonal or monoclonal)</td>
</tr>
<tr>
<td>Acute motor axonal neuropathy</td>
<td>GM1, GM1b, GD1a, GalNAc-GD1a</td>
<td>IgG</td>
</tr>
<tr>
<td>Miller Fisher syndrome</td>
<td>GQ1b, GT1a</td>
<td>IgG</td>
</tr>
<tr>
<td>Bickerstaff’s brainstem encephalitis</td>
<td></td>
<td></td>
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<tr>
<td>Ataxic Guillain–Barré syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngeal-cervical–brachial weakness</td>
<td>GT1a (GQ1b)</td>
<td>IgG</td>
</tr>
</tbody>
</table>

**Table 2 Glycolipid-mimicking structures identified on neuropathy-associated microorganisms**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Glycolipid mimicked</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>GM1, GM1b, GD1a, GalNAc-GD1a, GD3, GT1a, GQ1b</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>GM1, GT1a</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>Galactocerebroside</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>GM2</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Glycolipid-mimicking structures identified on neuropathy-associated microorganisms</th>
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<tbody>
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<td>Galactocerebroside</td>
<td><em>Mycoplasma pneumoniae</em></td>
</tr>
<tr>
<td>GM2</td>
<td>Cytomegalovirus</td>
</tr>
</tbody>
</table>
Neurology, 1998). Chiba et al. (1992) first reported that all six patients they studied with MFS had anti-GQ1b IgG antibodies during the acute phase of the illness, and this strong association was confirmed by other studies (Willison et al., 1993b; Yuki et al., 1993b; Carpo et al., 1998). It is now widely accepted that well over 90% of patients with MFS have this autoantibody. Equally significant is the complete absence of anti-GQ1b IgG antibodies from normal and other disease control groups, indicative of a high level of specificity for this disease association. The antibody titres peak at clinical presentation, decaying rapidly with the course of clinical recovery. Anti-GQ1b antibodies almost invariably cross-react with the structurally similar ganglioside GT1a (Chiba et al., 1993). Some MFS sera also demonstrate reactivity with other gangliosides such as GD3 and GD1b (Yuki, 1996). Anti-GQ1b IgG antibody titres are also elevated in the acute phase sera of some patients with ‘GBS with ophthalmoplegia’ (Chiba et al., 1993; Yuki et al., 1993b). The anti-GQ1b IgG antibody marker also identifies a cluster of closely related syndromes, often considered *forms frustes* of MFS, that have in common the presence of external ophthalmoplegia or cerebellar-like ataxia, as described below.

Biochemical analysis of the ganglioside fractions obtained from cranial nerves and the ventral and dorsal roots showed that the oculomotor, trochlear and abducens nerves, which innervate into the extraocular muscles, have a relatively higher proportion of GQ1b (Chiba et al., 1997). In addition, an immunohistochemical study on human tissues using an anti-GQ1b monoclonal antibody showed that GQ1b is specifically and densely localized in the paranodal myelin of the extramedullary portion of these three cranial nerves, but not in that of other cranial or peripheral nerves (Chiba et al., 1993). The close association between the anti-GQ1b IgG antibody and ophthalmoplegia is most probably explained by this uniquely concentrated localization of GQ1b to these sites.

**Bickerstaff’s brainstem encephalitis**

There has been a long-standing nosological discussion about the nature of this interesting syndrome and its relationship to MFS (Ropper, 1983). This debate has been rekindled by the finding that some cases of Bickerstaff’s brainstem encephalitis have anti-GQ1b IgG antibody (Yuki et al., 1993a; Odaka et al., 2001b; Winer, 2001). Bickerstaff first reported eight cases of ‘brain-stem encephalitis’ as a definitive syndrome (Bickerstaff and Cloake, 1951; Bickerstaff, 1957). Seven of the eight original patients had ophthalmoplegia, all eight had ataxia and four had areflexia or hyporeflexia, these features being suggestive of MFS. However, in addition to these features, all eight cases also showed impaired consciousness, and two had long-tract sensory disturbance. In 1982, Bickerstaff and colleagues reported on further cases in which they argued a central origin and close similarity to MFS (Al-Din et al., 1982, 1985). It has also been considered that Bickerstaff’s brainstem encephalitis should be a diagnosis reserved for patients showing coma, brisk reflexes or long-tract sensory disturbance in addition to the MFS triad (Yuki et al., 1993a). With respect to anti-GQ1b antibodies, three patients with Bickerstaff’s brainstem encephalitis associated with high anti-GQ1b IgG antibody titres that decreased with their clinical improvement have been found (Yuki et al., 1993a). In a larger study, 15 out of 25 patients (60%) with Bickerstaff’s brainstem encephalitis had anti-GQ1b IgG antibody (Odaka et al., 2001b). The finding that Bickerstaff’s brainstem encephalitis and MFS share common autoantibodies suggests that elements of the autoimmune mechanism are common to both, and that they are not distinct but are closely related conditions. Further work is needed to clarify the clinical and pathophysiological overlap in this interesting area (Winer, 2001).

**Acute ophthalmoparesis**

Occasional cases of extraocular muscle paralysis arising in the absence of ataxia or areflexia have been described which are of acute onset and self-limiting. Some patients have received prior immunizations or been exposed to antecedent infections. Chiba et al. (1993) found anti-GQ1b IgG in patients with acute post-infectious ophthalmoplegia without ataxia, which they designated ‘atypical MFS’. This clinical picture was described as ‘acute ophthalmoparesis’ in a further eight patients who were reported with anti-GQ1b IgG (Yuki, 1996). The features in a further 21 cases were summarized recently in a review, and illustrate that clinical overlap is frequent (Yuki et al., 2001a). Seventeen (81%) of the 21 patients had symptoms of antecedent infection. Five (24%) had had gastrointestinal infections, including *C.jejuni*. Four (19%) had mild, bilateral facial paresis, and two (9%) had oropharyngeal palsy. Lower cranial nerve paresis often occurred together with acute ophthalmoparesis, indicating that the latter is often not entirely isolated (Sakurai et al., 1998). Tendon reflexes were normal in nine out of 21 patients (43%), hypoactive in eight (38%), absent in three, and brisk in one; thus loss of tendon jerks is a supportive but not essential marker for acute ophthalmoparesis. In this series, eight out of 21 patients (38%) also complained of paraesthesias in the arm(s) or leg(s). Ropper (1994) reported a patient with sixth nerve palsies and paraesthesiae and proposed that this condition be termed a regional variant of GBS. Acute ophthalmoparesis also could be classed as a mild form of MFS or a regional variant of GBS. However, the term ‘atypical MFS’ is not sufficiently distinctive for anti-GQ1b-positive ophthalmoplegia without ataxia, since there are anti-GQ1b-positive patients with the reverse features, i.e. ataxia without ophthalmoplegia, as described below. From a clinical point of view, it is important to consider that cases of isolated ocular motor nerve palsies may have an autoimmune basis and that anti-GQ1b antibodies should be sought in these circumstances.
Ataxic Guillain–Barré syndrome

The presence or absence of ataxia in GBS is obscured by the profound limb weakness that generally occurs. However, a number of case series have reported patients with ataxic GBS, sensory ataxic GBS and pure sensory GBS in which ataxia is the dominant clinical feature, in the absence of either limb or craniofascicular weakness. Many of these cases have distal paraesthesias and areflexia, suggestive of peripheral nerve involvement, yet the ataxia may have clinical features more in keeping with cerebellar-type than peripheral-type ataxia. This has fuelled considerable debate on a central (i.e. cerebellar and spinocerebellar) or peripheral nerve (i.e. primary 1a afferent) origin for ataxia in these syndromes. This example of the neurologist’s preoccupation for creating dichotomous divisions within continuous clinical spectra has been pursued most vigorously in MFS, where limb weakness is absent, and ataxia a prominent feature. In the context of this review, the inter-relationships of these ataxic syndromes is complicated further by the variable and overlapping presence in all these subgroups of anti-glycolipid antibodies directed to GQ1b, GD1b and related disialylated structures.

The earliest report of patients having severe ataxia of the cerebellar type at the onset of GBS, without dominant limb weakness or ophthalmoplegia, is now 40 years old and led the debate for the cerebellar origin of the ataxia in this subgroup (Richter, 1962). However, the presence of distal paraesthesias, areflexia and elevated CSF protein levels suggested that the disease should be classified as a variant of GBS. Many of these patients experience some weakness, and the condition may progress to typical GBS, whereupon the ataxia may be obscured. In addition, occasional patients present with acute ataxia and areflexia without ophthalmoplegia, which have neither limb weakness nor sensory dominant limb weakness or ophthalmoplegia. During the acute phase of the illness, one such case was reported who had high titre anti-GQ1b IgG antibodies (Mori et al., 1999a). In a large series of 149 patients who had anti-GQ1b IgG without marked limb weakness, five showed acute, self-limiting ataxia without ophthalmoplegia (Kusunoki et al., 1999a). In a further series of 340 consecutive patients who had anti-GQ1b IgG, six cases had no external ophthalmoplegia, and one had minimal external ophthalmoplegia, in the absence of limb weakness, and the clinical features of these patients were thus consistent with an ataxic form of GBS (Yuki et al., 2000b). Anti-GQ1b IgG antibodies from these patients, as is usually seen in those with typical MFS, cross-reacted with GT1a. The observation that ataxic forms of GBS and MFS have a common autoantibody with the same fine specificity suggests that they most probably form a continuous spectrum. Why different clinical phenotypes occur in the presence of the same autoantibody remains unknown.

The anatomical lesion responsible for ataxia in MFS remains unproven, but Fisher (1956) first proposed selective involvement of 1a afferent neurones in his original paper. This explanation for ataxia based on abnormal conduction in 1a spinocerebellar afferent fibres originating in muscle spindles has some physiological support, as reflected by abnormalities of silent periods (Ropper and Shahani, 1983). Postural body sway analysis findings have also suggested selective involvement of 1a afferents in patients with MFS and ataxic GBS (Kuwabara et al., 1999; Mori et al., 1999a). One autopsy case of ataxic GBS showed marked degeneration of the fibre system of Clarke’s column, but no lesions in the cerebellum (Richter, 1962). Some large neurones of human DRG can be immunostained with anti-GQ1b monoclonal antibody (Kusunoki et al., 1999a), although the physiological role of these anti-GQ1b-positive neurones is not known. Direct staining of 1a afferents or their central connections with anti-GQ1b antibody has not however been demonstrated.

The interest in GD1b and sensory ataxia has been heightened by the recently developed rabbit model of this syndrome and the localization of GD1b to primary sensory neurones in the human PNS (discussed below). In a large survey looking at sensory features in 445 patients with GBS, nine cases were identified with monospecific anti-GD1b IgG antibodies, and all these patients had sensory disturbance. Cerebellar ataxia was observed in one case, and disturbance of deep sensation in three others (Miyazaki et al., 2001). In other case reports on patients with GBS and monospecific anti-GD1b IgG antibodies, the main clinical features were cerebellar ataxia in two patients and sensory ataxia in one patient. Thus, patients with ataxic GBS have been reported whose serum IgG reacts with GD1b alone but without reactivity to GQ1b (Yuki et al., 2000c). In another such patient with an acute, self-limiting neuropathy consisting of sensory ataxia without ophthalmoplegia or limb weakness, transient high-titre serum IgG antibodies reacted solely with GD3 and GD1b (Willison et al., 1994). Thus there are strong arguments in favour of both a central and a peripheral cause for ataxia in GBS being associated with either GQ1b or GD1b antibodies, and that the relative contributions of these factors are likely to vary between patients.

Pharyngeal–cervical–brachial weakness

Ropper (1986) was the first to describe patients who had acute oropharyngeal, neck and shoulder weakness in the absence of significant limb weakness. Other features included facial palsy, blepharoptosis, absence of sensory disturbance and preserved tendon jerk in the legs. This clinical presentation was noted to resemble botulism, apart from the absence of autonomic features, as has been also commented on for MFS. Ropper’s cases had elevated CSF protein and electrophysiological features including denervation and slow conduction velocities, leading him to speculate that these patients had a variant of GBS, which he referred to as ‘pharyngeal–cervical–brachial weakness resembling botulism or diphtheria’. One of the patients in the Italian series of cases who received intramuscular administration of bovine brain ganglioside developed pharyngeal–cervical–brachial weakness, indicating that immune responses to gangliosides possibly...
were involved in the development of this condition (Emilia-Romagna Study Group on Clinical and Epidemiological Problems in Neurology, 1998).

Mizoguchi et al. (1994) first reported serum anti-ganglioside antibodies in a patient with pharyngeal–cervical–brachial weakness although the case was not pure in that there was also some leg weakness. They detected two types of monospecific IgG antibody reactive with GT1a and GD1a, but that did not cross-react with each other, indicating that they were different antibody species. Because anti-GT1a and anti-GD1a IgG antibodies are present frequently in patients with MFS and AMAN, respectively, this pattern was not obviously remarkable. One might speculate that the anti-GT1a and anti-GD1a antibodies might have contributed to the development of pharyngeal–cervical–brachial weakness and generalized weakness, respectively. Monospecific antibodies to GT1a that do not react with GQ1b, both of which share a disialosyl residue in common at their non-reducing terminals, are highly unusual. Because GT1a is a rare ganglioside only available in small quantities, this subtlety has been relatively difficult to investigate in detail. Two further patients with clinically pure pharyngeal–cervical–brachial weakness, who had high anti-GT1a IgG antibody activity without anti-GQ1b antibody, were then reported (Kashiwara et al., 1998; Koga et al., 1998a). Absorption studies confirmed the monospecificity by showing that the anti-GT1a IgG antibodies from these patients could not be absorbed by GQ1b. These rare cases indicate that anti-GT1a IgG antibodies that lack cross-reactivity with GQ1b seem to be relatively specific for the pharyngeal–cervical–brachial GBS variant. Other patients with the pharyngeal–cervical–brachial weakness have anti-GT1a IgG that cross-reacts with GQ1b, in the absence of ophthalmoplegia (Koga et al., 1999b). Thus there is clearly some latitude in these associations. Three patients with acute oropharyngeal palsy, one of whom had mild limb weakness, were reported who were positive for both anti-GT1a and anti-GQ1b antibodies (O’Leary et al., 1996). Again this suggests that some clinical and serological overlap exists. One explanation for the site selectively could be that certain gangliosides are enriched in different areas of the nervous system. Arguing along these lines, biochemical studies by Chiba et al. (1993, 1997) sought but did not find GT1a in human cranial nerves; however, a recent immunochromic study using a monospecific anti-GT1a antibody has shown that GT1a is present more abundantly in the lower cranial nerves than in the upper ones (Koga et al., 2002). It thus seems likely that acute oropharyngeal palsy is a milder, more localized form of the pharyngeal–cervical–brachial GBS variant, that may be relatively associated with anti-GT1a antibodies.

**Chronic neuropathy syndromes with anti-ganglioside antibodies**

A large body of evidence supports the view that chronic inflammatory demyelinating polyneuropathy and polyneuropathies associated with IgG, IgM and IgA paraproteinaemia (also termed monoclonal gammopathies of unknown significance) have an autoimmune basis. Some of the clinical and electrophysiological features of IgG and IgA paraproteinaemic neuropathy are very similar to those of chronic inflammatory demyelinating polyneuropathy without monoclonal gammapathy, in contrast to IgM paraproteinaemic neuropathies that often have more distinct clinical phenotypes, as described below. Many IgM paraproteins react with neural glycolipid antigens, whereas the antigen specificities of IgG and IgA paraproteins to a large extent remain unknown and are not believed to be due to immunity to glycolipids, that have been widely sought. These latter disorders will not be considered further here, although they remain highly interesting (Miescher and Steck, 1996).

**Anti-MAG IgM paraproteinaemic neuropathy**

The first IgM paraproteins in which the neural specificity was identified were found to react with carbohydrate determinants present on a variety of peripheral nerve glycoproteins and glycolipids bearing the carbohydrate epitope, HNK-1. Using IgM paraproteins from neuropathy cases, Braun et al. (1982) found reactivity with MAG. Anti-MAG reactivity accounts for ~50% of IgM paraproteins occurring with sensorimotor neuropathy (Latov, 1994; Quarles, 1997). The presence of this reactive epitope on MAG led to the discovery of two peripheral nerve acidic glycolipids termed SGPG and SGLPG (Chou et al., 1986; Ariga et al., 1987), which contain the same antigenic site centred on the terminal 3-sulfate-glucuronic acid residue. Some anti-MAG antibodies may also cross-react with sulfatide. However, a considerable body of clinical evidence suggests that antibodies to sulfatide may be associated with predominantly sensory neuropathies in their own right (Lopate et al., 1997). In addition, some anti-MAG paraproteins also react with neural cell adhesion molecule, J1 glycoprotein and the peripheral nerve myelin glycoproteins, P0 and PMP22 (Bollensen et al., 1988).

The clinical phenotype of anti-MAG paraproteinaemic neuropathy is usually that of a chronic and slowly progressive sensory dominant demyelinating neuropathy (Nobile-Orazio et al., 1994; Van den Berg et al., 1996). There is a strong male preponderance and the onset is late. Upper limb postural tremor is a characteristic clinical feature. On sural nerve biopsy, there is chronic segmental demyelination, often with accompanying axonal degeneration. Immunohistochemistry usually reveals IgM and complement deposition associated with the striking feature visible on electron microscopy of widely spaced myelin. Since the majority of experimental and clinical data indicate that anti-MAG IgM is directly pathogenic (Quarles and Weiss, 1999), immunomodulating therapies have been tested extensively in these patients but rarely have been subjected to randomized trials (Nobile-Orazio et al., 2000). However, most patients have a good prognosis and are elderly, and the modest clinical benefit of
prolonged treatment with toxic immunotherapy should be balanced carefully against the high rate of adverse events.

**Chronic sensory ataxic neuropathy with anti-disialosyl antibodies**

In 1985, the first case of IgM paraproteinaemic neuropathy was reported in which the paraprotein reacted with NeuAc (α2–8) NeuAc (α2–3) Gal configured disialylated gangliosides (Ilyas et al., 1985), and this was followed by other single case reports (Arai et al., 1992; Daune et al., 1992; Obi et al., 1992; Willison et al., 1993a; Herron et al., 1994). The clinical phenotype of this paraproteinaemic neuropathy syndrome is a highly characteristic chronic sensory ataxic neuropathy as detailed in a recent large series of 18 cases (Willison et al., 2001). All cases have serum IgM antibodies that react principally with NeuAc (α2–8) NeuAc (α2–3) Gal configured disialosyl epitopes common to many gangliosides including GD1b, GD3, GT1b and GQ1b. Examples of this pattern of reactivity are shown in Fig. 3. The IgM accounting for the anti-ganglioside activity is almost invariably in the form of a benign IgM paraprotein which may also have cold agglutinating activity.

The clinical picture comprises a chronic neuropathy with marked sensory ataxia and areflexia, and with relatively preserved motor function in the limbs. In addition, many cases have motor weakness affecting oculomotor and bulbar muscles as fixed or as relapsing–remitting features. When present in their entirety, these clinical features have been described previously under the acronym CANOMAD: chronic ataxic neuropathy, ophthalmoplegia, IgM paraprotein, cold agglutinins and disialosyl antibodies (Willison et al., 1996). This distribution of clinical features is reminiscent of MFS, in which acute phase anti-disialylated ganglioside IgG antibodies are found. Clinical electrophysiology and nerve biopsy show both demyelinating and axonal features. A partial response to intravenous immunoglobulin and other treatments is reported in some cases.

The acute relapsing pattern that occasionally occurs in this syndrome is a curious phenomenon, since the antibody is present chronically. One such patient had rapidly developed marked sensory ataxia without weakness following an upper respiratory tract infection (Yuki et al., 1992b). The symptoms reached a maximum in a few days, with subsequent gradual improvement over a few weeks. Unsteady gait persisted for 15 years, during which time there were 10 similar relapses. Sensory nerve sensory action potentials were absent, and sural nerve biopsy showed a marked loss of large myelinated fibres. The patient was classified as having a relapsing form of ‘acute sensory neuropathy syndrome’, as proposed by Windebank et al. (1990). The serum contained a high titre of an IgM paraprotein directed against the disialylated gangliosides GD2, GD1b, GT1b and GQ1b. These so-called ‘b-series’ gangliosides are the predominant gangliosides in rat DRG, and the patient’s IgM antibody was shown to induce cell death in an in vitro system of rat DRG neurones (Ohsawa et al., 1993). His IgM also reacted with GQ1b (Tagawa et al., 1997). A monoclonal antibody specific for GQ1b and GT1a was immunostained laminae I and III of the dorsal horn but did not react with motor neurones (Kusunoki et al., 1993a). Propriospinal fibres project to lamina I. GQ1b may modulate deep sensation and be a target molecule for serum IgM antibodies in some patients with sensory ataxic neuropathy (Tagawa et al., 1997).

**Motor neuropathy with anti-GM1 and anti-GM2 antibodies**

The reactivity of chronic neuropathy sera with GM1 ganglioside has been an unresolved research focus for 15 years. The first reports were of anti-GM1 IgM paraproteins occurring in unusual forms of motor neurone disease (Freddo et al., 1986; Latov et al., 1988). Isolated patients were also described with neuropathies and anti-GM1 IgM monoclonal antibodies, on occasion co-existing with anti-MAG antibodies as a bicalonal gammopathy (Ilyas et al., 1988a). A multifocal acquired demyelinating motor and sensory neuropathy distinct from chronic inflammatory demyelinating polynévropathie had been described by Lewis et al. (1982) and further defined by Parry and Clarke (1988), the main characteristic being areas of focal conduction block. The relationship with anti-glycolipid antibodies grew from the report in 1988 of a multifocal motor neuropathy with demyelinating conduction block occurring with anti-GM1 IgM antibodies (Pestronk et al., 1988).

Three main patterns of anti-GM1 antibody specificity have been identified. First, antibodies may react with the terminal Gal (β1–3) GalNAc structure and will therefore cross-react with GD1b and asialo-GM1. Secondly, antibodies may react with GM1 and GM2 via shared internal sialylated sugar moieties. Thirdly, some antibodies are monospecific to GM1 (Pestronk 1991; O’Hanlon et al., 1996). Effort was made to correlate these variations in fine specificity with clinical subgroups and to search for a more general relationship between anti-GM1 antibodies and motor neurone disease: although such approaches were important, no unifying patterns emerged. Chronic motor neuropathies were thus identified in association with polyclonal or monoclonal IgM antibodies directed to GM1 and other Gal (β1–3) GalNAc-bearing glycolipids and found in ~25–50% of cases of multifocal motor neuropathy with conduction block depending upon the clinical definition and detection methodology used (van Schaik et al., 1995; Pestronk and Choksi, 1997; Leger et al., 2001).

The clinical picture of multifocal motor neuropathy is usually of slowly progressive, asymmetrical limb weakness with minimal or no sensory impairment, often with distal onset in an upper limb. It is characterized electrophysiologically by partial and focal motor conduction block across a segment of nerve with sparing of sensory fibres. Over time,
the clinical pattern may become confluent, with significant motor axonal degeneration. Multifocal motor neuropathy usually responds well to intravenous immunoglobulin which needs to be given regularly. Other agents, including cyclophosphamide, have not been shown in systematically controlled trials to improve outcome, although case series have been reported.

Anti-GM2 antibodies are also found in cases of chronic motor or motor dominant neuropathy, in addition to their presence in GBS, as described above. In one recent study, two chronic neuropathy cases were identified with highly elevated anti-GM2 IgM titres (O’Hanlon et al., 2000). One case with GM1/GM2 cross-reactive antibodies (titre 1: 78 000) had multifocal motor neuropathy with conduction block of 20 years duration, principally affecting the distal right arm. The second case, with GM2/GalNAc-GD1a/GalNAc-GM1b cross-reactive antibodies (titre 1: 70 000), had a demyelinating motor neuropathy of 8 years duration, principally affecting the proximal lower limbs. This latter case had a pattern of serological cross-reactivity shown by thin-layer chromatography overlay in Fig. 4, first recognized by Ilyas et al. (1988c). In view of their association with motor neuropathy, anti-GM2 antibodies have also been sought extensively in motor neurone disease. Occasional patients are identified with an apparent relationship (Yuki et al., 1991; O’Hanlon et al., 2000), but similar antibodies are also found occasionally in control subjects (O’Hanlon et al., 2000). Thus there is a relationship between anti-GM2 IgM antibodies and chronic neuropathy syndromes that remains to be clarified fully.

Origins and immunological characteristics of anti-glycolipid antibodies

In one of many detailed studies examining the serological evidence for 16 antecedent pathogens in GBS patients and sex- and age-matched controls, four pathogens were identified as related to the onset of GBS; C. jejuni (32%), CMV (13%), Epstein–Barr virus (10%) and M. pneumoniae (5%) (Jacobs et al., 1998). However, there is a wealth of evidence from smaller series and individual case reports that a wide range of other infections and vaccinations can also precipitate GBS. In chronic neuropathies associated with anti-glycolipid antibodies, precipitating infections are less evident although they may well play a clinical role. One of the major mechanisms by which anti-ganglioside antibodies are thought to arise in GBS is through molecular mimicry with microbial oligosaccharide structures. This has been studied particularly thoroughly in the outer core oligosaccharide structures on lipopolysaccharide (LPS) borne by C. jejuni species, as discussed in detail below. Chemical and structural analysis of LPS from C. jejuni serotypes isolated from GBS and non-GBS cases have identified sialylated moieties with configurations identical to several gangliosides. For example, LPSs from C. jejuni O:19, a serotype commonly associated with GBS (Kuroki et al., 1993; Yuki et al., 1997b), have been shown to contain GM1, GD1a, GD3 and GT1a-like motifs (Yuki et al., 1993c; Aspinall et al., 1994), and molecular mimicry at a functional level is supported by the finding that immunization of experimental animals with these LPSs produces the corresponding anti-ganglioside antibody response (Wirguin et al., 1997; Goodyear et al., 1999; Ang et al., 2000a). Serotyping studies have identified that certain C. jejuni, including O:19, have a greater potential for triggering GBS, and this may be due to quantitative differences in ganglioside-like LPS epitopes compared with non-GBS associated strains.

Whereas C. jejuni is one of the most common causes of acute diarrhoea worldwide, affecting >1% of the population per annum, GBS has a much lower incidence of 1.5 per 100 000 population and it is thus estimated that only 0.01% of C. jejuni infections trigger GBS. Although the absence of ganglioside mimics on some strains of C. jejuni LPSs may be part of the explanation for this, clinical studies have demonstrated that even when humans are exposed to C. jejuni strains possessing ganglioside-like structures (estimated to be present in <1% of C. jejuni infections), their presence is not sufficient in itself to trigger the production of anti-ganglioside antibodies. The host and microbial factors that determine whether any one individual will mount an immune response to core oligosaccharide structures that mimic self-gangliosides are likely to be multifactorial and
IgG2 in man (Garcia Guijo et al., 1992; Willison and Veitch, 1994). The class switching to the T cell-dependent IgG1 and IgG3 isotypes suggests that anti-ganglioside antibodies found in GBS have arisen from conventional B2 cells and been able to recruit T cell help or other accessory signals. Whether the help comes from intermolecular cooperativity (uptake of carbohydrate–protein complexes by carbohydrate-specific B cell receptors and subsequent presentation of peptides to conventional T helper cells), presentation via CD1, LPS signalling via lipid A and Toll receptors, or via other non-cognate pathways is unknown, but it clearly indicates dependence on T cell help in the induction of anti-ganglioside antibodies in GBS, rather than a purely T cell-independent origin as is usually associated with oligosaccharide antigens, including LPS. Little work has been performed on determining the nature of the T cell response to C. jejuni protein antigens that might provide this T cell help for anti-carbohydrate antibody responses.

The extent of affinity maturation in class-switched anti-ganglioside antibodies is also unknown. Since gangliosides are widely distributed throughout the body, there should be a high degree of B cell tolerance to ganglioside-mimicking core oligosaccharide structures that would limit affinity maturation. In GBS, irrespective of whether anti-ganglioside antibodies are pathogenic or not, tolerance to self-gangliosides is clearly broken in the small proportion of patients who develop such antibodies upon exposure to a particular ganglioside-mimicking strain of C. jejuni. Again, the mechanisms by which this occurs have not been addressed experimentally to any extent.

Although the data suggest that anti-ganglioside antibodies in GBS after C. jejuni infection arise through molecular mimicry, other explanations for the appearance of anti-glycolipid antibodies remain possible. One hypothesis is that T cell-mediated attack on peripheral nerves may lead to inflammatory destruction of neural tissue as occurs in the principal animal model of GBS, experimental allergic neuritis, with subsequent induction of anti-ganglioside antibodies as a secondary event (Hartung et al., 1995a, b). It is also possible that anti-ganglioside antibodies, forming a component of the natural autoantibody repertoire, could be expanded non-specifically by LPS acting as a polyclonal B cell activator, rather than via core oligosaccharide antigen-specific B cell receptor cross-linking. This ganglioside-reactive B cell pool activated non-specifically by LPS would be expected to produce low affinity IgM antibodies rather than class-switched IgG antibodies, and this may be more relevant to the chronic neuropathy syndromes associated with IgM antibodies that have a propensity to form paraproteins.

An alternative explanation is that the microbial infection directly targets nerve and that its persistence induces the inflammatory neuropathy, either in isolation or in conjunction with a post-infectious autoimmune response. Such an explanation may be difficult to conceive as an alternative to the more attractive molecular mimicry model for bacterial infections such as C. jejuni. However, one of the most common classes of viral infection that precede GBS are the family of herpes viruses that are known to exhibit marked neurotropism. Thus acute or latent viral infection could induce altered presentation of self-components, bystander activation or an inflammation-induced break in tolerance. Viruses may acquire host membrane glycolipids during replication or budding, or, alternatively, could have their proteins glycosylated with ganglioside mimics by host glycosyl- or sialyltransferases. As discussed below, some evidence suggests that CMV in particular can be glycosylated with GM2-like epitopes, of particular interest since post-CMV GBS is associated with anti-GM2 antibodies. Some of these issues have been explored experimentally. For example, the CMV genome has been sought in sural nerve biopsy material from GBS cases, although it has not been found to date (Hughes et al., 1992). With these considerations in mind, the following sections will describe some further details of the common antecedent infections and other events in relation to anti-glycolipid antibodies.

**Antecedent events as inducers of anti-glycolipid antibodies**

**Ganglioside administration**

Since their introduction in 1975, gangliosides extracted from bovine brain were prescribed extensively in Italy, ironically for the treatment of peripheral neuropathies (Govoni et al., 1997). Some evidence indicated that they played an important neuroprotective role and promoted nerve repair by enhancing nerve sprouting (Roisen et al., 1981). However, GBS cases following ganglioside administration were reported, and the number of affected subjects mounted (Figueras et al., 1992; Landi et al., 1993; Illa et al., 1995). A causal association between
The most widely studied and most common antecedent infectious agent in GBS is the Gram-negative bacterium *C jejuni*, a leading cause of acute enteritis in developed countries. Kaldor and Speed (1984) first reported that GBS patients who have had *C jejuni* infection have a more severe form of GBS. Rees et al. (1995c) showed that *C jejuni* infection often precedes GBS and is associated with axonal degeneration, slow recovery and severe residual disability. Thirteen (52%) of 25 patients with serological evidence of recent *C jejuni* infection had anti-GM1 IgG and/or IgM antibodies compared with 11 (15%) of 71 patients without evidence of infection, a statistically significant result (Rees et al., 1995a). Jacobs et al. (1996) showed that GBS patients with antecedent *C jejuni* infection more often had a severe pure motor variant of GBS. The presence of anti-GM1 antibody was significantly associated with preceding *C jejuni* infection. In control groups, patients with *C jejuni* enteritis who did not develop GBS or other neurological disorders had no anti-GM1 IgG antibodies (Yuki et al., 1990; Rees et al., 1995b).

The reports of patients who developed GBS after receiving bovine brain ganglioside mimics prompted the search for ganglioside mimics on preceding infectious agents (Figueras et al., 1992; Landi et al., 1993; Ila et al., 1995). LPSs were first extracted from *C jejuni* isolated from a patient with GBS who had anti-GM1 IgG antibody (Yuki et al., 1993c). The LPS reacted with cholera toxin B-subunit, a highly specific ligand for GM1 oligosaccharide. Gas–liquid chromatography–mass spectrometric analysis showed that the purified LPS contained galactose (Gal), N-acetylgalactosamine (GalNAc) and N-acetylneuraminic acid (NeuAc); the oligosaccharide components of GM1 ganglioside. $^1$H nuclear magnetic resonance showed that the oligosaccharide structure (Gal $β1–3$ GalNAc $β1–4$ [NeuAc $α2–3$ Gal]-) protrudes from the LPS core. This terminal structure is identical to that of the terminal tetrasaccharide of the GM1 ganglioside. The study was the first to demonstrate the existence of molecular mimicry between nerve tissue and the infectious agent isolated from a patient with GBS. In other studies, the oligosaccharide structures of GM1, GD1a, GD3 and GT1a-like LPSs have been determined in other *C jejuni* strains isolated from patients with GBS using similar methods (Aspinall et al., 1994; Prendergast et al., 1998). The immunological significance of this mimicry has been shown by demonstrating that *C jejuni* LPS can induce anti-ganglioside IgM and IgG antibodies in rats (Wirguin et al., 1997), mice (Goodyear et al., 1999) and rabbits (Ang et al., 2000a). However, in many of these studies, limb weakness or other clinical features were not evident in immunized animals despite the development of high titres of anti-glycolipid antibodies. The explanation for such findings is discussed in more detail below.

In a series of Chinese patients with GBS, serological evidence of *C jejuni* infection correlated with anti-GM1b and anti-GalNAc-GD1a IgG antibodies (Yuki et al., 1999a). The GM1b and GalNAc-GD1a epitopes have also been identified by serological means in the LPSs of *C jejuni* isolated from patients with AMAN, who had anti-GM1b and anti-GalNAc-GD1a IgG antibodies (Yuki et al., 1999b, 1997a). A single strain of *C jejuni* may express core oligosaccharide molecules...
bearing several ganglioside-like structures, and the corresponding serum may contain several species of anti-ganglioside IgG antibodies (Yuki et al., 1994a, 1995). Thus infection by a C. jejuni strain may induce a single anti-ganglioside IgG in some patients and a combination of antibodies in others.

**Antecedent events in Miller Fisher syndrome**

As is the case in GBS, a wide range of pathogens including C. jejuni, Haemophilus influenzae, Streptococcus pyogenes, Staphylococcus aureus, M. pneumoniae, Coxiella burnetii, CMV, Epstein–Barr virus, varicella-zoster virus and mumps virus have been reported as antecedent agents in MFS (Takano and Yuki, 1995). A comprehensive Japanese study on the antecedent infectious agents in MFS showed that 18% of patients with MFS were seropositive for recent C. jejuni infection, and that the frequency was lower than that of patients with GBS (31%) (Koga et al., 1998). Two C. jejuni have been isolated from Japanese patients who had anti-GQ1b IgG antibody in the acute phase of MFS (Yuki et al., 1994b), both of which had anti-GQ1b monoclonal antibody-reactive LPS fractions. This indicates that the LPSs from MFS-associated C. jejuni bear a GQ1b epitope. This molecular mimicry between GQ1b and C. jejuni LPS has also been observed by others (Jacobs et al., 1997a; Neisser et al., 1997). One oligosaccharide structure in an LPS from C. jejuni isolated from an MFS patient has been chemically determined and found to be identical to the terminal trisaccharide of GD3 (Salloway et al., 1996). Some patients with MFS have anti-GD3 IgG antibody, which cross-reacts with GQ1b, suggesting a mechanism by which infection with C. jejuni bearing GD3-like LPS can induce anti-GQ1b IgG antibodies in some cases (Koga et al., 1999a).

An interesting family outbreak of GBS after C. jejuni infection led to the isolation of the OH4384 bacterial strain. One of the affected cases exhibited coma and external ophthalmoplegia, suggesting Bickerstaff’s brainstem encephalitis (Yuki and Tsujino, 1995). The LPS of this OH4384 strain was shown to have a GT1a-like structure by structural analysis (Aspinall et al., 1994). GT1a is very similar in structure to GQ1b, and immunization of mice with the LPS from OH4384 has led to the isolation of monoclonal antibodies with GQ1b and LPS reactivity (Goodyear et al., 1999). These findings strongly support the view that molecular mimicry between these closely related structures is the mechanism by which cross-reactive anti-ganglioside/LPS antibodies are induced in MFS.

**Cytomegalovirus infection**

About 10% of GBS patients have serological evidence of recent CMV infection (Visser et al., 1996; Ogawara et al., 2000). Patients with CMV-related GBS have a different clinical pattern compared with C. jejuni or other infections (Visser et al., 1996). CMV-related GBS patients are significantly younger than other GBS groups, have a severe early course with a high frequency of respiratory insufficiency and often develop cranial nerve involvement and severe sensory loss. Electrophysiologically, the pattern is of AIDP. These clinical and electrophysiological findings have also been observed by others (Yuki and Tagawa, 1998). With respect to anti-ganglioside antibodies in CMV-associated GBS, Irie et al. (1996) first detected anti-GM2 IgM and IgG antibodies in three patients. None of 48 GBS patients without preceding CMV infection had anti-GM2 antibodies, although one of six non-GBS control patients with acute CMV infection had anti-GM2 antibodies. Jacobs et al. (1997b) also reported that anti-GM2 IgM antibodies were present more frequently in patients with GBS with CMV infection (22%) than in patients without the infection (2%), and others have reported similar observations (Khalili-Shirazi et al., 1999). It remains, however, that acute CMV infection, with and without GBS, is clearly associated with anti-GM2 IgM antibody (Yuki and Tagawa, 1998). This calls into question whether anti-GM2 IgM antibody plays a role in the development of GBS or represents an epiphenomenon related to CMV infection, and that CMV exerts its neuritogenic effects through other mechanisms. The mechanism by which CMV induces anti-GM2 antibodies is unknown. Ang et al. (2000b) have demonstrated by absorption studies that CMV-infected fibroblasts express the GM2 epitope. Since viruses do not encode glycosylating enzymes, infection of human fibroblasts with CMV may alter the distribution of GM2 in the cell membrane, which the virus then acquires in its membrane at some stage of its infective cycle. These data support the notion that anti-GM2 antibodies in acute CMV infection are induced specifically but do not yet provide a mechanism by which they induce GBS.

Ogawa-Goto et al. (1994) reported that sera from infants with symptomatic congenital CMV infection had anti-SGPG antibody activity, suggesting that human CMV infection can also lead to the induction of anti-SGPG antibodies. A correlation between CMV infection and anti-MAG/SGPG IgM antibody-associated polyneuropathy has been shown (Yuki et al., 1998), but this finding at present is controversial (Lunn et al., 1999; Irie et al., 2000).

**Mycoplasma pneumoniae infection**

Prior infection with M. pneumoniae is found in ~5% of GBS patients (Jacobs et al., 1998; Ogawara et al., 2000). Patients with GBS after M. pneumoniae infection appear to be younger than other GBS patients, but their clinical patterns do not otherwise have unique features. Kusunoki et al. (1995) detected IgG and IgM antibodies to the myelin glycolipid galactocerebroside in four out of four patients with GBS following M. pneumoniae. However, six out of 33 patients with mycoplasma infection without neurological signs also had this antibody in a subsequent study (Kusunoki et al., 1996c). Anti-galactocerebroside antibodies have also been detected in three out of three cases of acute disseminated encephalomyelitis after M. pneumoniae infection, as well as eight out of 32 patients with M. pneumoniae infection without neurological diseases (Nishimura et al., 1996). Thus a clear
disease-specific association has yet to be elucidated fully, although it is clear that the galactocerebroside epitope is present in *M. pneumoniae* (Kusunoki et al., 2001). Anti-Hex-LM1 IgM and anti-GM1b IgG antibodies have also been found in patients with GBS subsequent to mycoplasma infection (Inuzuka et al., 1988; Kitazawa et al., 1998), indicating that there is not an exclusive relationship between mycoplasma and anti-galactocerebroside antibody.

**Haemophilus influenzae infection**

Mori et al. (1999b) reported an axonal GBS patient with a history of acute bronchitis, in whom the small Gram-negative rod *H. influenzae* was grown in sputum and throat swab cultures. They went on to show that six out of 46 (13%) patients with GBS had serological evidence of recent *H. influenzae* infection and that their clinical and laboratory features were fairly uniform, comprising relatively pure motor axonal degeneration and infrequent cranial or sensory nerve involvement. Their cases were often positive for anti-GM1 IgG antibodies (Mori et al., 2000). Since *H. influenzae* is a commensal organism in the normal upper respiratory tract flora of 80% of humans, it is difficult to draw firm conclusions about its role in GBS. Furthermore, in the case–control study by Jacobs et al. (1998), prior infection by this bacterium was only 1% in GBS. The discrepancy between the results of these two studies might be due to differences in the sensitivity of serological assays used for detecting this infection that are notoriously difficult, and further studies are necessary to determine the nature of this association. It seems clear that the GM1 epitope does exist on LPS from *H. influenzae*, shown in an isolate from a patient with AMAN (Mori et al., 2000), and thus lends weight to a molecular mimicry hypothesis for *H. influenzae* infection similar to that seen with *C. jejuni*.

It is interesting to reflect that in the original publication by Fisher (1956), the isolation of *H. influenzae* from the sputum of one of the three patients was described. The affected patient had a history of cough and fever before neurological onset, and on the basis of a chest X-ray pneumonia had been diagnosed. Koga et al. (2001) has studied antecedent *H. influenzae* infection in 70 consecutive patients with MFS and found serological evidence of infection in 7%, all of whom had a history of antecedent respiratory tract infection. Anti-GT1a IgG which was cross-reactive with GQ1b was present in these patients. Furthermore, these authors showed that an anti-GT1a monoclonal antibody bound to the LPS extracted from the *H. influenzae* sample, indicative that this LPS carries the GT1a epitope. Again, as is the case with *C. jejuni*, molecular mimicry for a range of ganglioside antigens is likely to be involved in the development of MFS after infection by *H. influenzae*.

**Anti-GD2 antibody administration**

A therapeutic trial for cancer with anti-GD2 antibodies inadvertently has created a passive immunization experiment that casts interesting light on the pathogenesis of peripheral neuropathy associated with anti-ganglioside antibodies. GD2 is expressed abundantly on the cell surfaces of neuroectodermal tumours and is a minor constituent of normal peripheral nerves. Subsequent to treatment of metastatic melanomas with 14G2a, a murine anti-GD2 monoclonal antibody, two patients developed sensorimotor polynuropathy and two patients developed the syndrome of inappropriate antidiuretic hormone secretion (Saleh et al., 1992). In immunocytochemical studies, this anti-GD2 antibody reacted with peripheral nerve myelin sheaths, as well as with the pituitary cytoplasm in the posterior lobe of the pituitary gland (Yuki et al., 1997c). The antibody can also mediate antibody-dependent cytotoxicity and complement-dependent lysis of neuroblastoma and melanoma cell lines *in vitro*; hence its use in therapeutic trials in melanoma (Mujoo et al., 1989). The binding of anti-GD2 monoclonal antibody to peripheral nerve myelin clearly might induce complement-mediated demyelination. Since antidiuretic hormone is transported along the supraoptical–hypophyseal tract to the posterior pituitary, the anti-GD2 antibody may also disturb the secretion of antidiuretic hormone and thereby induce the syndrome of inappropriate secretion of antidiuretic hormone, that is also associated with GBS, albeit very rarely. This ‘human model’, along with the ‘ganglioside therapy’ cases described above, suggests very strongly that anti-ganglioside antibodies are also likely to be pathogenic in humans in other circumstances, such as post-infectious GBS. Such human experiments are both unusual and unwanted and, in the main, models of anti-glycolipid antibody-mediated neural injury have been investigated in experimental animals, as discussed below.

**Physiological models of anti-glycolipid antibody-mediated neural injury**

Determining whether autoimmune neuropathy sera are capable of causing neural injury has occupied researchers for many decades, particularly with the wide appreciation of the need to fulfil Witebsky’s postulates and their subsequent modifications as cardinal proof of nervous system autoimmunity (McFarlin, 1990; Rose and Bona, 1993; Sheikh and Griffin, 2001). It can thus be proposed that several lines of enquiry would need to be satisfied to gain proof that anti-glycolipid antibodies arise through molecular mimicry and then proceed to mediate autoimmune neuropathy. First, passive transfer of pathogenic anti-glycolipid antibody, or possibly T cells in the case of as yet unidentified CD1-restricted responses, would need to induce the autoimmune disease. Secondly, the autoimmune disease would need to be reproduced by immunization with, or exposure to the putative microorganism and be shown under such circumstances to be mediated principally by the anti-glycolipid autoantibody. Thirdly, but likely to be more circumstantial, overwhelming evidence could be sought from clinical data to prove an unambiguous relationship.
Apart from the anti-GD2 antibody studies and the GBS cases that occurred following 'ganglioside therapy' described above, no direct evidence for a primary pathogenic role for anti-ganglioside antibodies is forthcoming from human studies, although the circumstantial clinical evidence for such a role seems to be accumulating. Thus a variety of in vivo animal models and ex vivo physiological systems, and cell culture-based models are available and need to be developed further in order to investigate the putative effects of anti-glycolipid autoantibodies. The route of access by which antibody penetrates nerve is an important consideration in these types of experiments. Organ bath preparations have advantages over in vivo models in that direct application of a known, fixed antibody concentration to desheathed nerve preparations can be studied, thereby obviating any protective effects of the blood–nerve barrier. Attempts have been made to bypass the blood–nerve barrier by direct intraneural injection into otherwise intact nerve in situ; however, such studies are prone to artefact unless conducted under highly controlled conditions. An even simpler system is to study the effects of antibodies on explant cultures, primary cultures or neural cell lines; these systems have an elegant simplicity, but equally are far removed from the normal pathophysiologial situation that their relevance to human disease is weakened. Despite a number of caveats and pitfalls, some significant advances have been achieved for anti-glycolipid antibodies in a variety of systems. Two of these are discussed in more detail below.

**Anti-GM1 antibodies and conduction block at the nodes of Ranvier**

Considerable efforts have been made to perturb function and morphology at the node of Ranvier in a variety of anti-GM1 antibody-mediated models. This emphasis has arisen because cholera toxin, antibody and lectin binding studies have clearly shown that GM1 is enriched in nodal and paranodal structures, as shown in Fig. 2 (Molander et al., 1997; Sheikh et al., 1999). Furthermore, autopsy studies of AMAN cases have shown that immunoglobulin and complement deposits frequently were localized at the node of Ranvier, where sodium channels are clustered, and at the internodal axolemma (Hafer-Macko et al., 1996a). Electrophysiological studies on anti-GM1 antibody-mediated nerve injury have shown divergent findings. In early studies in this area, intraneural injection of antibody into rat nerve showed that human anti-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 into rat tibial nerves of human anti-GM1 immunoglobulin, and histological examination of the injected nerves did not show demyelination, despite the binding of the anti-GM1 antibody to the nodes of Ranvier. 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 anti-GM1 antisera (Arasaki et al., 1993). Takigawa et al. (1995) also found that rabbit anti-GM1 antibodies increased potassium current elicited by step depolarization, and in the presence of active complement blocked sodium channels irreversibly. However, another study showed that application of high titres of human and rabbit anti-GM1 antisera did not cause acute conduction block or block sodium channels (Hirota et al., 1997).

In a study by Paparounas et al. (1999), the acute physiological effects of antibodies to GM1 (and other gangliosides) were examined in the presence of complement on nerve conduction in an isolated sciatic nerve organ bath preparation over a 6 h period. No abnormal effects were observed despite the fact that antibody and complement products were being deposited, albeit possibly not at saturated levels, at a proportion of nodes of Ranvier during the time course of these recordings. When considered collectively, these data infer that the node of Ranvier is relatively resistant to complement-dependent anti-GM1 antibody-mediated injury over the short time frames used in organ bath studies. These studies contrast sharply with results from anti-galactocerebroside antibody application that clearly causes acute conduction failure following bath application over similar time frames (Lafontaine et al., 1982). It thus remains possible, indeed in our view likely, that the node may be sensitive to more prolonged or aggressive injury mediated via anti-GM1 antibodies. Indeed, this has been shown recently in a whole-animal immunization model, as described below.

**Anti-GQ1b antibodies and the neuromuscular junction**

Of the many anatomical sites potentially targeted in anti-ganglioside antibody-mediated neuropathy, the motor nerve terminal at the NMJ is particularly appropriate for study for a number of reasons: (i) the nerve terminal lacks a blood–nerve barrier, thereby allowing antibodies easy access to the neuronal and glial membranes that are the presumed sites of injury; (ii) it is the site for other paralytic antibody-mediated diseases (e.g. myasthenia gravis); (iii) it is rich in gangliosides including GQ1b, GM1 and GD1a (Willison and O’Hanlon, 1999); (iv) it is the binding site for bacterial toxins that also use gangliosides as ectoacceptors (Oldfors, 1986; Senda et al., 1995); (v) it is an affected site in clinical and pathophysiological studies of GBS/MFS (Ho et al., 1997; Uncini and Lugaresi, 1999); and (vi) it can be studied in the mouse with relative ease. Several ex vivo physiological studies provide very strong supportive evidence that anti-GQ1b and other anti-disialylated ganglioside antibodies can mediate pathophysiological changes at the NMJ (Roberts et al., 1994; Buchwald et al., 1995, 1998; Willison et al., 1996; Goodyear et al., 1999; Plomp et al., 1999). It had been widely viewed that anti-GQ1b antibodies in MFS would be most likely to cause the
clinical motor manifestations via segmental demyelination of extraocular and craniobulbar nerve trunks in a pattern similar to that classically described in GBS. In vitro conduction studies on isolated, desheathed mouse sciatic nerve have demonstrated anti-disialosyl antibody binding and complement fixation at the nodes of Ranvier occurring in the absence of conduction abnormalities, thus indicating that in this model, the site is relatively resistant to acute physiological failure as described above (Paparounas et al., 1999). This has not, however, been the case at the NMJ. Using the ex vivo mouse hemi-diaphragm preparation, anti-GQ1b-positive MFS sera and IgG were first found to cause a temporary and moderate increase of spontaneous quantal acetylcholine release at NMJs, as assessed by a rise in miniature end plate potential frequencies, and subsequently induced nerve terminal paralysis. Passive immunization studies in the mouse using a human affinity-purified and cloned CANOMAD-associated IgM anti-disialosyl antibody demonstrated extensive in vivo deposits of IgM at motor nerve terminals in conjunction with electrophysiological evidence of nerve terminal dysfunction (Willison et al., 1996).

In a series of more extensive studies, the in vitro effects of MFS sera, MFS IgG fractions and a human monoclonal anti-GQ1b IgM antibody on mouse NMJs were re-examined (Plomp et al., 1999). Here it was demonstrated that anti-GQ1b antibodies bind at NMJs where they induced massive quantal release of acetylcholine from nerve terminals and eventually blocked neuromuscular transmission in a purely presynaptic fashion closely resembling the effect of the paralytic neurotoxin, α-latrotoxin (Okamoto et al., 1971; Duchen et al., 1981). Furthermore, through the use of different complement-deficient sera, the effect of anti-GQ1b antibodies was shown to be entirely dependent on activation of complement components. Since neither classical pathway activation nor the formation of membrane attack complex was required, the effect could be due to involvement of the alternative pathway and intermediate complement cascade products such as C3a and C5a, although this has yet to be clarified fully.

In a series of studies performed by Buchwald and colleagues using a perfused macropatch clamp electrode technique on the phrenic nerve hemi-diaphragm preparation, IgG from anti-GQ1b positive- as well as anti-GQ1b-negative MFS patients blocked evoked acetylcholine release and depressed the amplitude of postsynaptic potentials, indicating both a pre- and postsynaptic blocking effect (Buchwald et al., 1995, 1998). This effect was fully reversible by washing out the perfusate, and occurred in an entirely complement-independent manner. Although the details of these observations may seem at variance with the electrophysiological results of Plomp et al. (1999), the studies collectively indicate that the motor nerve terminal and NMJ should be viewed seriously as one of the potential targets for antibody-mediated attack in MFS. This should not undermine the likely importance of other sites including nodes of Ranvier, where GQ1b is known to accumulate. Clinical electrophysiological studies could investigate this site more thoroughly in affected patients, as recently reported (Uncini and Lugaresi, 1999). Similarly, the terminal motor nerve may be a pathogenic site in more generalized paralytic syndromes, as occurs in AMAN (Ho et al., 1997).

The role of anti-GQ1b antibodies arising as a result of C.jejuni infection with cross-reactive LPS core oligosaccharides has also been investigated for these α-latrotoxin-like effects. Mice were immunized with GT1a/GD3-like C.jejuni LPS and thereafter monoclonal antibodies were isolated that reacted with both the immunizing LPS and GQ1b/GT1a/GD3 gangliosides. In immunohistological studies, the monoclonal antibodies bound to ganglioside-rich sites including the NMJ (Goodyear et al., 1999). In ex vivo electrophysiological studies in the phrenic nerve hemi-diaphragm preparation, application of antibodies either ex vivo or in vivo via passive immunization induced massive quantal release of acetylcholine, followed by neurotransmission block in an identical fashion to the MFS sera and IgG fractions, and the anti-disialosyl antibodies from CANOMAD cases. Again the effects were complement dependent and associated with extensive deposits of IgM and C3c at nerve terminals. At both the light and electron microscopic level, morphological destruction of the nerve terminal in this model was observed (O’Hanlon et al., 2001). In addition to strengthening the electrophysiological data using human anti-ganglioside antibodies, these data also provide strong support for the molecular mimicry hypothesis as a mechanism for the induction of cross-reactive pathogenic anti-ganglioside/LPS antibodies in MFS.

**Experimental neuropathies induced by active or passive immunisation**

As addressed above, although a number of human diseases are believed to have an autoimmune aetiology, it is apparent that only a few, such as myasthenia gravis, formally fulfil Witebsky’s criteria (McFarlin, 1990). Other diseases, such as rheumatoid arthritis and multiple sclerosis, do not, even though notable immunological reactivity clearly occurs. The missing information in these two diseases and others relates to the lack of an identified autoantigen, as defined in Witebsky’s second criterion (Witebsky, 1959); in certain subtypes of the glycolipid antibody-associated peripheral neuropathies described above, in particular MFS and GQ1b ganglioside, the antigenic targets have now been characterized convincingly for some observers. Moreover, whole-animal models of experimental neuropathies induced by active or passive immunization have now been created, as described below, and more progress in this area is anticipated soon. Thus we are close to fulfilling at least some of the criteria required to establish GBS as a well-proven autoimmune disease.
Experimental neuropathy induced by galactocerebroside

Over 20 years ago, Saida and colleagues established an animal model of demyelinating neuropathy by sensitization of rabbits with galactocerebroside, a glycolipid hapten common in CNS and PNS myelin. Rabbits immunized repeatedly with bovine brain galactocerebroside developed a neurological disorder manifested by flaccid quadriparesis, limb hypaesthesia and respiratory paralysis (Saida et al., 1979). Autopsied animals had small multiple perivascular foci of demyelinating lesions in roots, DRG, proximal peripheral nerve adjacent to ganglia and, less frequently, in distal nerves. No change was found in the CNS. Demyelination started around venules, with splitting and vesiculation of the outer myelin sheaths of adjacent fibres, and later progressed to form confluent lesions. The lesions were associated with infiltration of phagocytic mononuclear cells, mostly macrophages, which insinuated themselves between myelin lamellae, phagocytyzed myelin and, subsequently, denuded axons. Perivascular infiltration of small lymphocytes was not encountered, suggesting that antibody-mediated effector mechanisms were dominant. The distribution of demyelinating lesions seemed to correspond to areas known to have a defective blood–nerve barrier. This whole-animal study was followed up by intraneural injection experiments with rabbit anti-galactocerebroside serum that produced focal demyelinating lesions in rat sciatic nerves (K. Saida et al., 1979). This lesion was characterized by Schwann cell changes and myelin destruction, followed by phagocytic mononuclear cell invasion and by a rapidly evolving acute inflammatory response. Local application of anti-galactocerebroside serum to rat ventral roots was also shown to induce acute conduction block (Lafontaine et al., 1982). In these studies, demyelinating activity was lost after complement inactivation, suggesting that the activity of anti-galactocerebroside serum is antibody dependent and complement mediated (Sumner et al., 1982). Despite these very convincing animal data, it remains statistically unproven in large clinical serological studies whether anti-galactocerebroside antibody is involved in human neuropathies, as described above.

Experimental neuropathy associated with anti-MAG/SGPG antibody

Local microinjection of anti-MAG IgM antibodies into feline nerves has been shown to cause focal demyelination at the injection site, characterized by macrophage-mediated myelin stripping (Hays et al., 1987; Willison et al., 1988). These effects only occurred when serum was added fresh or with a fresh source of complement, suggesting that antibodies caused demyelination by complement fixation, as is the case with galactocerebroside. In a series of interesting passive immunization experiments with purified anti-MAG IgM into young chickens, demyelination of nerve with prominent widening of myelin lamellae was observed, closely resembling the ‘widely spaced myelin’ that is so characteristic of human biopsies (Tatum, 1993). These abnormalities in chickens were obtained in the absence of an external source of complement, leading to the explanation that anti-MAG antibodies might act in part by inhibiting myelin turnover or by separating or impeding the fusion of apposed leaflets of intraperiod lines. However, it was never clarified in these whether human IgM was able to activate chicken complement. Demyelination with widely spaced myelin lamellae can also be induced in rabbit nerve by intraneural injection of either anti-MAG IgM or the terminal complement complex (Monaco et al., 1995). In nerves treated with anti-MAG IgM, the abnormalities were consistent with the activation of endogenous rabbit complement, resulting in the formation of the complement terminal attack complex. Another series of studies in the rat comprising intraneural injections of rat anti-SGPG antibody induced demyelination in rat sciatic nerve, along with mild to moderate clinical symptoms (Maeda et al., 1991).

Active immunization experiments have also been performed with SGPG. Kohriyama et al. (1988) immunized rabbits with SGPG, resulting in an immune response with anti-SGPG antibody induction. The rabbits showed weight loss and mild weakness, predominantly in their hind feet. Electrophysiological studies showed a slowed nerve conduction velocity in the sciatic nerve. Rats have also been immunized with SGPG, and show minor but clear clinical signs of neuropathy, consisting of mild tail muscle tone loss and walking disabilities (Yamawaki et al., 1996). Electrophysiological examination of the sciatic nerves revealed nerve conduction abnormalities that consisted of conduction block and a mild decrease in conduction velocity. In this study, the anti-SGPG antibodies were shown to react with human MAG, but not with rat MAG which does not bear the sulfated glucuronic acid epitope common to MAG and SGPG in some other species including man (O’Shannessy et al., 1985). The target molecule in rat nerve in these studies is therefore not MAG, but likely to be SGPG. Pathological evidence of demyelination unfortunately is lacking in both the rabbit and rat studies following inoculation with SGPG.

Experimental sensory ataxic neuropathy induced by GD1b

One of the clearest animal models of neuropathy has been pioneered by Kusunoki and colleagues. As discussed above, IgM paraproteins that bind to a group of disialylated gangliosides, including GD1b, are strongly associated with a chronic sensory ataxic neuropathy (reviewed in Willison et al., 2001). Immunohistochemical observations have shown the localization of GD1b to the neurones of human DRG (Kusunoki et al., 1993b), thus suggesting that GD1b in the
DRG neurones might be a target antigen for IgM paraproteins in this form of sensory ataxic neuropathy. GD1b is also localized to large neurones of rabbit DRG, but it is not present in the paranodal myelin of rabbit peripheral nerve and it was therefore assumed that sensitization of rabbits with GD1b might induce a sensory ataxic ganglionopathy (Kusunoki et al., 1996b). Indeed, this developed in ~50% of the rabbits immunized with GD1b. The affected animals lay on the floor with splayed limbs, often in a peculiar posture, and moved their limbs awkwardly. Muscle power and pain sensation appeared largely preserved. In these animals, anti-GD1b antibody titres were markedly raised. The isotype was reported as IgM alone in the original paper (Kusunoki et al., 1996b), but was described as both IgM and IgG in the following one (Kusunoki et al., 1999b). Rabbits responded to more than one epitope on GD1b, which contains both a disialosyl epitope and a Gal (β1–3) GalNAc epitope, the latter also being present on GM1 and asialo-GM1. It was clearly shown that the ataxic rabbits had a higher level of serum IgG mono-specific to GD1b than the unaffected ones, indicating the critical importance of the fine specificity of the immune response for discrete carbohydrate structures.

Pathological examination of the affected rabbits showed axonal degeneration in the dorsal roots, the dorsal column of the spinal cord and the sciatic nerve, and some neuronal cell bodies in the DRG had either degenerated or disappeared. The ventral root was entirely spared. Lymphocytic infiltration was not observed in the affected regions, indicating that an entirely antibody-mediated injury had taken place. This was supported further by demonstrating that degeneration of rabbit sensory neurones could be induced by passive transfer of anti-GD1b antiserum (Kusunoki et al., 1999c). Thus, the anti-GD1b antibody is likely to be an important pathogenic factor causing specific degeneration of primary sensory neurones that convey deep sensation.

This is the first well-defined model of autoimmune neuropathy mediated by an anti-ganglioside antibody, although its clinical and serological features are somewhat different from those of human sensory ataxic neuropathy, as might be expected when crossing species and time courses. However, this model confirms the assumption that anti-ganglioside antibodies may determine the clinical phenotypes of autoimmune neuropathies by binding to the respective ganglioside antigens that have a unique distribution in the PNS. The loss of primary sensory neurones that mediate proprioceptive sensation prompted Kusunoki’s group to investigate the expression of trkC in DRG in the animal model because this type of neurone is thought to depend mainly on neurotrophin-3-mediated trkC signalling (Hitoshi et al., 1999). TrkC expression was reduced in DRG of diseased rabbits in acute phase. This interesting result suggests that the anti-GD1b antibody-mediated downregulation of trkC expression may be one of the pathogenic mechanisms by which anti-GD1b antibodies mediate this experimental sensory ataxic neuropathy.

Animal models of acute motor axonal neuropathy induced by GM1

There have been two reports in the older literature of GM1-immunized rabbits developing paralysis or subclinical peripheral neuropathy (Nagai et al., 1976; Thomas et al., 1991), but this has not been observed convincingly in rodents, despite efforts. These findings suggest that failure to induce neuropathy by sensitization with gangliosides might depend in part on species and strain susceptibility and partly on the immunization procedure used. In a recent series of studies, rabbits were sensitized with a bovine brain ganglioside mixture according to the procedure of Kusunoki et al. (1996b), and it was observed that these rabbits developed high anti-GM1 IgG antibody titres, accompanied by a flaccid limb weakness of acute onset with a monophasic illness course, as illustrated in Fig. 5 (Yuki et al., 2001b). Interestingly, these animals subsequently have been screened for anti-galactocerebroside IgG antibodies that were not detected, supporting the view that the immune response is specific for GM1 and has not arisen as a bystander event secondary to peripheral nerve injury. The converse experiment has also been conducted (i.e. immunization with galactocerebroside and screening for anti-GM1) and draws the same conclusion (N. Yuki et al., unpublished results). In rabbits immunized with GM1, pathological findings in peripheral nerves showed predominant Wallerian-like degeneration with neither lymphocytic infiltration nor demyelination. IgG was deposited on the axons of the anterior roots, a site where GM1 was proved to be present. Sensitization with purified GM1 also induced this motor axonal neuropathy, indicating that GM1 was the immunogen in the mixture. Thus a rabbit model of AMAN associated with anti-GM1 IgG anybody has now also been clearly established.

A point worth noting is that GM1 and galactocerebroside are expressed in both the PNS and CNS, but sensitization with these molecules produces only peripheral neuropathy (T. Saida et al., 1979). The blood–nerve barrier that protects the PNS may not be as tight as the blood–brain barrier. Thus it seems probable that small amounts of circulating IgG, which cannot enter the CNS, can penetrate the endoneurial space in the PNS. This relative leakiness may make the PNS, especially the nerve roots, nerve terminals and DRG, more vulnerable than constituents of the CNS to IgG antibody-mediated disorders.

Fulfilling the criteria for proof of anti-glycolipid antibody-mediated autoimmunity

Do anti-GD1b antibody-mediated sensory ataxic neuropathy and anti-GM1 antibody-mediated AMAN now meet the criteria for an autoimmune disorder? To fulfil the Witebsky’s postulates formally, the data to satisfy the first criterion are needed: clinical and pathological disease should be induced by passive transfer of anti-GM1 or anti-GD1b IgG antibody from AMAN and sensory ataxic neuropathy patients, neither
of which have yet been achieved (Sheikh and Griffin, 2001). The other three criteria, however, have been satisfied for GM1 in that one of the autoantigens in AMAN has been identified as GM1, it is expressed on human peripheral nerve, antibodies have been raised in experimental animals and pathological changes have appeared in the corresponding tissue of this actively sensitized animal, consistent with those seen in human AMAN cases. The explanation as to why the GM1- and GD1b-mediated models only occur so floridly in certain types of experimental animal (i.e. the Japanese rabbit) still remains elusive and clearly requires further investigation.

Chickens, rats and mice have all been studied following immunization with GM1 or exposure to anti-GM1 antibodies through a number of routes and formats, and the results have often been either negative, or resulted in a milder phenotype, as described above. Many factors may be relevant, one of the most likely being the relative lack of activation of the complement system, due to either antibody or species characteristics, or a combination of both. In the near future, we should expect to see further animal models described with these and other antigens in a variety of species, and a deeper exploration of the pathogenic events, focusing on opportunities for therapeutic interventions.

**Therapeutic considerations**

An anti-glycolipid antibody-mediated immune attack can be monophasic or self-limited, can have acute exacerbations and remissions, or be chronic and slowly progressive. Any therapeutic approach thus depends on the severity and duration of the immune attack, and needs to take account of the antibody characteristics, including isotype and complement-fixing properties. GBS manifests an acute onset and a monophasic course, suggesting that early aggressive treatment is needed. The first treatment to be proven clinically effective was plasma exchange, which shortens the duration of disability in GBS in comparison with conventional supportive treatment (Guillain–Barré Syndrome Study Group, 1985; French Cooperative Group on Plasma Exchange in Guillain–Barré Syndrome, 1987). The therapeutic effect seems to relate to the removal of circulating autoantibodies, whose target may include ganglioside in at least some cases. Effective therapies have yet to be proven in clinical trials for MFS and related acute conditions, although it seems likely that these would be similar to GBS treatments. Experimental studies demonstrating pathogenic effects of anti-GQ1b antibodies lend support to the notion that the removal of anti-GQ1b antibodies is reasonable and useful.

In GBS, intravenous immunoglobulin therapy is as effective as plasma exchange (van der Meche et al., 1992; Plasma Exchange/Sandoglobulin Guillain–Barré Syndrome Trial Group, 1997). This has led to the widespread use of intravenous immunoglobulins for patients with MFS and related conditions, although the mechanisms by which immunoglobulins act remain unclear and are likely to be multifactorial (Stangel et al., 1999). Interestingly, a subgroup of anti-GM1-positive GBS patients responded well to treatment with immunoglobulins but not to plasmapheresis (Jacobs et al., 1996; Kuwabara et al., 2001). Future treatment studies using in vitro and in vivo animal models are underway in many laboratories and are likely to identify new immunomodulating drugs, neuroprotective agents that act at common points in the pathways mediating antibody-mediated complement-dependent tissue injury, and drugs that promote nerve regeneration.

With respect to chronic neuropathies, the clinical situation is rather different. Pioneering studies by Latov et al. (1980) and Pestronk et al. (1988) have informed us that patients with anti-MAG/SGPG IgM antibody or anti-GMI IgM antibody, respectively, are treatable and should be considered separately from other chronic neuropathies. In both these conditions, IgM class antibodies are present, in contrast to the longer lived IgG that is believed to be pathogenic in GBS.
IgM has a very short serum half-life of only a few days, and thus plasma exchange is unlikely to be effective unless performed aggressively and very frequently, a situation that is impracticable in most clinical circumstances. The paraproteinaemic neuropathy with anti-MAG/SGPG IgM antibody is a representative example of such an indolent, slowly progressive disease. Treatment of patients with anti-MAG/SGPG IgM antibody is directed primarily at lowering the autoantibody level. This has been attempted using several immunosuppressive regimens, either alone or in combination, including prednisolone, plasma exchange, cytotoxic agents and high-dose intravenous immunoglobulins (Nobile-Orazio et al., 2000). Current immunotherapies are effective temporally in half of the patients, but are associated with considerable side effects, which limit their prolonged use and efficacy. Thus after over a decade of attempted treatments, many specialists now advise that the anti-MAG neuropathy remains untreated. Similar dilemmas arise in other chronic syndromes including multifocal motor neuropathy associated with anti-GM1 IgM antibody. This latter condition, however, is often exquisitely sensitive to intravenous immunoglobulin therapy which is now very widely used as long-term recurrent therapy and should be used as a trial therapy in all patients with significant disability. Intravenous immunoglobulin is not without side effects, including the practical and theoretical risks of transmissible diseases, and is an expensive option when used recurrently (Nobile-Orazio et al., 2000). Safer and more effective therapy is required to reduce the level of the autoantibodies. Depleting B cells using rituximab, a monoclonal antibody directed against the B cell surface marker CD20, is a new, attractive approach that has been used principally to treat lymphoproliferative B cell disorders; to date, its use in neuropathy has not been especially successful, but it may offer benefit in some carefully selected cases (Levine and Pestronk, 1999).

Conclusions

The last 20 years have seen the emergence of abundant clinical data clearly showing a disease-specific correlation between peripheral neuropathies and anti-glycolipid antibodies. Many interesting clinico-serological patterns have emerged that support the view that anti-glycolipid antibodies play a key active role in pathogenesis. Experimental evidence obtained from human and animal studies continues to support the model of post-infectious neuropathy as a disease involving molecular mimicry between bacterial and neural oligosaccharides. Many key issues remain unresolved across the whole pathogenesis spectrum, ranging from the genetic and/or environmentally derived host factors that confer disease susceptibility to a small proportion of infected individuals, details of the site-specific nature of pathology according to antibody specificity, the extent to which T cell factors are involved in roles other than providing T cell help to antibody production, and the nature of the immunological injury that lesions the blood–nerve barrier. Once these issues have been resolved, the opportunity hopefully will arise to develop specific rationales for targeted immunotherapy, in order that we can prevent or limit the devastating injury that can result from autoimmune-mediated neuropathy.

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