Detection of antibodies in neuropathy patients by synthetic GM1 mimics

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Antibodies to the ganglioside GM1 are associated with various forms of acute and chronic immune-mediated neuropathy, including Guillain–Barré syndrome (GBS) and multifocal motor neuropathy. In diagnostics and research, these antibodies are usually detected by GM1 preparations derived from bovine brain tissue, which are non-covalently attached to solid carriers such as enzyme-linked immunosorbent assay (ELISA) plates. Such brain-derived GM1 preparations are potentially contaminated with other glycolipids. In the current study, uncontaminated mono- and divalent synthetic analogs of the ganglioside GM1 were successfully attached via covalent bonds onto the surface of ELISA plates. The resulting modified diagnostic tool showed strong affinities and good specificities for binding of monoclonal mouse and human anti-GM1 antibodies and cholera toxin, as well as for the anti-GM1 antibodies in serum samples from neuropathy patients. While these proof-of-principle experiments reveal the potential of synthetic ganglioside mimics in diagnostics, they show the necessity of further studies to overcome certain limitations, specifically the non-specific interactions in the negative control assays with synthetic GM1.

Keywords: antibody / diagnostics / ganglioside mimics / GM1 ganglioside / neuropathy

Introduction

The presence of serum antibodies to the ganglioside GM1 is associated with various forms of immune-mediated neuropathy, including the Guillain–Barré Syndrome (GBS), multifocal motor neuropathy (MMN) and monoclonal gammopathy and polyneuropathy (Willison and Yuki 2002). GBS is the most common form of acute neuromuscular paresis, and 10–40% of GBS patients have serum anti-GM1 antibodies, depending on the geographical region of the patients and the techniques used to detect these antibodies. Anti-GM1 antibodies in GBS are predominantly of the immunoglobulin (Ig) G subclass and associated with a severe motor variant of the disease (Jacobs et al. 1996). In GBS, these antibodies are primarily raised to microbial carbohydrate antigens and produced during a preceding infection (Willison 2005b). Due to molecular mimicry (Yuki 2005) between these microbial carbohydrates and the pentasaccharide unit of GM1, these antibodies can cross-react to native GM1 in peripheral nerve membranes, where they induce complement-dependent nerve dysfunction (Yuki 2001). These antibodies usually disappear within weeks from the serum, after which the then surviving GBS patients recover. MMN is a chronic form of neuropathy in which 30–50% of the patients have serum anti-GM1 antibodies, predominantly of the IgM subclass, and the presence of these antibodies is associated with a severe form of the disease (Nobile-Orazio et al. 2005; Van Asseldonk et al. 2005; Kleinschnitz and Reiners 2006; Kaji 2008; Rajabally 2008). Similar to GBS, the anti-GM1 antibodies in patients with MMN are neurotoxic in animal models. Patients with GBS and MMN show a partial response to treatment with intravenous immunoglobulins (IVIg). At present, there is no specific treatment focused on anti-GM1 antibodies, although they likely have a dominant contribution to the peripheral nerve damaging.

Antibodies to GM1 are diagnostic markers (Marcus 1990) for GBS, MMN and other forms of immune-mediated neuropathy and a possible target for future specific treatment. A number of protocols for enzyme-linked immunosorbent assays (ELISAs) for measuring serum anti-GM1 antibodies have been described (Kornberg et al. 1994; Vanschaik et al. 1994, 1995), as well as a standardized INCA T-ELISA (Kuijf et al. 1996), as well as a standardized INCAT-ELISA (Kuijf et al. 2005; Willison et al. 1999) protocol aimed to facilitate the comparison of results from different laboratories. The detection of anti-GM1 antibodies in these protocols is, however, restricted by the use of bovine brain-derived gangliosides, which are difficult to purify, expensive, potentially infected...
and usually contaminated with other glycolipids. These drawbacks may partly explain the variation and limited sensitivity and specificity of currently used ELISAs to detect the antibodies. Development of a specific treatment based on selective removal of anti-GM1 antibodies from the blood of the patients by immunoabsorption would require covalent attachment of the GM1 to a surface carrier at the aglycone part, thus leaving accessible the carbohydrate moiety of GM1 to which the antibodies are directed. Bovine brain-derived GM1, however, cannot be used for this purpose considering its impurity, potential risk of infection and unsuitability of the ceramide tail for the covalent attachment. Fully pure GM1 ganglioside (or its mimics) would be required to solve these problems. Moreover, depletion from the serum may also be hampered by the relatively low affinity of the anti-GM1 antibodies.

In recent years, there is a rapidly increasing interest in a new class of neuropathy-associated autoantibodies. These antibodies only bind to complexes of gangliosides and have no affinity to either ganglioside individually (Kaida et al. 2004, 2005, 2006a, 2006b; Willison 2005a, 2006; Kaida, Kanzaki, Morita, Kamakura, Motoyoshi and Kusunoki 2006; Kaida, Kanzaki, Sada, Hirakawa, et al. 2007; Kaida, Kanzaki, Sada, Motoyoshi, et al. 2007; Kaida, Morita, et al. 2007; Kuijf et al. 2007; Kaida and Kusunoki 2008; Kanzaki et al. 2008; Kusunoki et al. 2008; Notturno et al. 2008, 2009; Ogawa et al. 2009). Direct evidence for the existence of ganglioside complexes in nature has been provided (Todeschini et al. 2008; Greenshields et al. 2009), and the formation of new methods for the assessment of serum antibody reactivity to ganglioside complexes in patients is of considerable interest. Thus, in addition to more traditional ELISA and thin-layer chromatography (TLC) with immuno-overlay (Kaida et al. 2004), an automated glycoarray-based combinatorial system for the analysis of interactions of proteins with ganglioside complexes has been proposed recently (Rinaldi et al. 2009). It is clear that the purity of the gangliosides applied in these techniques is crucial for their specificity and sensitivity, and therefore, the demand for the individual uncontaminated compounds, rather than bovine brain-derived ganglioside fractions, is high.

Application of synthetic ganglioside mimics may circumvent the limitations of bovine GM1 fractions. First, these synthetic analogs can be produced in completely pure form, preventing the binding of antibodies to contaminants. Second, GM1 mimics can be synthesized with a group identical to the GM1 carbohydrate group, but with alternative apolar tails tailor-made for covalent binding to scaffolds. This covalent attachment also takes care of attachment of the GM1 sugar onto the biosensor in only one orientation (Manova et al. 2011). Third, polyvalent ganglioside analogs can be synthesized to increase the binding of the low-affinity (Deisenhammer et al. 1996) antibodies. These polyvalent gangliosides can be designed to contain either two or more identical carbohydrate groups (Pukin et al. 2007), or the oligosaccharide parts of different gangliosides linked to one dendrimeric scaffold. This would allow binding of antibodies that are reactive against ganglioside complexes. Thus, such structurally analogous and completely pure ganglioside mimics are able to be specifically recognized by the same antibodies, while at the same time they are equipped with functional groups that allow chemical tuning of the properties and composition of the diagnostic tools. These innovations therefore have the potential for significant advantages over currently employed methods for antibody binding.

In the present study, we investigated the proof of principle of whether covalently attached pure synthetic mono- and divalent analogs of GM1 can bind to anti-GM1 antibodies. These analogs were covalently attached to Nunc Immobilizer™ Amino ELISA plates and tested for binding with cholera toxin and mouse and human monoclonal anti-GM1 antibodies. In addition, sera from patients with GBS, MMN and monoclonal gammopathy and polynuropathy were screened for the presence of anti-GM1 antibodies using these synthetic GM1 analogs.

Results

Attachment of ganglioside analogs on the surfaces of ELISA plates

In the present study, Nunc Immobilizer™ Amino ELISA plates were used for modifications with the GM1 analogs. These plates are specifically designed for the covalent attachment of peptides and proteins via the reaction of free amino or thiol groups thereof.

The immobilization of the synthetic monovalent GM1 analog 1 (Figure 1) was achieved in two steps. First, plates were incubated with propargylamine (HC≡C-CH2-NH2) under the recommended conditions for protein immobilization via amino groups. The protocol for such an immobilization is provided with the plates by the manufacturer. No auxiliary reagent was needed for the attachment onto the plate surfaces. Alkyne-coated surfaces formed in this way were incubated at the next step with 1 in the presence of CuSO4 and sodium ascorbate. During this incubation, the Huisgen-Meldal-Sharpless cycloaddition “click” reaction (Bock et al. 2006) between alkyne moieties on the surface and the azido group of 1 occurred under very mild conditions, thus resulting in diagnostic tools functionalized with this covalently attached GM1 analog. The attachment of dimer 2 on the ELISA plates was performed in one step following the recommended protocol for peptide immobilization via amino groups.

Detection of cholera toxin and monoclonal anti-GM1 antibodies in ELISA using the modified plates

In order to establish general principles, we first examined five monoclonal antibodies (mAbs) and the cholera toxin B subunit (CT-B) in respect of their binding to bovine GM1 (coated on the Maxisorp plates) and covalently immobilized synthetic GM1 analogs. Among the mAbs used in this study, two mouse antibodies, DG-1 and DG-2 (Goodyear et al. 1999), and one human antibody, Sm-1 (Paterson et al. 1995), recognize GM1; DG-1 is monospecific for GM1, whereas DG-2 has been shown to bind, apart from GM1, also GA1 and GD1b (Townson et al. 2007; Greenshields et al. 2009). The GM1-negative mouse mAb EG-1 and the human mAb L were tested as negative controls.
The ganglioside-binding characteristics of the anti-GM1 mouse mAbs DG-1 and DG-2 and human mAb Sm-1 are presented in Figure 2A–C. As can be observed, GM1-positive mouse mAbs bind to the synthetic GM1 analogs in a slightly different manner when compared with their binding to the bovine GM1. Thus, DG-1 reacts with the bovine GM1 with a half-maximal binding value of 0.45 µg/mL, while analysis of its binding to 1 and 2 gives 1.54 and 2.14 µg/mL, respectively. At the same time, for the synthetic gangliosides, the saturation of the binding is reached at a much higher concentration of DG-1 antibodies. Thus, for this antibody, the sensitivity of the assay with natural GM1 is higher than the assay with synthetic analogs, but the potential to discriminate between samples of different concentrations increases upon use of covalently attached GM1.

For the DG-2 mAbs (Figure 2B), the half-maximum binding value of 0.85 µg/mL for binding to natural GM1 is decreased to 0.4 µg/mL in the case of 2 and even lower to 0.2 µg/mL for the GM1 analog 1, while the maximum signal is reached at similar concentrations for all the three gangliosides. In other words, the synthetic GM1 display a higher sensitivity than the natural GM1 toward DG-2.

The human anti-GM1 mAb Sm-1 reacted with synthetic GM1 analogs with both the half-binding values and the saturation points similar to those observed for the binding to GM1 (Figure 2C), thus indicating 1 and 2 to be very good mimics of natural GM1 with respect to the binding to the human mAbs.

The performance of the ELISAs based on the natural and synthetic gangliosides in the detection of cholera toxin is demonstrated in Figure 2D. As can be clearly seen, for both mono- and divalent GM1 analogs, the saturation of the binding is reached at significantly lower concentrations of the toxin when compared with this value for the bovine GM1. The calculated half-maximum binding values for the synthetic gangliosides are also considerably lower than for the natural GM1, showing that synthetic GM1 is in this case far superior over natural GM1.

The ganglioside-binding characteristics were also studied for the negative-to-GM1 mAbs EG-1 and L, as controls for non-specific binding. These mAbs did not bind to any of the gangliosides, thus giving a proof of the specificity of the observed binding of the anti-GM1 mAbs.

**Detection of anti-GM1 antibodies in the serum from neuropathy patients**

Next, the performance of the routine ELISA (based on the bovine brain GM1 non-covalently adsorbed on Nunc Maxisorp™ ELISA plates) and of the ELISAs based on the plates modified by covalent attachment of monomeric 1 and dimeric 2 GM1 analogs were compared in detecting IgM and IgG antibodies in the serum from normal controls and patients with neuropathies. After coating of the plates, the same validated INCAT protocol (Jacobs et al. 1996; Willison et al. 1999; Kuijf et al. 2005) was used in all the types of ELISAs. Sera from a series of 33 neuropathy patients strongly positive for anti-GM1 antibodies in routine INCAT-ELISA (15 GBS patients, 15 MMN patients, 3 monoclonal gammopathy and polyneuropathy patients), as well as 5 healthy control sera were examined for antibody reactivity to the synthetic GM1 mimics covalently bound in an ELISA. The comparison with the routine INCAT and monovalent GM1-based assays is presented in Figure 3. The GBS patients were selected on the basis that they displayed a strong IgG antibody activity in INCAT-ELISA (in practice: 1.1 ± 0.2), which for bovine GM1 maxes out at ca. 1.3 (Figure 3A). The MMN patients and monoclonal gammopathy with polyneuropathy patients were selected for their IgM antibody activity in INCAT-ELISA (1.15 ± 0.25 with the maximum at ca. 1.4).

The figure demonstrates that within the tested serum variations, both IgG and IgM antibodies behave very similar in the modified ELISAs and the standardized INCAT-ELISA. Indeed, statistical analysis of the data using the Spearman rank correlation shows that the relationship between the optical density (OD) values in these two assays is significant (Spearman’s ρ = 0.75, p = 2 × 10⁻⁷). The high value of ρ indicates the strong correlation between the affinity of the antibodies toward natural GM1 and their affinity to the synthetic analogs, clearly pointing to the potential of the synthetic analogs in diagnostic detection of the anti-GM1 antibodies in sera.
Fig. 2. Ganglioside-binding characteristics of the anti-GM1 antibodies and CT-B. Circles, binding to natural GM1; inverted triangles, binding to monovalent GM1 analog 1; squares, binding to divalent GM1 analog 2.

Fig. 3. Comparison of ELISAs with covalently bound monovalent pure GM1 analog 1 vs non-covalently bound bovine impure GM1 with respect to detecting IgM and IgG antibodies in the serum from neuropathy patients and healthy controls. The values on the axes represent d-OD in the assays obtained by subtracting the OD in the blank wells from the OD in the GM1 (analog)-coated wells.
Significant attention has been directed recently to study the antibodies to ganglioside complexes (Kaida et al. 2004, 2005, 2006a, 2006b; Willison 2005a, 2006; Kaida, Kanzaki, Morita, Kamakura, Motoyoshi and Kusunoki 2006; Kaida, Kanzaki, Sada, Hirakawa, et al. 2007; Kaida, Kanzaki, Sada, Motoyoshi, et al. 2007; Kaida, Morita, et al. 2007; Kuijf et al. 2007; Kaida and Kusunoki 2008; Kanzaki et al. 2008; Kusunoki et al. 2008; Notturno et al. 2008, 2009; Ogawa et al. 2009). Such antibodies bind much stronger, if not exclusively, the mixtures of gangliosides (e.g. GM1/GD1a mixture) rather than single gangliosides, and therefore it is likely that multivalent interactions are important in the recognition of ganglioside complexes. In order to establish whether the multivalency based on the identical ganglioside structures plays a role in the antibody binding, the ELISAs based on the monovalent (1) and divalent (2) GM1 analogs were compared (Figure 4) in the detection of the anti-GM1 antibodies in the same set of serum samples as was used to compare antibody reactivity to 1 and bovine GM1.

Figure 4 shows the relative performance of the assays based on the synthetic gangliosides 1 and 2. As can be clearly seen, for both IgG and IgM antibodies, the majority of the tested samples showed virtually the same difference in OD (d-OD) in both assays.

Discussion

Identification of anti-GM1 antibodies in the serum from patients with immune-mediated neuropathies is complicated by the natural origin of the bovine GM1 fractions used to detect these antibodies. It is almost impossible to obtain 100% pure gangliosides from natural sources, and the contaminants are—due to the way these compounds are isolated—likely to be other glycolipids that could interfere with the determination of the specificity of the anti-ganglioside antibodies. Several studies have shown that the commercially available bovine GM1 fractions are contaminated with other gangliosides, which may also bind antibodies in the serum from the patients. Moreover, the binding of serum antibodies to GM1 may be enhanced or diminished by the presence of other gangliosides. Some patients with GBS may even have antibodies to combinations of gangliosides, the so-called ganglioside complexes, instead of to single gangliosides (Kaida et al. 2004, 2005, 2006a, 2006b; Willison 2005a, 2006; Kaida, Kanzaki, Morita, Kamakura, Motoyoshi and Kusunoki 2006; Kaida, Kanzaki, Sada, Hirakawa, et al. 2007; Kaida, Kanzaki, Sada, Motoyoshi, et al. 2007; Kaida, Morita, et al. 2007; Kuijf et al. 2007; Kaida and Kusunoki 2008; Kanzaki et al. 2008; Kusunoki et al. 2008; Notturno et al. 2008, 2009; Ogawa et al. 2009). Recognition of the fine specificity of these antibodies is relevant because of the distinct association between the antibody specificity and defined clinical subgroups in GBS and MMN (Willison and Yuki 2002). Pure ganglioside preparations are therefore essential to determine antibody specificities, including ganglioside complexes, especially when techniques like ELISA and arrays are used.

In the current study, antibodies were detected by ELISAs using synthetic GM1 analogs with the same terminal pentasaccharide as in the natural GM1. These synthetic mono- and divalent GM1 mimics contained one or two of these carbohydrate moieties. By the way of comparison of the performance of the mono- and divalent GM1 analogs, a primary assessment of the role of multivalency in antibody binding could be made. For the attachment of the ganglioside analogs, the ELISA plates designed for the attachment of proteins were used successfully. Since this worked unproblematically, this indicates a broader scope for the application of these plates, in particular, the possibility to attach smaller molecules. The current experimental data even show that multistep chemical
transformations are compatible with low-noise ELISAs. Thus, the attachment of the azide-terminated GM1 analog \( I \) required a two-step process with application of a copper salt at the second step. Performing this reaction sequence did not result in any loss of performance of the ELISA plates.

Importantly, the synthetic GM1 analogs were completely pure individual compounds. Binding studies with cholera toxin and specific mouse and human mAbs to GM1 demonstrated that the targets for antibody binding in the test systems were preserved. Moreover, the CT-B showed considerably stronger binding to the synthetic gangliosides than to bovine GM1, which implies that the synthetic ganglioside-based ELISA would be a much more sensitive tool for the detection of cholera toxin. The sensitivity of the ELISA with synthetic GM1 mimics to detect the presence of mAbs was in general very similar to that of the routine ELISA using the bovine GM1 preparation. Mouse and human control monoclonals showed no binding, excluding the possibility of aspecific binding. These results delivered a proof of principle that synthetic GM1 mimics can be used as a sensitive and specific target to demonstrate anti-ganglioside antibodies by ELISA.

Next, we aimed to detect anti-GM1 antibodies in the serum from patients with immune-mediated neuropathies using the mono- and divalent synthetic GM1 analogs and compared the results to those using the bovine GM1. Serum IgM antibodies were tested in healthy controls, MMN and monoclonal gammopathy patients and serum IgG antibodies in healthy controls and acute stage GBS patients. A high correlation between the serum IgM and IgG activities to the synthetic GM1 mimics and to the native bovine GM1 was found. This correlation was demonstrated by the Spearman rank-correlation calculations, which unequivocally show a correlation between antibody activities to bovine GM1 and \( I \) (Spearman’s \( \rho = 0.75; \ p = 2 \times 10^{-7} \)). The ELISA with the synthetic GM1 mimic demonstrated IgM antibody activity in the majority of MMN patients and IgG activity in the majority of GBS patients, while the healthy controls were usually negative. These data show the potential of these synthetic GM1 mimics to be used in ELISA to detect IgM and IgG antibodies in the serum from patients with both MMN and GBS. It seems that the application for this purpose of a dimeric GM1 analog with two identical carbohydrate units is not really beneficial over the monomeric one. Thus, the mono- and divalent GM1 mimics showed highly comparable results (Figure 4), indicating that the divalency does not improve the detection system for patient sera. Sera with antibody reactivity to bovine GM1, but weak or no antibody reactivity to synthetic monovalent GM1, also had weak or no antibody reactivity to the divalent GM1 analog. This may indicate that multivalency is of little to no importance in the antibody binding, i.e. binding of serum anti-GM1 antibodies does not depend on the presence of multivalent targets, or that the density of the monovalent GM1 mimics on the surface is sufficient to provide multivalent interactions.

Although the results with the ELISAs with bovine GM1 preparation and synthetic ganglioside mimics were highly correlated, a significant difference in the assay outcomes of natural and synthetic GM1 is the scatter of the data observed for the synthetic GM1s. This is most clearly seen for the negative controls, which are expected to give a result close to zero for all five data points from healthy patients, but this is only seen for the bovine-derived GM1 assay. This is not caused by experimental scatter (“noise”), as, for example, plots of the responses of the synthetic GM1’s \( 1 \) and \( 2 \) display a correlation with little noise (Figure 4). Apparently, aspecific interactions are present in the assays with synthetic GM1s, which would hamper accurate quantification of the antibody concentration in sera.

Part of this scatter can be explained by the manner in which the samples were selected for this study. Thus, for the comparison of the routine and modified ELISAs, serum samples were chosen that previously not only tested positive in the traditional ELISA, but also showed d-OD values close to the experimentally found maximum of 1.3. This selection criterion also revealed a positive aspect of the use of synthetic GM1. As a result of the highly desirable higher dynamic range of the synthetic GM1-based tests, the plateau corresponding to the binding saturation therein is reflected in higher d-OD values than those in the routine ELISA, and thus the differences in activities of the antibodies with high affinities can be observed better. This, of course, may give rise to observed variations (with d-OD sometimes significantly >1.3) with the synthetic GM1 for cases where bovine GM1 gives a near-constant response.

A subgroup of MMN and GBS patients was clearly positive only for reactivity to the bovine GM1 preparation (Figure 3), giving remarkably low signals in the synthetic GM1-based assays. This subgroup with serum reactivity preferentially to bovine GM1, consisted of a heterogeneous group of patients with MMN or GBS with various preceding infections, clinical features and outcome and titers to bovine GM1. This subgroup did not differ significantly from the other MMN and GBS patients regarding these features. Interpretation of this finding is hampered by the absence of a golden standard for the detection of anti-GM1 antibodies. Previous results with other techniques (Kornberg et al. 1994; Vanschaik et al. 1994, 1995; Willison et al. 1999; Kuifj et al. 2005) have shown that the binding of anti-GM1 antibodies is highly influenced by the type of ELISA plates, the presence of Tween, temperature and other test characteristics. In addition, cross-reactivity of these antibodies with other gangliosides (possibly present as contaminants) may also yield positive results for the bovine-derived GM1 that are absent in pure, synthetic GM1 derivatives. Further studies with application of a 100% pure natural GM1 in ELISA (which in our view may become a golden standard) are needed to clarify this situation.

The synthetic GM1 ELISA may be less sensitive than the bovine GM1 ELISA for detecting serum antibodies in these particular cases, e.g. because the random orientation of the glycan part of non-covalently attached bovine GM1 sugars and their flexibility in a rather disorganized layer may allow orientations that are optimally suited to interact with an (comparatively large) antibody (see Figure 5A for a simplified depiction of this hypothesis). In comparison, the better ordered layer of covalently attached GM1 may in fact be packed too densely, which diminishes the orientations with
which the oligosaccharide part may present itself to the antibody, and thereby making the oligosaccharide moiety topologically less available for the antibody binding (Figure 5B).

Alternatively, the antibodies binding to the bovine GM1 preparation may partly bind to contaminants or to complexes (Kaida et al. 2004; Willison 2005a; Kuijf et al. 2007; Notturmo et al. 2008; Ogawa et al. 2009) between GM1 and these contaminants. The higher dynamic range of synthetic GM1—as was observed for mouse antibody DG-1 (Figure 2A)—could actually point to differences in affinities of different antibodies that do not clearly show up in the current ELISAs due to their limited dynamic range. Clearly, while these data show a proof of principle, more research is needed to minimize the aspecific response and to determine the value of the synthetic GM1 mimics in clinical practice. Although the direct response of the synthetic GM1-based ELISA test is lower in the case of several serum samples, availability of completely pure ganglioside analogs allows the controlled formation of a stable layer of the covalently bound antigen, which is attractive for the investigation of the fine specificity of antibodies to both individual gangliosides and ganglioside complexes. Future studies with GM1 derivatives need to be directed to variation of the spacers (length and polarity) between the substrate and the oligosaccharide moiety and to the density of GM1 molecules on the surface. With tailor-made syntheses both can, in principle, be controlled to a very high degree. This could enhance a major advantage of using the synthetic GM1 mimics compared with bovine-derived products, namely the highly defined circumstances under which the antibody binding can be studied.

Conclusions
We have successfully performed the covalent attachment of mono- and divalent GM1 analogs onto Nunc Immobilizer™ Amino ELISA plates. Thus, obtained diagnostic tools have shown strong affinities to the CT-B and monoclonal mouse and human anti-GM1 antibodies. Detection of the anti-GM1 IgG and IgM antibodies in sera from neuropathy patients demonstrated that natural GM1 and synthetic mimics bind analogously to these antibodies, which shows the potential of the synthetic GM1 analogs in practical anti-GM1 antibody detection. The main differences are the increased scatter observed with these synthetic analogs, and the higher dynamic range obtainable with these synthetic sugars. These differences in binding behavior between natural and synthetic oligosaccharides show the necessity of more detailed studies. In general, these proof-of-principle experiments reveal the potential to obtain selective and sensitive diagnostics based on synthetic ganglioside mimics and to provide a valuable alternative to the currently used methods.

Materials and methods
General information
Nunc Immobilizer™ Amino 96-well ELISA plates were used for the coatings with synthetic GM1 analogs. The ganglioside GM1 was obtained from Sigma (Schnelldorf, Germany, purity ≥95% TLC according to the supplier) and coated on the 96-well Nunc MaxiSorp™ ELISA plates as described previously (Jacobs et al. 1996; Willison et al. 1999). The ganglioside analogs 1 and 2 were synthesized during the design of inhibitors of cholera toxin (Pukin et al. 2007, 2008).
Human serum samples were obtained from patients with acute and chronic neuropathies and controls. The study included patients with GBS (n = 15), MMN (n = 15), monoclonal gammopathy and polyneuropathy (n = 3) and healthy controls (n = 5).

Attachment of the monovalent GMI analog onto the ELISA plate
To the wells of a Nunc Immobilizer Amino ELISA plate, a 10 mM solution of propargylamine in 0.1 M sodium carbonate buffer (pH 9) was added (200 µL per well). The plate was incubated at room temperature for 4 h with shaking. The solution was discarded by flicking and the wells were washed three times with water (each time 400 µL per well). After that, the following solutions were added to each well in the following order: 6 µM solution of copper (II) sulfate in water (50 µL per well), 3 µM solution of the ganglioside analog 1 in water (100 µL per well), 12 µM solution of sodium ascorbate in water (50 µL per well). The plate was incubated overnight at room temperature with shaking. The wells were emptied by flicking and washed three times with water (each time 400 µL per well).

Attachment of the divalent GMI analog onto the ELISA plate
To the wells of a Nunc Immobilizer Amino ELISA plate, 0.75 µM solution of the ganglioside analog 2 in 0.1 M sodium carbonate buffer (pH 9) was added (200 µL per well). The plate was incubated overnight at room temperature with shaking. The wells were emptied by flicking and washed three times with water (each time 400 µL per well).

Enzyme-linked immunosorbent assay
Serum IgM and IgG anti-GM1 ganglioside antibodies were determined by ELISA according to the method previously described (Jacobs et al. 1996) and standardized by the INCAT group (Willison et al. 1999; Kuijf et al. 2005). To determine anti-GM1 reactivity, the mean d-OD of two GM1-coated wells and two reference wells were used.

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Conflict of interest
None declared.

Abbreviations
CT-B, cholera toxin B subunit; d-OD, difference in optical densities; ELISA, enzyme-linked immunosorbent assay; GBS, Guillain–Barré syndrome; IVlg, intravenous immunoglobulin; mAb, monoclonal antibody; MMN, multifocal motor neuropathy; OD, optical density; TLC, thin-layer chromatography.

References


