Ganglioside Agglutination Immunoassay for Rapid Detection of Autoantibodies in Immune-Mediated Neuropathy

Armin Alaedini,* Itzhak Wirguin, and Norman Latov

Department of Neurology, Columbia University, New York, New York

Elevated levels of serum autoantibodies directed against gangliosides are closely associated with acute and chronic autoimmune neuropathies. An agglutination immunoassay using polystyrene microparticles coated with a total extract of brain gangliosides was used to test patient sera for the presence of anti-ganglioside antibodies. Results were compared with those obtained by ELISA for anti-GM1 and anti-GQ1b ganglioside antibodies. Eight of the twelve sera from patients with multifocal motor neuropathy and seven of the thirteen sera from patients with Guillain-Barré syndrome were

positive for the presence of anti-ganglioside antibodies by the ganglioside agglutination immunoassay. The assay compared favorably with the ELISA system in sensitivity and specificity, while requiring a fraction of the time and cost to perform. The new assay can serve as a rapid and effective method for detecting or screening for anti-ganglioside antibodies in patients with acute or chronic immune-mediated neuropathies. It would be particularly useful for detecting antibodies that react with multiple gangliosides, or with minor or as yet uncharacterized gangliosides. *J. Clin. Lab. Anal.* 15:96–99, 2001. © 2001 Wiley-Liss, Inc.

Key words: autoantibodies; gangliosides; peripheral neuropathies; immunoassays; Guillain-Barré syndrome

INTRODUCTION

Antibodies to gangliosides are implicated in the pathogenesis of several autoimmune neuropathic syndromes, including the Guillain-Barré syndrome (1,2), and a number of chronic peripheral neuropathies (3). These antibodies react with oligosaccharide determinants of major or minor gangliosides, which are highly concentrated in the peripheral nerves.

In several cases, the antibodies recognize oligosaccharide determinants that are shared by different gangliosides. For example, anti-GM1 ganglioside antibodies in motor neuropathy often react with the Gal(β 1–3)GalNAc epitope, which is shared by GD1b (4); antibodies to GD1b in sensory ataxic neuropathy recognize disialosyl epitopes shared by GD2, GD3, GT1b, and GQ1b (5,6); antibodies to GD1a in motor dominant neuropathy recognize the NeuAc(α 2–3)Gal(β 1–3) moiety shared with GT1b and GM3 (7); and anti-GQ1b ganglioside antibodies in the Miller Fisher variant of the Guillain-Barré syndrome react with the disialosyl moiety which also characterizes GD3 and GD1b gangliosides among others (8).

At present, anti-glycolipid antibodies are routinely detected by ELISA, which in its present format, measures serum antibody binding to purified individual glycolipids coated onto microwells (9). This assay system is relatively cumbersome, requires several days to perform, and takes place under nonphysiologic conditions of temperature and serum dilution. In addition, routine testing is limited to single major ganglio-

sides, and therefore may miss sera with antibodies that react with minor gangliosides, or with as yet uncharacterized gangliosides.

We recently reported a rapid agglutination system for detecting anti-GM1 antibodies using GM1 ganglioside-coated latex microspheres (10). In this paper, we report that using microspheres coated with a preparation of total ganglioside extract, other anti-ganglioside antibodies, including anti-GQ1b antibodies, can be detected as well. The newly developed assay system may be useful for rapid screening of patient sera for reactivity against several different gangliosides, including minor gangliosides, or glycolipid antigens that have not yet been characterized.

MATERIALS AND METHODS

Serum Samples

Serum samples were obtained from 45 patients: 12 with multifocal motor neuropathy (MMN), 13 with Guillain-Barré syndrome (GBS), 10 with chronic inflammatory demyelinating polyneuropathy (CIDP), 6 with amyotrophic lateral scler-

*Correspondence to: Armin Alaedini, Ph.D., Department of Neurology, Columbia University, 650 W. 168th St., BB 3-323, New York, NY 10032. E-mail: aa819@columbia.edu

Received 5 October 2000; Accepted 24 October 2000

rosis (ALS), and 4 with demyelinating neuropathy associated with anti-myelin-associated glycoprotein (anti-MAG) antibodies. Criteria used for patient classification have been described before (11–14). In addition, serum samples from 10 normal subjects were evaluated as controls. All sera were stored at -20°C .

Preparation of Latex Particles

Preparation of the microparticles was optimized, particularly with regard to the amount of antigen coated on the surface of the particles and the type of medium employed in the initiation of the reaction, such that normal sera would test negative in the final assay. Latex beads were coated with a total ganglioside preparation (Ca^{2+} salt) by passive adsorption. A 2 mg/mL solution of gangliosides (Sigma Chemicals, St. Louis, MO) was prepared by combining 105 μL of a 4.76 mg/mL stock solution of gangliosides in H_2O with 20 μL of methanol and 125 μL of 100 mM 2-(N-morpholino)-ethanesulfonic acid (MES) buffer (pH 6.1). A 1% suspension of 0.3 μ blue polystyrene latex particles (Seradyn Particle Technology, Indianapolis, IN) was prepared from the 2.5% stock suspension by adding H_2O . Adsorption of gangliosides to the beads was initiated by addition of 125 μL of microparticle suspension to the ganglioside solution, followed by gentle stirring for 4 hours at room temperature. The suspension was then incubated for 72 hours at 4°C . The particles were washed twice with a solution of 1% bovine serum albumin (BSA) in 25 mM MES buffer (pH 6.1) by centrifugation at 9,800g and 4°C , and resuspended in the same solution. The coated beads were incubated for 48 hours at 4°C before use.

Agglutination Reaction

On a 3-ring glass slide (Cel-Line, Newfield, NJ), 5- μL aliquots of serum were applied. Then, 5 μL of the coated latex beads was added to each ring and mixed thoroughly with a plastic applicator. The slide was rocked gently for 30 to 40 seconds. Positive agglutination, characterized by blue clumps of beads, indicated the presence of anti-ganglioside antibodies. Colored latex beads were used instead of white beads because of the ease with which positive agglutination results could be visualized. Strong results were clearly visible with the naked eye. Weak results could be visualized by holding the slide to a light source and observing for agglutination from underneath. In order to minimize inter-operator variability, all results were confirmed using a microscope ($\times 40$ magnification). Results were scored from 1 to 3 according to the degree of agglutination, where 1 denotes weak agglutination and 3 strong agglutination. In the absence of agglutination, the reaction was considered to be negative.

Enzyme-linked Immunosorbent Assay (ELISA)

The presence of antibodies directed against GM1 and GQ1b in sera was determined by the enzyme-linked immunosorbent

assay, following the previously described procedure (15), with minor modification. Wells in 96-well round-bottom polystyrene microtiter plates (Becton Dickinson, Franklin Lakes, NJ) were coated with 0.5 μg of the individual gangliosides (Sigma Chemicals) in 100 μL of methanol. Wells to which no ganglioside was added served as controls. After evaporation of the methanol, all wells were blocked by incubation with 300 μL of 1% BSA in 10 mM phosphate-buffered saline (154 mM NaCl, pH 7.4) (PBS) for 4 hours at 4°C . The plates were incubated overnight at 4°C , and then washed with the BSA/PBS solution. This was followed by the addition of 100 μL of peroxidase-conjugated goat anti-human IgM or IgG secondary antibody (ICN Biomedicals, Costa Mesa, CA) after 1:1000 and 1:800 dilution respectively in BSA/PBS solution (a final concentration of 2.14 $\mu\text{g}/\text{mL}$ for both antibodies) to each well, and incubation for 2 hours at 4°C . Plates were then washed as before and 100 μL of developing solution comprised of 27 mM citric acid, 50 mM Na_2HPO_4 , 5.5 mM *o*-phenylenediamine, and 0.01% H_2O_2 (pH 5–5.5) was added to each well. The plates were incubated at room temperature for 30 minutes, before measuring absorbance at 450 nm. The titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 units greater than in the corresponding control well. Sera with titers of 800 or less were considered to be negative for the presence of clinically significant amounts of antibodies against GM1, as such titers are also seen in normal subjects (9,11). Similarly, only sera with titers of 100 and above were considered positive for anti-GQ1b antibodies.

RESULTS

Sera from a total of 55 individuals were examined for anti-ganglioside antibodies by the agglutination immunoassay and ELISA. Of the 12 sera from MMN patients, 8 were positive by the agglutination assay for anti-ganglioside antibodies. The same patient sera were positive by the ELISA system for anti-GM1 antibodies. Of the 13 sera from GBS patients, 7 were positive for anti-ganglioside antibodies by the agglutination assay, while only 4 of these were positive by the ELISA system for antibodies directed against GM1 or GQ1b. All sera from patients with CIDP, ALS, and demyelinating neuropathy associated with MAG antibodies, in addition to those from normal subjects were found to be negative (Table 1).

TABLE 1. Analysis of patient sera with ELISA and ganglioside agglutination assay

Group	Number of specimens	Number positive by ELISA		Number positive by agglutination assay
		GM1	GQ1b	
MNN	12	8	0	8
GBS	13	2	2	7
CIDP	10	0	0	0
ALS	6	0	0	0
Anti-MAG neuropathy	4	0	0	0
Normal	10	0	0	0

With regard to sera from patients with MMN where antibodies are directed against GM1 ganglioside, the agglutination assay showed equally good sensitivity when compared to the ELISA system. It gave positive results in all 8 of the 12 patients with MMN and elevated titers of anti-GM1 antibodies as determined by ELISA, with titers ranging between 1,600 and 102,400. All serum samples from MMN patients with titers of 800 or less tested negative by the agglutination assay.

In analysis of sera from GBS patients, where the presence of several different anti-ganglioside antibody species have been reported, more patient sera were positive by the agglutination assay than by the ELISA system (Table 1). The two sera with elevated levels of IgG anti-GM1 antibodies and the two with elevated levels of IgG anti-GQ1b antibodies, with titers ranging from 100 to 25,600, as determined by ELISA, also tested positive with the agglutination assay. In addition, three other sera, which were found to be negative for antibodies against GM1 and GQ1b by ELISA, were positive for anti-ganglioside antibodies by the agglutination assay. The remaining six serum samples were negative by both assays.

With the limited number of samples examined, the new assay demonstrated high specificity for patients with MMN and GBS, as none of the other patients or normal subjects exhibited positive results. Four sera with elevated levels of serum IgM and increased titers of anti-MAG antibodies tested negative for reactivity to gangliosides with the agglutination assay. Solutions of nonspecific human IgM and IgG in MES buffer (1 mg/mL) also yielded negative results when tested with the assay.

DISCUSSION

Measurement of serum anti-ganglioside autoantibody levels is increasingly used in the evaluation of patients with immune-mediated neuropathies. The currently available ELISA systems, however, are relatively time consuming and costly, and their use is limited due to issues of methodology, laboratory variability, and interpretation (16–18). Furthermore, in using these methods in their current format, testing against only a few standard gangliosides may miss some of the reactivities, whereas testing against every putative ganglioside antigen is inefficient and not always possible. In this study, a simple and quick agglutination assay capable of detecting a functional antibody-antigen interaction is described.

In patients with MMN, where the target antigen is the GM1 ganglioside, the ganglioside agglutination assay and ELISA detected the presence of antibodies in the same patients. The degree of agglutination, however, was not found to correspond well to antibody titers as determined by ELISA, possibly due to differences in assay conditions. In contrast to the ELISA system, which measures binding of highly diluted serum at 4°C, the agglutination assay is performed under more physi-

ologic elements of temperature and serum concentration, and measures a more functional interaction. The agglutination assay may thus better represent the antibody-antigen interaction that takes place in the human body.

In patients with GBS, the higher positivity rate for the agglutination assay (7/13) in comparison with ELISA (4/13) may be explained by the fact that the new assay detects the presence of multiple anti-ganglioside antibodies present in the serum, regardless of specificity or isotype. Sera from patients with GBS may cross-react with or have antibodies to multiple gangliosides, including minor ones (19–21), and although most of the antibodies are IgGs, antibodies of the IgM and IgA isotype have also been reported (22). When using ELISA, we tested the sera against GM1 and GQ1b, which are the most common antigens described, but testing for all other possible gangliosides was beyond the scope of this study.

Although the designed ganglioside agglutination immunoassay does not measure antibody titers, it offers several advantages to the currently used ELISA system. The assay can be especially useful as a rapid screening method, with the capability to detect the presence of antibodies to different gangliosides without the use of any instruments, while requiring only a few minutes to complete, and being more economical. It would be particularly helpful in situations where rapid diagnosis and therapy are essential, as in the Guillain-Barré syndrome.

REFERENCES

- Asbury AK. New concepts of Guillain-Barré syndrome. *J Child Neurol* 2000;15:183–191.
- Hughes RAC, Hadden RDM, Gregson NA, Smith KJ. Pathogenesis of Guillain-Barré syndrome. *J Neuroimmunol* 1999;100:74–97.
- Latov N. Pathogenesis and therapy of neuropathies associated with monoclonal gammopathies. *Ann Neurol* 1995;37(S1):S32–S42.
- Nobile-Orazio E, Carpo M, Legname G, Meucci N, Sonnino S, Scarlato G. Anti-GM1 IgM antibodies in motor neuron disease and neuropathy. *Neurology* 1990;40:1747–1750.
- Ilyas AA, Quarles RH, Dalakas MC, Fishman PH, Brady RO. Monoclonal IgM in a patient with paraproteinemic polyneuropathy binds to gangliosides containing disialosyl groups. *Ann Neurol* 1985;18:655–659.
- Willison HJ, Almemar A, Veitch J, Thrush D. Acute ataxic neuropathy with cross-reactive antibodies to GD1b and GD3 gangliosides. *Neurology* 1994;44:2395–2397.
- Oga T, Kusunoki S, Fujimura H, Kuboki T, Yoshida T, Takai T. Severe motor-dominant neuropathy with IgM M-protein binding to the NeuAc α 2-3Gal β - moiety. *J Neurol Sci* 1998;154:4–7.
- Carpo M, Pedotti R, Lolli F, et al. Clinical correlate and fine specificity of anti-GQ1b antibodies in peripheral neuropathy. *J Neurol Sci* 1998;155:186–191.
- Pestronk A. Motor neuropathies, motor neuron disorders, and antiglycolipid antibodies. *Muscle Nerve* 1991;14:927–936.
- Alaedini A, Latov N. Agglutination immunoassay for rapid detection of anti-GM1 antibodies in patients with neuropathy. *Ann Neurol* 2000;48:439.
- Kinsella LJ, Lange DJ, Trojaborg W, Sadiq SA, Younger DS, Latov N. Clinical and electrophysiologic correlates of elevated anti-GM1 antibody titers. *Neurology* 1994;44:1278–1282.

12. Briani C, Brannagan TH 3rd, Trojaborg W, Latov N. Chronic inflammatory demyelinating polyneuropathy. *Neuromuscul Disord* 1996;6:311–325.
13. Van den Berg L, Hays AP, Nobile-Orazio E, et al. Anti-MAG and anti-SGPG antibodies in neuropathy. *Muscle Nerve* 1996;19:637–643.
14. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 1990;27:S21–S24.
15. Sadiq SA, Thomas FP, Kilidireas K, et al. The spectrum of neurologic disease associated with anti-GM1 antibodies. *Neurology* 1990;40:1067–1072.
16. Marcus DM, Latov N, Hsi BP, Gillard BK. Measurement and significance of antibodies against GM1 ganglioside. Report of a workshop, 18 April 1989, Chicago, IL, USA. *J Neuroimmunol* 1989;25:255–259.
17. Pestronk A. Testing for serum IgM binding to GM1 ganglioside in clinical practice. *Neurology* 2000;54:2353–2354.
18. Zielasek J, Ritter G, Magi S, Hartung HP, Toyka KV. A comparative trial of anti-glycoconjugate antibody assays: IgM antibodies to GM1. *J Neurol* 1994;241:475–480.
19. Ho TW, Willison HJ, Nachamkin I, et al. Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barré syndrome. *Ann Neurol* 1999;45:168–173.
20. O'Leary CP, Veitch J, Durward WF, Thomas AM, Rees JH, Willison HJ. Acute oropharyngeal palsy is associated with antibodies to GQ1b and GT1a gangliosides. *J Neurol Neurosurg Psychiatry* 1996;61:649–651.
21. Vriesendorp FJ, Triggs WJ, Mayer RF, Koski CL. Electrophysiological studies in Guillain-Barré syndrome: correlation with antibodies to GM1, GD1b and *Campylobacter jejuni*. *J Neurol* 1995;242:460–465.
22. Koga M, Yuki N, Takahashi M, Saito K, Hirata K. Close association of IgA anti-ganglioside antibodies with antecedent *Campylobacter jejuni* infection in Guillain-Barré and Fisher's syndromes. *J Neuroimmunol* 1998;81:138–143.