Immunoglobulin (Ig) M Antibody Against Myelin Associated Glycoprotein (MAG): A Comparison of Methods

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The presence of immunoglobulin (Ig)M antibody against myelin associated glycoprotein (MAG) has been associated with autoimmune demyelinating, sensorimotor neuropathies. Approximately 50% of patients with IgM paraproteinemia and associated peripheral neuropathy possess antibodies against MAG. These autoantibodies are thought to interfere with the process of myelination, myelin maintenance, or axon-Schwann cell interaction. The detection of these autoantibodies is useful to the clinician and is suggestive of active demyelination in a peripheral neuropathy. Our objective in this study was to compare the results obtained using three different methods (dual enzyme immunoassay [EIA], immunofluorescent antibody [IFA] and Western blot [WB]) for detecting IgM antibody against MAG in patients suspected of having autoimmune demyelinating neuropathies. Since the dual EIA

utilized two different antigens, results from this assay were separated into two groups: MAG and sulfate-3-glucuronyl paragloboside (SGPG). When compared to WB (gold standard), percent agreement, sensitivity, and specificity for EIA and IFA are as follows: MAG EIA (68.3, 100.0, and 60.6); SGPG EIA (95.1, 100.0, and 93.9); and myelin IFA (97.6, 100.0, and 97.0). The authors conclude that the SGPG EIA and myelin IFA compared well with the standard WB method when detecting IgM antibody against MAG (100 kD). Many sera demonstrated reactivity on the MAG EIA that were negative by WB (100 kD glycoprotein). The authors recommend screening for MAG IgM in suspected patient sera by SGPG EIA or myelin IFA and utilizing these same methods to titer sera confirmed positive by WB. *J. Clin. Lab. Anal.* 18:247–250, 2004. © 2004 Wiley-Liss, Inc.

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INTRODUCTION

Autoimmune responses to the peripheral nervous system that can present as peripheral neuropathies are manifestations associated with autoantibodies against various neural glycoconjugates. These neuropathies can be acute or chronic and can involve axonal degeneration or demyelination. Autoimmune neuropathies can be further divided into those associated with monoclonal gammopathies and polyclonal inflammatory polyneuropathies such as Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, and paraneoplastic neuropathies. In these diseases, there is significant overlap of the involved autoantigens that mediate the immune mechanism. The following peripheral nerve-specific autoantibodies may

be found in the neuropathies listed above (1–6): anti-myelin associated glycoprotein (MAG), anti-sulfate-3-glucuronyl paragloboside (SGPG), anti-gangliosides, anti-compact myelin associated proteins like P0, P2, and peripheral myelin protein 22 (PMP22). These antibodies are grouped into the L2/HNK-1 family and react with related carbohydrate epitopes commonly found on cell adhesion molecules (7–9).

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The distribution of MAG is restricted to the periaxonal Schwann cell membranes, Schmidt-Lanterman incisures, and lateral loops, whereas P0 and PMP-22 are the components of compact myelin (5,10). SGPG has a wider distribution being present in both Schwann cells and neuronal membranes including myelin and the axolemma (11).

The epitope recognized by human MAG antibodies is a sulfated oligosaccharide shared by additional neural glycoconjugates such as SGPG, P0, and PMP22 (12). Cross-reactivity of MAG antibodies with other neural glycoconjugates has been well documented (12–14).

Anti-MAG neuropathies associated with IgM paraproteinemia are usually a heterogeneous disease group that are slowly progressive with evidence of demyelination and a variable degree of axonal loss. They are often associated with gait ataxia. Of all peripheral neuropathy cases associated with IgM paraproteinemia, 50% possess autoantibodies against MAG (15–17).

It is thought that antibodies against MAG interfere with the process of myelination and myelin maintenance and may be involved in axon-Schwann cell interaction. The detection of these autoantibodies is useful for the clinician, therefore, as it suggests active demyelination in a peripheral neuropathy.

MATERIALS AND METHODS

Clinical Sera

Sera from 21 patients suspected of having demyelinating neuropathies (submitted for MAG IgM testing) and 20 control sera from normal random blood donors were included in the study. All patient information was eliminated from the samples included in this study according to our institutional review board (IRB) approved protocol in order to meet the requirements of the Health Insurance Portability and Accountability Act (HIPAA). All sera were stored at 4°C until all testing was complete.

Anti-MAG IgM by Western Blot (WB)

MAG IgM WB immunoassays were donated by IMMCO diagnostics (Buffalo, NY). This assay detects IgM antibody against the 100 kD MAG antigen. Other IgM autoantibodies against various nerve myelin associated antigens may also be detected. Strips are incubated with diluted (1:100) patient serum and incubated at room temperature (RT) on a rocker for 1 hr. If present, specific IgM antibodies will bind to the 100 kD MAG antigen on the strip. Each strip is then washed 4 × with 2 mL wash buffer, incubating at RT for 5 min on a rocker for each wash cycle. Wash buffer is then aspirated and 1 mL of goat anti-human IgM

conjugate is added to each strip and incubated for 30 min at RT on a rocker. Strips are then washed again as described above and incubated with 1 mL enzyme substrate at RT for 15 min on a rocker. The enzymatic reaction is stopped by washing each strip twice with 2 mL wash buffer. A positive reaction appears as a blue-violet band at 100 kD. Specimens that yield no immunoreactivity on the blot strip at 100 kD are negative for MAG IgM antibody.

Anti-Myelin IgM by immunofluorescent antibody (IFA)

Anti-myelin IgM IFA immunoassays were also donated by IMMCO diagnostics. This assay is intended for the detection of IgM antibody against associated antigens within the myelin layer of monkey sciatic nerve tissue. Sera were diluted 1:10 in phosphate buffered saline (PBS), and 50 µL was applied to tissue slides and allowed to incubate 30 min at RT in a moist chamber. Slides were rinsed and submerged in PBS for 10 min at RT with agitation. Slides were then rinsed with distilled water, and 50 µL of IgM fluorescein-labeled conjugate was applied and allowed to incubate for 30 min at RT in a moist chamber. Slides were then washed in the same manner as described above and cover slips were mounted onto slides with mounting media. Slides were viewed at 400 × using a Nikon Eclipse E400 (S&M microscopes, Colorado Springs, Co.) fluorescent microscope. Fluorescence was calibrated using a MAG positive control that demonstrated myelin sheath fluorescence of approximately 2+. Sera that demonstrated a fluorescence of ≥ 1+ of the myelin sheath were considered positive for myelin IgM antibody.

Anti-MAG and Anti-SGPG IgM by enzyme immunoassay (EIA)

This dual (MAG/SGPG) EIA was performed at an off-site reference laboratory and detects IgM antibody against partially purified MAG and highly purified SGPG in separate test wells. The reference range for the MAG/SGPG assay is <16 U/mL and <1:400, respectively. No further information is available on this assay.

RESULTS

When compared to the MAG IgM WB (100 kD; gold standard), the myelin IFA and SGPG EIA showed acceptable sensitivities and specificities (Table 1). The MAG EIA demonstrated poor specificity (60.6%) when compared to MAG IgM WB. (Table 1). One patient serum demonstrated weak fluorescence (1+) at a dilution of 1:10 when tested by the myelin IFA, but WB did not confirm the presence of MAG (100 kD)

Table 1. Comparison of MAG EIA, SGPG EIA, and myelin IFA to MAG IGM WB in 41 sera

		MAG IgM EIA		SGPG IgM EIA		Myelin IgM IFA	
		+	-	+	-	+	-
MAG IgM	+	8	0	8	0	8	0
WB	-	13	20	2	31	1	32
% Agreement =		68.3		95.1		97.6	
% Sensitivity =		100.0		100.0		100.0	
% Specificity =		60.6		93.9		97.0

Fig. 1. IFA staining of the myelin sheath (primate peripheral nerve; 400 ×) using anti-MAG IgM positive serum.

antibody. This serum was considered negative by myelin IFA at a titer of 1:20.

Eight patient sera demonstrated typical myelin staining (Fig. 1) when using myelin IFA. These same eight sera also demonstrated typical MAG immunoreactivity (Fig. 2) against a 100 kD glycoprotein by WB analysis.

Two patient sera that were SGPG positive/WB negative gave titers (1:400) at the cut-off for the SGPG EIA. One of these sera showed 1+ fluorescence by IFA at the 1:10 dilution as mentioned above. The majority of SGPG EIA positive sera possessed titers of 1:800 or greater. The MAG EIA detected IgM antibody in 13 patient sera that were negative by MAG WB analysis. The majority of these sera gave MAG EIA values well above the cut-off established for the assay. As expected, the eight MAG EIA/WB positive sera also showed reactivity on the SGPG EIA. All methods showed 100.0% sensitivity when compared to MAG WB (Table 1).

DISCUSSION

Patients with antibodies against MAG predominantly have sensory or sensory motor neuropathies character-

Fig. 2. Positive (100 kD) and negative immunoreactivity using MAG IgM WB.

ized by demyelination. This can be demonstrated by performing nerve conduction studies or nerve biopsies, which are time consuming or invasive procedures. An alternative to these procedures involves the detection of antibodies directed against glycoproteins of the myelin sheath. Antibodies against MAG have been associated with active demyelination. Detection of these antibodies, therefore, may be of diagnostic significance. Unfortunately, at a clinical level, there are several overlapping syndromes among the various polyneuropathies, which can complicate a correct diagnoses. Moreover, due to the variations in the sensitivity and specificity of the available immunological diagnostic test methods, accurate identification and confirmatory diagnosis of the presence of anti-MAG antibodies poses a real challenge. We have shown here how the available methods for detecting MAG antibody compare to WB, which is accepted as the gold standard. In general, the sensitivity of the three different assays is 100% when compared to WB. In contrast, we found significantly decreased specificity (60.6%) with the MAG IgM EIA. However, specificity with the SGPG IgM EIA and myelin IgM IFA were excellent (93.9% and 97.0%, respectively). Our finding of lower specificity in the EIA utilizing partially purified MAG is in agreement with the report from Pestronk et al. (18). Some of these false positives can be attributed to antibodies that bind to impurities found in partially purified MAG antigen (18).

In our study, the SGPG EIA and myelin IFA proved to be better screening assays for MAG antibodies (100 kD specific). Since EIA is objective and can be automated, this method would be the best choice to screen for MAG IgM antibodies. Burger et al. (19) has reported on the purification of SGPG and the high specificity of SGPG IgM EIA for detecting antibody against MAG. The SGPG EIA and myelin IFA can also be used for titrating sera confirmed positive for MAG (100 kD) IgM by WB, but the EIA can be performed for about half the cost of IFA and should, therefore, likely be the screening method of choice.

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