Antiganglioside Antibodies in Multifocal Acquired Sensory and Motor Neuropathy

Armin Alaedini, PhD; Howard W. Sander, MD; Arthur P. Hays, MD; Norman Latov, MD, PhD

Background: Multifocal acquired demyelinating sensory and motor neuropathies are considered autoimmune and responsive to immunotherapy. In the absence of demyelination, however, they are considered idiopathic if no other cause is found.

Objective: To determine whether patients with multifocal acquired sensory and motor neuropathy of an otherwise unknown cause have antiganglioside antibodies, regardless of whether they are classified as demyelinating or axonal, indicating a possible immune-mediated origin.

Patients and Methods: Serum samples from 25 patients with multifocal acquired sensory and motor neuropathy of an otherwise unknown cause were tested for antibodies to gangliosides using an agglutination immunoassay. Reactive serum samples were further tested by enzyme-linked immunosorbent assay against individual gangliosides. Electrophysiologic studies were reviewed for evidence of demyelination.

Results: Increased levels of ganglioside antibodies were detected in 12 (48%) of the 25 patients using the agglutination immunoassay, and in 7 (58%) of the 12 agglutination-positive patients by the enzyme-linked immunosorbent assay. Serum samples from these 7 patients had IgG antibodies to 1 or more gangliosides; none had elevated levels of IgM antiganglioside antibodies. Three of the patients fulfilled 2 of the American Academy of Neurology electrophysiologic criteria for demyelination, but none fulfilled the 3 of the 4 possible criteria required for the diagnosis of demyelinating neuropathy. A sural nerve biopsy specimen in 2 patients revealed axonal degeneration.

Conclusion: Multifocal sensory and motor neuropathies of an otherwise unknown cause may be associated with antiganglioside antibodies, regardless of whether they exhibit demyelinating features.

Arch Neurol. 2003;60:42-46

**MULTIFOCAL** acquired sensory and motor neuropathies are usually classified as demyelinating or axonal. The demyelinating neuropathy, also referred to as “multifocal motor and sensory demyelinating neuropathy,” “multifocal acquired demyelinating sensory and motor neuropathy,” or the “Lewis-Sumner syndrome,” is thought to be a variant of chronic inflammatory demyelinating polyneuropathy, and to be immune mediated.1,4 In the absence of evidence for demyelination, the multifocal neuropathy is thought to be axonal, and may be associated with vasculitis, diabetes mellitus, sarcoid, and Lyme disease, among others. If no cause is found, however, the neuropathy is considered idiopathic.3

Immune-mediated neuropathies can be associated with increased levels of antiganglioside antibodies. Elevated titers of IgM anti-GM1 antibodies are associated with multifocal motor neuropathy,9 and IgM antibodies to GD1b or other gangliosides with disialosyl epitopes are associated with ataxic sensory neuropathy,7 among others.8 In contrast to the chronic neuropathies that are associated with antiganglioside antibodies of the IgM isotype, IgG antiganglioside antibodies that recognize 1 or more gangliosides are typically associated with acute onset neuropathies.9 Acute motor axonal neuropathy is associated with IgG antibodies to GM1, and the Miller Fisher syndrome is closely associated with IgG antibodies to GQ1b.10,11 In some cases, the antiganglioside antibodies have been shown to cause neuropathy in experimental systems.12-15

To determine whether multifocal acquired sensory and motor neuropathies of an otherwise unknown cause are associated with autoantibodies, we tested patients using the ganglioside agglutination immunoassay and enzyme-linked immunosorbent assay (ELISA) for the
presence of reactivity to gangliosides. The patients’ electrophysiologic studies were also reviewed for evidence of demyelination.

METHODS

PATIENTS AND SERUM SAMPLES

Serum samples were obtained from 25 patients with multifocal acquired sensory and motor neuropathies of an otherwise unknown cause, followed up at the Neuropathy Center of the Weill Medical College of Cornell University, New York, NY, over the latter 6 months of 2001. The patients had asymmetric weakness or sensory loss in a multifocal distribution, without known cause for neuropathy. The patients were followed up and patients were examined by 2 of us (H.W.S. and N.L.). The patients’ medical records and the results of neurological examinations were reviewed. Two of the patients underwent sural nerve biopsies. In the other cases, a biopsy was recommended but declined by the patients, either because of the risk of complications such as lingering pain or because they preferred to have a trial of therapy first. Study results in all cases were either negative or showed no abnormality for other causes of neuropathy, with normal chest radiograms, as well as blood tests for diabetes mellitus, vasculitis, monoclonal gammopathy, anti–myelin-associated glycoprotein and antisulfatide antibodies, Lyme disease, and hepatitis B and C infection. Genetic analysis for hereditary neuropathy with liability to pressure palsies was done in 4 patients, where the result was negative. The other patients declined the test, but none had a family history of neuropathy, exercise-induced exacerbations, lesions localized to common compression sites, or characteristic electrophysiologic changes of hereditary neuropathy with liability to pressure palsies. Serum samples were kept frozen at −20°C until used. Serum samples from 6 patients with amyotrophic lateral sclerosis, 20 patients with multiple sclerosis, and 40 healthy subjects were examined in parallel as controls.

ELECTROPHYSIOLOGIC STUDIES

Electrophysiologic studies were conducted at our center, or by the referring physician, in which case the studies were reviewed. Evidence of focality or multifocality was considered present based on the following criteria: (A) side-side compound muscle action potential (CMAP) amplitude asymmetry exceeding 50%; (B) disparity between ipsilateral median and ulnar CMAP amplitudes exceeding 50%; (C) side-side sensory nerve action potential amplitude asymmetry exceeding 50%; and (D) conduction block exceeding 50% without temporal dispersion.

To assess the presence of demyelination, the American Academy of Neurology (AAN) AIDS Task Force electrophysiologic criteria were used as follows: (E) partial conduction block exceeding 20% without temporal dispersion, or the presence of temporal dispersion; (F) absent or severely prolonged F-wave minimal latencies in 2 or more nerves; (G) severe reduction in conduction velocity in 2 or more motor nerves; and (H) severe distal latency prolongation in 2 or more motor nerves.

The AAN AIDS Task Force methodology was used, except where waveforms for some patients were unavailable for review. In instances where waveforms were unavailable, lack of a report of temporal dispersion was assumed to indicate absence of this finding.

GANGLIOSIDE AGGLUTINATION IMMUNOASSAY

Polystyrene beads were coated with a total ganglioside extract as described previously, with minor modification. A 3-mg/mL solution of gangliosides was prepared by combining 750 µg of a bovine ganglioside extract (calcium salt) (Sigma-Aldrich, St Louis, Mo) dissolved in 105 µL of water with 20 µL of methanol and 125 µL of 100mM 2-(N-morpholino)-ethanesulfonic acid buffer (pH 6.1) in a 1.7-µL polypropylene conical tube (Corning Life Sciences, Corning, NY). Adsorption of gangliosides to latex beads was initiated by addition of 250 µL of a 1% suspension of 0.3-µm blue polystyrene particles (Seradyn Particle Technology, Indianapolis, Ind) to the ganglioside solution, followed by gentle stirring for 4½ 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 in 25mM 2-(N-morpholino)-ethanesulfonic acid buffer (pH 6.1) by centrifugation at 9800g and 4°C, and resuspended in the same solution. The micro-particles were incubated for 48 hours at 4°C before use.

The agglutination test was carried out on a 3-ring glass slide (Cel-Line, Newfield, NJ). On each ring, 5-µL aliquots of coated micro-particles were added to 5 µL of serum and mixed with a plastic applicator. The slide was rocked gently for 15 seconds. Positive agglutination, characterized by blue clumps of beads, indicated the presence of antiganglioside antibodies.

RESULTS

Twelve (48%) of the 25 patients with multifocal sensory and motor neuropathy had increased titers of antiganglioside antibodies as determined by the ganglioside agglutination immunoassay. Their clinical or electrophysiologic features were similar to those without antibodies. By ELISA, 7 (58%) of the 12 patients had elevated titers of IgG antibodies to 1 or more gangliosides, whereas the
other 5 (42%) of the 12 patients did not (Table 1). Three had increased antibodies to GM1 alone; 1 had antibodies to GD1a and GQ1b; 1 to GM1 and GD1a; 1 to GM1, GM2, and GD1b; and 1 to GM1, GD1a, and GQ1b. All patients were negative for antibodies to GT1b. None of the patients had increased titers of IgM antiganglioside antibodies; none of the control serum samples showed reactivity in the agglutination assay or ELISA.

The salient clinical features of the 12 patients, including the electrophysiologic studies, are listed in Table 2. All had clinically apparent weakness and sensory loss in an asymmetric distribution. Ten of the 12 patients had sustained improvement in response to immunotherapy, as evidenced by increased strength of 1 or more points in 1 or more muscle groups on the Medical Research Council scale. Genetic testing for hereditary neuropathy with liability to pressure palsies was done in 4 patients (patients 1, 9, 10, and 11), and was negative in all 4.

Electrodiagnostic studies of the 12 patients were performed either at our center or at large, tertiary care medical center electromyography laboratories. In 8 of the 12 patients who tested positive for antibodies, at least 3 limbs were electrophysiologically examined. In the other 4 patients, 2 limbs were examined. At a minimum, 4 motor nerves and 2 sensory nerves were examined. Four patients had more than 1 electrodiagnostic study performed. Needle electromyography was done in 10 patients. In all patients there was evidence of sensory and motor neuropathic dysfunction. In 7 patients the findings were severe with at least 2 lower extremity nerves demonstrating absent or severely (<80% of the lower limit of normal) low-amplitude CMAPs. Needle electromyography was abnormal in the 10 patients examined, with varying degrees of active and/or chronic denervation.

There was electrodiagnostic evidence of focality or multifocality in 9 of the 12 patients. One patient in whom focality was not demonstrated had completely absent leg responses. The findings suggesting focality were side-side CMAP amplitude asymmetry (7 patients), amplitude disparity in ipsilateral median and ulnar CMAPs (4 patients), conduction block (3 patients), and side-side sensory nerve action amplitude asymmetry (1 patient). Proximal stimulation, including either axilla or Erb point stimulation was performed in 3 of the patients (patients 7, 10, and 12). In one of these patients (patient 7) Erb point stimulation demonstrated a radial nerve conduction block.

In 9 patients the F-waves studies met the AAN AIDS Task Force criteria for demyelination with 2 or more absent or severely prolonged F waves. However, the F waves were absent in 7 of the 9 patients (patients 1, 3, 4, 5, 8, 10, and 11), and prolonged in only 2 (patients 7 and 9). In 1 of the 9 patients (patient 9), the F waves were absent in 1 nerve and severely prolonged in 1 nerve. In another patient, the F waves were severely prolonged in 2 nerves (patient 7). Apart from the above mentioned 9 patients, there was 1 patient (patient 2) with absent F waves in a single nerve.

None of the patients met the required 3 (of the 4 possible) electrodiagnostic criteria of the AAN AIDS Task Force for demyelination or chronic inflammatory demyelinating polyneuropathy. Three patients met 2 of the criteria (patients 3, 7, and 9). The arms were more affected than the legs in these patients. One other patient (patient 4) had evidence of bilateral tibial nerve conduction blocks; however, the AAN AIDS Task Force criteria exclude this nerve. A total of 4 patients, therefore, had some features suggestive, but not diagnostic, of a demyelinating origin. None of the patients met the AAN AIDS Task Force distal latency or conduction velocity criteria.

Sural nerve biopsy specimens were obtained in 2 of the 12 patients who tested positive for antibody (patients 1 and 10). In patient 1 transverse semithin sections showed marked loss of myelinated fibers, with scattered foci of myelin debris. Teased fiber analysis revealed myelin ovoids in 63% of the fibers, indicating active axonal degeneration. A few scattered perivascular lymphocytes were seen in the epineurium. There was no evidence for vasculitis. In patient 10, semi-thin sections showed diffuse reduction of large and small my-
eliminated nerve fibers. Myelin sheaths were of appropriate thickness, and no myelin stripping or onion bulbs were observed.

**COMMENT**

In this study, antiganglioside antibodies were detected in 12 (48%) of 25 patients with multifocal acquired sensory and motor neuropathy by the agglutination assay. Seven of the 12 patients who tested positive for agglutination also had elevated levels of IgG antibodies to 1 or more gangliosides by ELISA. The association with antiganglioside antibodies, and improvement following immune therapy in most of the patients, are consistent with immune-mediated pathogenesis. The antibody titers were lower than those seen in patients with IgM antiganglioside antibodies, but in the same range as those seen in some patients with the Guillain-Barré syndrome. The ganglioside agglutination immunoassay appears to be more sensitive than the ELISA system as it detects antibodies to multiple gangliosides, or to minor gangliosides such as GM1b or GalNAc-GD1a that are not commercially available, and is carried out at more physiologic conditions of temperature and concentration.

The presence of IgG antibodies to gangliosides distinguishes patients with multifocal acquired sensory and motor neuropathy from those with multifocal motor neuropathy, which is associated with IgM anti-GM1 ganglioside antibodies. The presence of IgG antibodies is suggestive of involvement of T cells, which are also implicated in the Guillain-Barré syndrome where multiple ganglioside antibodies have been described. Recent studies indicate that ganglioside specific T cells can recognize more than 1 ganglioside, possibly explaining the presence of antibodies to multiple gangliosides in some of the patients. Antiganglioside antibodies were not seen in all patients with multifocal acquired sensory and motor neuropathy, possibly because the disease in some patients may be mediated by T cells without antibodies, or antibody levels might fluctuate depending on disease activity, or other disease mechanisms may be present. In addition, titers of IgG antiganglioside antibodies may be affected by intravenous immunoglobulin, which has been reported to increase autoantibody clearance.
None of the patients would have been diagnosed as having demyelinating neuropathy or chronic inflammatory demyelinating polyneuropathy, as they did not fulfill 3 of the 4 possible electrophysiologic criteria, as required by the AAN AIDS Task Force criteria. However, it is likely that the disease involved both axons and myelin sheaths in some of the patients, as 3 patients had features fulfilling 2 of the 4 possible criteria for demyelination. It may be that additional proximal stimulation studies might have revealed more regions of conduction block, but such studies are less reliable given the difficulty in ascertaining supramaximal stimulation at proximal sites, and that they were negative in 2 of the 3 patients in which they were performed. The presence of axonal degeneration in the nerve biopsy studies also does not rule out demyelination at other sites but makes demyelination less likely. Gangliosides are present in myelin and axons, so both might become involved depending on the antibody specificities, distribution of the antigenic targets, or consequences of the antibody binding.

CONCLUSIONS

The findings in this study indicate that antiganglioside antibodies are present in a substantial number of patients with multifocal acquired sensory and motor neuropathy of an otherwise unknown cause, regardless of whether the neuropathy is classified as demyelinating or axonal. The presence of these antibodies suggests that immune mechanisms may be involved in the pathogenesis of the disease. Affected patients may benefit from immune therapy.

Accepted for publication July 18, 2002.

Author contributions: Study concept and design (Drs Alaedini, Sander, and Latov); acquisition of data (Drs Alaedini, Sander, Hays, and Latov); analysis and interpretation of data (Drs Alaedini, Sander, Hays, and Latov); drafting of the manuscript (Drs Alaedini, Sander, Hays, and Latov); administrative, technical, and material support (Drs Alaedini, Sander, and Hays); study supervision (Drs Alaedini, Sander, and Latov).

This study was supported in part by grant NS11766 from National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md, and by generous private donations from James and Linda Gardner, New York, NY; Winston Wolfe, Memphis, Tenn; George Fisher, Rochester, NY; Peter Bing, Los Angeles, Calif; and The Morris and Alma Schapiro Foundation, New York.

Corresponding author and reprints: Armin Alaedini, PhD, 525 E 68th St, LC-807, Department of Neurology and Neuroscience, Cornell University, New York, NY 10021 (e-mail: ara2004@med.cornell.edu).

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