Antiganglioside Antibodies in Multifocal Acquired Sensory and Motor Neuropathy

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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.

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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 gammapathy, 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. 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 the cases 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 workflows for some patients were unavailable for review. In instances where workflows 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-mL 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 4.5 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 microparticles 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 were confirmed using a light microscope (×40 magnification) and scored from 1 to 3 according to the degree of agglutination, where 1 denoted weak agglutination and 3 strong agglutination. In the absence of agglutination, the reaction was considered negative.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Serum samples were also tested by ELISA for the presence of IgG and IgM antibodies to GM1, GM2, GD1a, GD1b, GT1b, and GQ1b gangliosides. Wells in 96-well round-bottom polystyrene microtiter plates (Corning Life Sciences) were coated with 0.5 µg of the individual gangliosides (Sigma-Aldrich) in 100 µL of methanol. No ganglioside was added to control wells. After evaporation of the methanol, all wells were blocked by incubation with 300 µL of 1% bovine serum albumin in 10mM phosphate-buffered saline (134mM sodium chloride, pH 7.4) solution for 4 hours at 4°C. The plates were incubated overnight at 4°C, and then washed with the bovine serum albumin/phosphate-buffered saline solution. Then, 100 µL of peroxidase-conjugated goat antimouse IgG (1:1000 dilution) or IgM (1:600 dilution) secondary antibody (ICN Biomedicals, Costa Mesa, Calif) was added to each well, and the plates were incubated for 2 hours at 4°C. The wells were then washed as before, followed by the addition of 100 µL of developing solution composed of 27mM citric acid, 50mM sodium phosphate, 5.5mM o-phenylenediamine, and 0.01% hydrogen peroxide (pH 5.5). The plates were incubated at room temperature for 30 minutes, before measuring absorbance at 450 nm. The titer for each sample was assigned as the highest dilution in which the absorbance was 0.1 U greater than in the corresponding control well. Serum samples with IgG titers of 100 and above were considered positive for the presence of clinically significant antibody levels, while serum samples with IgM titers of 1600 and higher were considered positive.

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 GT1b; 1 to GM1 and GD1a; 1 to GM1, GM2, and GD1b; and 1 to GM1, GD1a, and GT1b. 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.

Table 1. Antiganglioside Antibody Profile for Patients Who Tested Positive for Antibodies by the Agglutination Assay

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Ganglioside Agglutination Assay</th>
<th>ELISA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 IgG100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1-2 IgG200, IgG200</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 IgG100</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 IgG100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 IgG200</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2 IgG100</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1 IgG100</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ELISA, enzyme-linked immunosorbent assay.

*Results were scored from 1 (weak) to 3 (strong) according to the degree of agglutination.
†Titer for each specimen was assigned as the highest dilution in which the absorbance was 0.1 U greater than in the corresponding control wells.

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-
Table 2. Clinical and Electrophysiologic Characteristics of Patients Who Tested Positive for Antibodies by the Agglutination Assay

<table>
<thead>
<tr>
<th>Patient/Age, y/ Duration of Illness</th>
<th>Weakness/Atrophy</th>
<th>Sensory Impairment</th>
<th>Response and Treatment</th>
<th>Electrodiagnostic Characteristics†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/46/12 y</td>
<td>L hand, R ankle dorsiflexion&gt;L/atrophy of L forearm and hand</td>
<td>Vibration at L hand and L large toe&gt;R, pin in hands and feet</td>
<td>Improved while receiving prednisone, etanercept, and plasmapheresis</td>
<td>A, B, C, and F</td>
</tr>
<tr>
<td>2/66/10 y</td>
<td>Biceps bilaterally, L finger extenders/bilateral atrophy of 1st dorsal interossei</td>
<td>Vibration at L large toe&gt;R, pin in L foot&gt;R</td>
<td>Improved while receiving IVIG</td>
<td>A (2)</td>
</tr>
<tr>
<td>3/47/6 mo</td>
<td>Bilateral upper extremities, hip flexors, R ankle/atrophy of shoulder girdle muscles, hands, and thighs</td>
<td>Vibration at R large toe&gt;L, position at R large toe, pin in hands and feet</td>
<td>Not improved while receiving IVIG, beginning pulse cyclophosphamide</td>
<td>A, B, E (ulnar), and F</td>
</tr>
<tr>
<td>4/61/18 mo</td>
<td>Biceps, leg flexion and extension, L deltoid&gt;R, R ankle dorsiflexion/...</td>
<td>Vibration L toe and ankle&gt;R, pin in hands and feet</td>
<td>Improved while receiving IVIG</td>
<td>D (2-tibial), and F</td>
</tr>
<tr>
<td>5/53/2.5 y</td>
<td>R ankle dorsiflexors&gt;L/...</td>
<td>Vibration at fingers, toes, and L ankle and knee&gt;R, pin in hands and feet</td>
<td>Improved while receiving IVIG</td>
<td>F</td>
</tr>
<tr>
<td>6/76/6 y</td>
<td>L hip flexors and leg flexors/...</td>
<td>Vibration in L large toe, pin in feet</td>
<td>Improved while receiving IVIG</td>
<td>A</td>
</tr>
<tr>
<td>7/64/12 y</td>
<td>R deltoid, biceps, triceps, and hand/...</td>
<td>Vibration in R hand, pin in hands and R foot</td>
<td>Improved while receiving IVIG</td>
<td>C, D (2-median, 1-radial), and F</td>
</tr>
<tr>
<td>8/57/7 y</td>
<td>L ankle dorsiflexors&gt;R, L ankle plantar flexors/atrophy of calves and R thigh</td>
<td>Vibration at L large toe&gt;R, pin at toes</td>
<td>Improved while receiving IVIG</td>
<td>F</td>
</tr>
<tr>
<td>9/54/25 y</td>
<td>L wrist flexion and extension, L ankle dorsiflexion&gt;R, L ankle plantar flexion/atrophy of R hand</td>
<td>Vibration and position at large toes, pin in hands and feet</td>
<td>No information</td>
<td>B, D (ulnar), E (median), and F</td>
</tr>
<tr>
<td>10/73/8 y</td>
<td>L ankle dorsiflexors/...</td>
<td>Vibration at L large toe&gt;R, pin in hands and feet</td>
<td>Improved while receiving etanercept</td>
<td>A, B, E (tibial), and F</td>
</tr>
<tr>
<td>11/58/10 y</td>
<td>R ankle dorsiflexors&gt;L/...</td>
<td>Vibration at R large toe&gt;L, pin in hands and feet</td>
<td>Improved while receiving IVIG</td>
<td>A and F</td>
</tr>
<tr>
<td>12†/47/8 mo</td>
<td>L wrist extension, L ankle dorsiflexion&gt;R, hamstrings/...</td>
<td>Vibration at toes, pin in calves</td>
<td>Improved while receiving IVIG</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviations: A, side-side compound muscle action potential amplitude asymmetry exceeding 50%; B, ipsilateral median and ulnar ipsilateral compound muscle action potential amplitude disparity exceeding 50%; C, side-side sensory nerve action potential amplitude asymmetry exceeding 50%; D, conduction block exceeding 50% without temporal dispersion; E, possible partial conduction block exceeding 20% or the presence of temporal dispersion; F, absent or severely prolonged F-ve wave minimal latencies in 2 or more nerves; IVIG, intravenous immunoglobulin.

*Ellipses indicate not applicable.
†For A through E the parenthetical numbers indicate the number of times the criterion was fulfilled if greater than 1.
‡Electrophysiologic studies on patient 12 revealed a predominately motor neuropathy, but did not fulfill criteria A through F.

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 immunotherapy 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.

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REFERENCES