Autoimmune Targets of Heart and Skeletal Muscles in Myasthenia Gravis

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Objective: To investigate the clinical, histological, and immunological features of patients with myasthenia gravis (MG) who also developed myocarditis and/or myositis.

Design: Observational and retrospective case series.

Setting: Keio University, Hanamaki General Hospital, Kanazawa University, Nagasaki University, and Juntendo University.

Patients: A cohort of 8 patients with MG with clinically defined inflammatory myopathies.

Interventions: Clinical and histological features were described. Serological analyses included MG-related antistriational autoantibodies (those to titin, ryanodine receptor, muscular voltage-gated potassium channel Kv1.4) and myositis-specific autoantibodies.

Results: Of 924 patients with MG, 8 (0.9%) had inflammatory myopathies. The mean (SD) onset age of MG was 55.3 (10.3) years. All patients showed severe symptoms with bulbar involvement; 5 patients had myasthenic crisis and 4 had invasive thymoma. Myocarditis was found in 3 patients and myositis in 6. Myocarditis, developing 13 to 211 months after the MG onset, was characterized by heart failure and arrhythmias. Myositis, developing before or at the same time as MG, affected limb and paraspinal muscles. Histological findings of skeletal muscles showed CD8+ lymphocyte infiltration. Seven patients had 1 of these antistriational autoantibodies but not myositis-specific autoantibodies. Immunomodulatory therapy was required for all patients and was effective for both MG and inflammatory myopathies, although 1 patient died.

Conclusions: Heart and skeletal muscles are autoimmune targets in some patients with MG. This autoimmunity has a broad clinical spectrum with antistriational autoantibodies.


The main disease mechanism in myasthenia gravis (MG) is a humoral immune attack on the acetylcholine receptor (AChR), leading to the characteristic defect transmission in the neuromuscular junction. In addition, heart and skeletal muscles are also speculated to be autoimmune targets in MG. It is known that some patients with lymphocyte-rich thymomas. However, inflammatory myopathies, ie, autoimmune-mediated myocarditis and/or myositis, developed in a few patients with MG, especially thymoma-associated MG. Inflammatory myopathies not only lead to the deterioration of muscular weakness, but also were the most serious complications in the disease course of patients with MG. Because inflammatory myopathies in patients with MG are rare, the clinical characteristics of case series have not been described. Patients with MG have autoantibodies to the molecules on heart and skeletal muscles. These are considered antistriational antibodies and include autoantibodies to titin, ryanodine receptor, and muscular voltage-gated potassium channel Kv1.4. These autoantibodies are used...
to classify the subsets of MG. On the other hand, polymyositis, a representative form of inflammatory myopathies, includes autoantibodies against nuclear or cytoplasmic antigens. They are known as myositis-specific autoantibodies (MSA) and include those directed against Jo-1, other aminoacyl-tRNA synthetases, and signal recognition particles. These autoantibodies are useful clinical markers associated with specific manifestations such as interstitial lung disease and skin rash. However, the associations between antistriational antibodies and MSA in inflammatory myopathies have not been studied.

Here we describe the clinical, histological, and immunological features of 8 patients with MG who also developed myocarditis and/or myositis.

**METHODS**

**PATIENTS**

We studied 924 unrelated Japanese patients with MG at Keio University, Hanamaki General Hospital, Kanazawa University, Nagasaki University, and Juntendo University between January 2000 and December 2007. The diagnosis of MG was based on associations between the following parameters: history and signs of fluctuating weakness in voluntary muscles, presence of serum anti-AChR antibody, definite clinical improvement on injection of anticholinesterase, and a decremental pattern of repetitive nerve stimulation. Anti–muscle-specific kinase antibodies were also examined in some AChR-negative patients with generalized MG. We defined at least 3 clinical features as necessary for diagnosis for MG. Severity of MG and postintervention status were evaluated according to the system proposed by the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America. The extent of thymoma and histological diagnosis at surgery were evaluated by the Masaoka system and the World Health Organization classification.

**IMMUNOLOGICAL EVALUATION**

Serum anti-AChR antibody was measured by a conventional radioimmunoassay. Antititin antibodies were detected with a commercially available enzyme-linked immunosorbent assay (ELISA) in which a recombinant MGt30 protein was used as the antigen (DLD Diagnostika, Hamburg, Germany). A ratio of optical densities to standard serum greater than 1.0 was regarded as positive. Anti–ryanodine receptor antibodies were detected with sandwich ELISA using native-form ryanodine receptor from rabbit striate muscle as the antigen. Titer of control samples were defined as positive. Anti-Kv1.4 antibody was determined by an immunoprecipitation assay using Sulphur 35–labeled rhabdomyosarcoma cellular extract as the antigen source. Serum samples that precipitated 70 kDa Kv1.4 from rhabdomyosarcoma cell extracts and not from leukemic cell (K562) extracts were regarded as positive. Myositis-specific autoantibodies were screened by RNA immunoprecipitation assay using K562 cellular extracts. This method can detect autoantibodies to Jo-1, other aminoacyl-tRNA synthetases, and signal recognition particles. Human leukocyte antigen class II DRB1 and DQB1 alleles were determined based on polymerase chain reaction and a restriction fragment method using genomic DNA extracted from peripheral blood leukocyte. All blood samples and clinical information were obtained after the patients had given informed consent, as approved by the institutional review board of each hospital.

**RESULTS**

Of 924 patients with MG, 8 (1 man and 7 women) developed inflammatory myopathies; thus, the prevalence of inflammatory myopathies in our series was 0.9%. The clinical features and immunological findings are summarized in the Table. The cohort included 4 patients evaluated in our previous studies. The mean (SD) age of MG onset was 35.3 (10.3) years. All patients showed severe symptoms with bulbar involvement (greater than class IIIb by Myasthenia Gravis Foundation of America classification), and 5 had episodes of myasthenic crisis. Because bulbar symptoms fluctuated and were reduced by anticholinesterase agents, these were considered symptoms of MG rather than myositis. Extended thymectomy was conducted in 6 patients, and thymoma was found in 4. Only 1 case (patient 5) developed MG and myositis 5 years after the removal of thymoma. All thymomas were invasive, requiring additional treatments in some patients. The histology of thymoma included types AB, B1, and B3.

Myocarditis was found in 3 patients and myositis in 6 (1 patient had both). The mean (SD) age at onset of inflammatory myopathies was 58.1 (8.2) years. Myocarditis, characterized by heart failure and arrhythmias, developed 13 to 211 months after MG onset. Electrocardiograms showed T-wave abnormality, atrioventricular dissociation, and wide QRS. In contrast, myositis developed before or at the same time as MG. Myositis affected various skeletal muscles with myalgia, limb swelling, and fever. Patient 4 had dropped head; anticholinesterase agents markedly improved her nasal voice and limb weakness, but not her dropped head (Figure 1). T2-weighted cervical magnetic resonance imaging showed high-intensity lesions and atrophy in the dorsal paraspinal muscles. Body computed tomography showed symmetrically diffuse atrophy of the paraspinal dorsal muscles from the cervical and lumbar levels. Serum creatine kinase levels were elevated in 7 patients.

Histological evaluations were performed in 6 patients, including muscle biopsy in 5 and autopsy in 1 (Figure 2). Inflammatory cell infiltration was observed in the heart and skeletal muscles, but the severity varied. The most severe case was the patient who died (patient 1); an autopsy was performed. The heart showed widespread inflammatory infiltrates containing multinucleated giant cells with massive myocardium degeneration. All muscle tissue specimens collected from the diaphragm, iliopsoas muscles, pectoralis muscle, intercostal muscle, and paraspinal muscle showed massive inflammatory cellular infiltrate. Muscle fibers showed variety in size as well as severe necrosis and degeneration. There were marked increases in atrophic fiber but they
did not exhibit perifascicular atrophy. Inflammatory cells included CD68⁺, CD8⁺, and, rarely, CD4⁺ cells. CD8⁺ cells were also observed among nonnecrotic muscle fibers. On the other hand, patient 4 had a mild case of myositis, as revealed by a biopsy of the left biceps brachii muscle. There was variation in muscle fiber size, with no perifascicular atrophy. Necrotic muscle fibers were observed. A limited number of mononuclear cells infiltrating the perimysial or perivascular region were CD8⁺. The muscle fibers, including normal-appearing ones, aberrantly expressed diffuse MHC class I on surface membranes.

Immunological evaluation showed that all patients were seropositive to AChR. Seven of 8 patients had at least 1 of the striational autoantibodies, and 4 had all of them. The frequencies of positivity for autoantibodies to titin, ryanodine receptor, and Kv1.4 were 63%, 75%, and 75%, respectively. All 3 patients with myocarditis had anti-Kv1.4 antibodies. However, MSA, including autoantibodies to Jo-1, other aminoacyl-tRNA synthetases, and signal recognition particles were not detected. Immuno- genetic background analysis revealed that there were no common antigens of DRB1 or DQB1 alleles.

All patients received immunomodulatory therapy. Eight received corticosteroids; 5, plasmapheresis; 5, tacrolimus; 2, intravenous immunoglobulin therapy, and 2, cyclosporin microemulsion. Acute exacerbation of symptoms of MG and/or inflammatory myopathies required a combination of corticosteroids, plasmapheresis, and intravenous immunoglobulin therapy. In contrast, tacrolimus and cyclosporin microemulsion were useful for reducing the dose of and complications due to corticosteroids. The clinical features were severe, but the prognoses were generally favorable. Seven patients had pharmacological remission or minimal manifestation of both MG and inflammatory myopathies.

In this study, we demonstrated that inflammatory myopathies developed in patients with severe MG and antistriational autoantibodies in patients aged from 40 to 60 years. Myocarditis occurred after MG onset, accompanied by thymoma and anti-Kv1.4 antibodies. In contrast, myositis appeared before or at the same time as MG onset. The clinical features of myocarditis and/or myositis varied widely. The combinations of immunomodulatory therapies were effective and resulted in remission of both MG and inflammatory myopathies.

Previous studies indicated that inflammatory myopathies in MG were characterized by thymoma, pathologically granulomatous or giant cell, and heart muscle in-

Table. Clinical and Immunological Characteristics of 8 Patients With Myasthenia Gravis and Inflammatory Myopathies

<table>
<thead>
<tr>
<th>Patient No./Sex</th>
<th>Onset Age, y/ Class*</th>
<th>Symptoms</th>
<th>Thymus Pathology Stageb</th>
<th>Onset Age, y/ Myocarditis/ Myositis</th>
<th>CK, IU/L</th>
<th>AChR, nM/Titin/ RyR/Kv1.4/MSAc</th>
<th>HLA Class II</th>
<th>Outcome/ Follow-up Period, moa</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M</td>
<td>45/V</td>
<td>Ptosis, dysarthria, limb weakness, 1 crisis</td>
<td>Type B1 thymoma/ IVa</td>
<td>62/+/+</td>
<td>Limb weakness, dyspnea, myalgia, ventricular tachycardia</td>
<td>9633</td>
<td>*0404</td>
<td>*0803</td>
<td>*0601</td>
</tr>
<tr>
<td>2/F</td>
<td>43/V</td>
<td>Plosis, limb weakness, dysphagia, 1 crisis</td>
<td>Type B1 thymoma/ III</td>
<td>53/−/−</td>
<td>Dyspnea, tachycardia, hypotension</td>
<td>397</td>
<td>*0101</td>
<td>*0406</td>
<td>*0302</td>
</tr>
<tr>
<td>3/F</td>
<td>44/V</td>
<td>Plosis, limb weakness, dysphagia, 2 crises</td>
<td>Type B3 thymoma/ II</td>
<td>45/+/−</td>
<td>Chest compression, tachycardia</td>
<td>67</td>
<td>*1201</td>
<td>*1401</td>
<td>*0303</td>
</tr>
<tr>
<td>4/F</td>
<td>68/Vib</td>
<td>Limb weakness, nasal voice, dysphagia, dyspnea</td>
<td>ND</td>
<td>66/−−</td>
<td>Dropped head, limb weakness</td>
<td>362</td>
<td>*0803</td>
<td>*1403</td>
<td>*0601</td>
</tr>
<tr>
<td>5/F</td>
<td>66/IIib</td>
<td>Nasal voice, dysarthria, limb weakness</td>
<td>Type AB thymoma/ II</td>
<td>66/−−</td>
<td>Limb weakness, myalgia</td>
<td>378</td>
<td>8/−/−/−/−</td>
<td>*1502</td>
<td>*0601</td>
</tr>
<tr>
<td>6/F</td>
<td>48/V</td>
<td>Limb weakness, dysphagia, 1 crisis</td>
<td>ND</td>
<td>48/−/−</td>
<td>Limb weakness, myalgia</td>
<td>3482</td>
<td>*1301</td>
<td>*1501</td>
<td>*0501</td>
</tr>
<tr>
<td>7/F</td>
<td>66/IIIb</td>
<td>Nasal voice, plosis, dysphagia</td>
<td>Atrophy</td>
<td>63/+/−</td>
<td>Limb weakness, fever, limb swelling</td>
<td>2286</td>
<td>*1403</td>
<td>*1501</td>
<td>*0602</td>
</tr>
<tr>
<td>8/F</td>
<td>62/V</td>
<td>Plosis, diplopia, facial weakness, dysarthria, limb weakness</td>
<td>Atrophy</td>
<td>62/−−</td>
<td>Limb weakness, myalgia, limb swelling</td>
<td>3193</td>
<td>*1501</td>
<td>*1501</td>
<td>*0602</td>
</tr>
</tbody>
</table>

Abbreviations: AChR, anti–acetylcholine receptor antibody; CsA, cyclosporine microemulsion; CK, serum creatine kinase; IVIg, intravenous immunoglobulin; Kv1.4, anti–Kv1.4 antibody; mPR, high-dose methylprednisolone; MM, minimal manifestation; MSA, myositis-specific autoantibodies; ND, not determined; PP, plasmapheresis; PR, pharmacological remission; PSL, prednisone; RyR, anti–ryanodine receptor antibody; Titin, anti–titin antibody; +, present; −, absent.

aAccording to the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America.13
bAccording to the World Health Organization classification and the Masaoka system.14,15

cAutoantibodies.

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volvement.2,5-8 Although the extent and severity of muscle involvement varied, widespread myocardial necrosis was associated with the most serious manifestation.2 In fact, some have found that myocarditis was likely to be lethal in the course of MG.2,5,7,8 Evoli et al21 described 7 of 50 patients with thymoma-associated MG who died suddenly, which might attributed to myocarditis. In this regard, in addition to patient 1, we also experienced 2 patients with thymoma-associated MG who died suddenly. There was no evidence of myocarditis; however, anti-Kv1.4 antibodies were detected in their serum. Regarding other antistriational antibodies, Mygland et al22 detected the anti–ryanodine receptor antibodies in 3 patients who had thymoma-associated MG with myocarditis.

Neck weakness is sometimes found in MG and usually fluctuates, with rest or anticholinesterase resulting in improvement. However, sustained dropped head can occur in some patients.1,23,24 Dropped head syndrome is observed in various neuromuscular disorders and is generally explained by the susceptibility to injury of the paraspinal musculature by kinetic postural changes and age-dependent loss of tissue elasticity.23 We speculate that the inflammatory process in the paraspinal muscles may be one of the causes of dropped head. Rodolico et al25 described a case similar to ours in which a 65-year-old woman had dropped-head symptoms with diffuse paraspinal muscle atrophy in thymoma-associated MG.

The predominance of CD8⁺ lymphocyte and aberrant MHC class I expression on muscle fibers are important findings in polymyositis12 and were also seen in inflammatory myopathies in our patients with MG. However, we emphasize that the clinical and immunological features of inflammatory myopathies associated with MG are different from those of polymyositis. First, myocardial involvement and thymoma are unusual in polymyositis.12 Second, inflammatory myopathies in MG are accompanied by antistriational autoantibodies, but without MSA, which were detected in approximately 40% of pa-

Figure 1. A patient with dropped head. A, Patient 4 could not raise her head against gravity. B, Axial T2-weighted cervical magnetic resonance image shows bilateral high-intensity lesions and atrophy in the dorsal paraspinal muscles (arrow). C, Body computed tomographic scanning reveals symmetrical and diffuse atrophy of the dorsal paraspinal muscles from the cervical and lumbar levels (arrows).

Figure 2. Histological findings of heart and skeletal muscles. A-F, Autopsy findings were obtained from patient 1. Macroscopic findings of myocarditis are shown. A, Widespread infiltration of the heart muscle with multinucleated giant cells was observed. B, Severe myositis was found in the iliopsoas muscle. C and D, Hematoxylin-eosin staining was used. The slides were stained with monoclonal antibodies to CD8 (E) and CD68 (F). G-J, Muscle biopsy findings of biceps brachii were obtained from patient 4 and were hematoxylin-eosin stained (G). Slides were stained with monoclonal antibodies to CD8 (H) and major histocompatibility complex class I antigen, comparing patient 4 (I) with a control participant (J).
tients with polymyositis.\textsuperscript{10} Although the pathogenesis of antistriational autoantibodies was not elucidated, we believe these autoantibodies are useful as clinical markers of developing myocarditis and/or myositis.\textsuperscript{22} Our previous study indicated that anti-Kv1.4 antibodies were not detected in the serum of patients with polymyositis.\textsuperscript{10} However, detection of these antibodies was not performed in patients with myositis who did not have MG. Recently, clinical concepts mediated by neuronal voltage-gated potassium channel autoantibodies (those to Kv1.1, 1.2 and 1.6) have been expanding.\textsuperscript{20,27} A broad clinical spectrum and paraneoplastic manner in thymoma are common characteristics of neuronal and muscular voltage-gated potassium channel autoimmunity.

In conclusion, our observations suggest that heart and skeletal muscles are autoimmune targets in some patients with MG.

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