Supplemental Data

Inflammatory myopathy with myasthenia gravis: thymoma association and polymyositis pathology

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Detailed clinical features of individual patients

1) IM with MG patients whose MG diagnosis preceded their IM diagnosis

Patient 1

A 59-year-old man was admitted to a local hospital because of progressive myalgia in shoulder girdle muscles and an elevated serum CK level (10,226 IU/L), which started 4 days before admission. At the age of 45, he was diagnosed as having an invasive thymoma, which was treated by a combination of therapies including surgical removal of the thymoma, radiotherapy and chemotherapy. He was diagnosed as having anti-AChR Ab positive ocular MG at the age of 54. His anti-AChR Ab positive ocular MG was kept under good control until 2 weeks before admission when he developed herpes zoster and had to discontinue his maintenance doses of immunosupressant (tacrolimus [TCR] 3 mg). At the age of 56, he had an episode of subacute exacerbation of ocular symptoms with elevation of his serum CK level (4,209 IU/L), which improved after an additional immunotherapy and his serum CK level became normal. His doctor in charge diagnosed the symptoms as an MG relapse associated with rhabdomyolysis of uncertain cause. On admission, neurological examinations showed dropped head, mild proximal muscle weakness with myalgia and swallowing and respiratory difficulties. There was no ocular symptom. He had a low-grade fever (37.2°C). His weakness exacerbated rapidly after hospitalization and he became tetraplegic. He showed decreased vital capacity (39%) and increased $PaCO_2$ (44 mmHg) in arterial blood gas analysis. He required mechanical ventilation. Needle electromyography (EMG) showed an irritable myopathy and RNS test showed normal responses. T2-weighted magnetic resonance imaging (MRI) revealed multifocal high-signal-intensities in the gluteus medius and

paraspinal muscles. Biopsy of the deltoid muscle confirmed the pathological diagnosis of IM. After the introduction of high-dose methylprednisolone therapy (IVMP) followed by oral prednisolone (PSL) 55 mg and TCR 3 mg, his weakness and myalgia gradually improved. This serum CK level normalized within 2 weeks after the start of treatment. One year later, he had no ocular or limb muscle symptom under maintenance treatment with PSL 5 mg and TCR 3 mg.

Patient 2

A 73-year-old woman with MG was admitted to a local hospital because of a one-month history of ptosis and diplopia with elevated serum CK levels. She was diagnosed as having MG (Myasthenia Gravis Foundation of America [MGFA] stage II) and an invasive thymoma at the age of 50. She was treated by surgical removal of the thymoma and with immunosuppressive drugs including PSL. Although she showed exacerbations of MG twice at the age of 53 and 56, which required an additional immunotherapy each time, her MG symptoms including ocular symptoms had been well controlled with oral PSL (3.75 mg). On admission, she presented with no muscle weakness except for bilateral ptosis and diplopia. Her serum CK level was 376 IU/L. Needle EMG showed an irritable myopathy and RNS showed normal responses. Biopsy of the biceps brachii muscle showed scattered necrotic and regenerating fibers and the sarcolemma of muscle fibers positive for diffusely upregulated MHC class I; these findings are consistent with IM. After increasing her oral PSL dose to 30 mg, her symptoms improved and her serum CK returned to normal level. Then, her PSL dose was tapered to maintenance levels. Three years after the onset of IM, she had no ocular symptom and her muscle strength was normal.

Patient 4

A 50-year-old woman was admitted to a hospital because of progressive weakness, increased fatigability and elevated serum CK levels. At the age of 38, she was diagnosed as having MG and an invasive thymoma (MGFA II). The titer of anti-AChR Ab was 50 nmol/L. The thymoma was surgically removed. She was administered immunotherapeutic drugs including oral PSL, which improved her symptoms. Her MG symptoms were well controlled during the following 7 years. At the age of 45, she developed generalized weakness and diplopia with elevation of her serum anti-AChR Ab titer (170 nmol/L). Although she was treated with steroids followed by repeated treatments with intravenous immunoglobulin (IVIg), her symptoms gradually worsened. She was found to have elevated serum CK levels (244 to 341 IU/L) for 3 years before admission. On examination, she presented with ptosis, swallowing difficulties and neck and proximal limb weakness (Medical Research Council [MRC] grade 4 in proximal muscles and 5 in distal muscles) with fatigability. Her serum CK was 341 IU/L. She was positive for the anti-AChR (300 nmol/L) and anti-titin Abs. RNS test demonstrated decremental responses and EMG showed an irritable myopathy. Echocardiography showed decreased left ventricular wall motion and ejection fraction (EF) (53%). Biopsy of the deltoid muscle showed lymphocyte infiltration and upregulation of MHC class I and class II antigens in the sarcolemma of non-necrotic muscle fibers; these findings confirmed the diagnosis of IM. She was treated with cyclosporine (200 mg/day), which improved her limb muscles power and normalized her serum CK levels. Follow-up echocardiography proved EF improvement. The patient remained in a stable condition until her last follow-up visit, which was 3 years after the IM diagnosis

Patient 5

This 57-year-old man was admitted to a hospital because of drop head and mild weakness in his four limbs, which started 1 month before admission. At the age of 43, he was diagnosed as having MG associated with an invasive thymoma. The thymoma was surgically removed and immunosuppressive therapy was introduced. On admission, he presented with dysphagia, head drop and mild proximal weakness in her four limbs (MRC grade 4 in proximal muscles and 5 in distal muscles) with no ocular symptoms. Laboratory examinations showed an elevated serum CK levels of 1,059 IU/L, elevated levels of serum liver enzymes and positivity for serum anti-AChR Ab (6.6 nmol/L). EMG showed an irritable myopathy and RNS test revealed normal responses. Chest computed tomography (CT) showed recurrence of the thymoma and diffuse panbronchiolitis. Biopsy of the deltoid muscle showed scattered regenerating fibers with scattered inflammatory cells and MHC class I diffusely up-regulated in the sarcolemma of the muscle fibers; these findings were consistent with IM. Liver biopsy revealed concurrent autoimmune cholangitis. After the start of treatment with an oral steroid (20 mg), his head drop and limb muscles weakness improved. However, he died of respiratory failure caused by the exacerbation of diffuse panbronchiolitis at the age of 58.

Patient 6

A 49-year-old woman was admitted to a local hospital because of a two-week history of progressive, upper proximal muscle pains associated with general malaise and fever. At the age of 38, she was diagnosed as having MG associated with an invasive thymoma and was treated with an immunosuppressant (TCR 1 mg) and surgical removal of the tumor followed by radiotherapy. On examination, she presented with severe pain in the upper proximal muscles and mild to moderate weakness in the four limbs. She had no

ocular symptoms. Her serum CK level was 5,102 IU/L. Initially, she was diagnosed as having rhabdomyolysis. However, her limbs muscle weakness progressed rapidly and became severe (MRC grade 1 in the proximal and distal muscles) with respiratory decompensation requiring mechanical ventilation. Laboratory examinations showed an elevated serum CK-MB level, electrocardiography revealed ventricular tachycardia and echocardiography revealed hypokinesis with thickening of cardiac walls, suggesting cardiac involvement. EMG showed an irritable myopathy and RNS test showed normal responses. Biopsy of the deltoid muscle showed muscle fiber size variation, necrotic and regenerating muscle fibers and focal endomysial inflammatory infiltrates. Needle biopsy of the myocardium revealed lymphocytic myocarditis with massive T cell infiltration (predominantly CD8 positive T cells). These findings suggested IM complicated by myocarditis. She was administered immunoabsorption therapy, IVIg and IVMP followed by oral PSL. Her serum CK level normalized rapidly (less than 3 weeks) after the start of treatment. Her symptoms including respiratory failure improved markedly and she was extubated. During the following 6 months, she showed further improvement of weakness in the four limbs (MRC grade 4 in the proximal and distal muscles).

2) IM with MG patients whose IM diagnosis preceded their MG diagnosis

Patient 7

A 47-year-old woman was admitted to a local hospital because of fever and muscle pain with swelling. The muscle symptoms started from the forearms and gradually extended to her four limbs over 1 month. On admission, she had a low-grade fever (37.6°C) and moderate weakness in the proximal part of all extremities with respiratory difficulties.

Within 1 week after hospitalization, her weakness rapidly worsened and became severe, and she required mechanical ventilator support. Her serum CK level was 7,315 IU/L. She was positive for anti-AChR Ab (12.0 nmol/L). Chest CT revealed an invasive thymoma. Needle EMG showed myopathic changes with spontaneous activities. The results of the edrophonium test, RNS test and single-fiber electromyography (SFEMG) showed no abnormalities. bs treated with three cycles of IVMP and two cycles of IVIg followed by oral PSL 60 mg, which was tapered to 20 mg and methotrexate (MTX) 12.5 mg/ week added. She received radiotherapy for thymoma, along with immunotherapies for IM. These treatments markedly improved her symptoms and she achieved an independent activity of daily living. Five years after her initial hospitalization, chest CT revealed the exacerbation of thymoma and she was hospitalized for chemotherapy. In the hospitalization, she showed fluctuating mild limb weakness without ocular symptoms. Her serum CK level was 463 IU/L on admission. Abnormal electrophysiological findings including decremental RNS responses and increased jitter and blocking on SFEMG in the muscle of the extremities led to the diagnosis of MG. She received IVIg for MG following chemotherapy for thymoma. However, after the introduction of IVIg, she developed respiratory failure requiring mechanical ventilation, with a marked elevation of the CK level (3,640 IU/L). Electrophysiological re-examination showed an irritable myopathy and improvement of decremental RNS responses, suggesting relapse of IM. She was treated with IVMP and oral PSL, which were effective; the CK levels normalized within a few days after the introduction of IVMP. After 8 months of hospitalization, she was discharged from the hospital again. She died of sepsis caused by repeated episodes of pneumonia 6.1 years after the IM diagnosis.

3) IM with MG patients who were diagnosed with IM and MG within 1 year

Patient 3

A 53-year-old man had a four-month history of fluctuating diplopia, ptosis and nasal voice. On admission, he showed bilateral ptosis and diplopia but no clear weakness in the four limbs. His serum CK level was 231 IU/L. He was positive for anti-AChR, anti-mitochondrial M2, anti-microsome and anti-thyroglobulin Abs. RNS test showed abnormal decremental responses. Chest CT revealed an invasive thymoma with pleural dissemination. Before muscle biopsy, treatment with oral PSL (50 mg/day) was started. During the next 3 months, he showed clear improvement of his symptoms; thus, the oral PSL dose was tapered. RNS test performed 2 months after the start of treatment showed normal responses. However, his serum CK levels remained elevated with fluctuation (325 to 1008 IU/L) regardless of the oral PSL dose. Three months after the start of oral PSL treatment, at which time the PSL dose was tapered to 35 mg/day, he developed rapidly progressive drop head and proximal limb muscle weakness within a week. Despite the severe limb weakness, he showed no ocular symptoms. He required mechanical ventilation because of carbon dioxide narcosis caused by the respiratory distress. Chest CT showed no remarkable changes. EMG showed an irritable myopathy and RNS test showed normal responses. Biopsy of the biceps bracii muscle showed inflammatory cell infiltrates, necrotic and regenerating fibers and upregulation of MHC class I in the sarcolemma of muscle fibers, suggesting IM. The PSL dose was increased to 50 mg/day and TCR (3 mg/day) was added. He showed gradual improvement of weakness and mechanical ventilation was discontinued. After that, the invasive thymoma was treated by radiotherapy and chemotherapy. Three years after, his activity of daily living was graded as independent.

Patient 8

A 78-year-old man was admitted to our hospital because of drop head. One and a half year before admission, he noticed weakness of abdominal muscles when he did sit-ups. A family physician found that he had an elevated serum CK level (518 IU/L). His symptom and elevated serum CK levels continued for 6 months and they normalized without treatment. Nine months before admission, he presented with gradually worsening drop head with back muscle pain and an elevated serum CK level again (235 IU/L). Although he stopped taking statins, these symptoms continued; thus he was hospitalized for examination. Neurological examinations showed mild neck extensor muscle and proximal dominant limb weakness. There were no clear ocular symptoms. His serum CK level was 263 IU/L. He was positive for anti-AChR (47.7 nmol/L) and anti-titin Abs. T2 weighted cervical MRI revealed multifocal high-signal-intensity areas in paraspinal muscles. RNS test showed decremental responses and EMG showed an irritable myopathy. Biopsy of the splenium muscle showed inflammatory cell infiltrates and upregulation of MHC class I in the sarcolemma of scattered muscle fibers. He started wearing a neck brace and taking pyridostigmine without steroids, which he refused. Drop head improved and he was discharged from the hospital. He was followed up for 2.6 years without exacerbation of symptoms.

Patient 9

A 70-year-old woman with chest pain was admitted to a local hospital emergently because of sever dyspnea, which developed after taking low-dose benzodiazepine for anxiety. She had been under medical care for red cell aplasia, which was diagnosed at a different hospital 3 weeks before admission. She required a mechanical ventilator

support for her respiratory failure. However, her limb muscle strength was relatively preserved. Laboratory examinations showed positivity for serum anti-AChR Ab and an elevated serum CK level (1,140 IU/L). Chest CT revealed normal findings. RNS test showed decremental responses. Needle EMG was not performed. Biopsy of the biceps brachii muscle showed endomysial infiltration of mononuclear cells surrounding non-necrotic fibers and increased expression levels of MHC class I and class II antigens on non-necrotic fibers, suggesting the concomitant existence of IM alongside MG. She was treated with three cycles of IVMP followed by oral PSL (40 mg). Although her serum CK level normalized after the first IVMP, her respiratory failure was refractory. She developed ventricular tachycardia (VT) and showed diffuse hypokinesis of the cardiac muscle on echocardiography. Two months after the start of treatment, she developed sepsis and died of multiple organ failure.

Patient 10

A 60-year old woman was admitted to our hospital because of frequent episodes of falling while walking, which started 3 months before admission. She presented with weakness and fatigability in proximal limbs muscles and bilateral tibialis anterior muscles, but no ptosis or diplopia. The serum CK level was 292 IU/L. She was negative for anti-AChR Ab. There was decremental RNS responses (30%). The edrophonium test revealed a marked improvement of her muscle strength. EMG showed myogenic changes in the proximal extremities. Pyridostigmine (120 mg /day) improved her muscle strength and enabled her to stand up from sitting position easily. Biopsy or the deltoid muscles showed infiltration of mononuclear cells predominantly in the endomysium with small amounts of necrotic fibers and regenerating fibers. MHC class I was diffusely up-regulated in the sarcolemma of muscle fibers. These findings

suggested that he had both IM and MG. He was treated with PSL 60 mg in addition to pyridostigmine resulting in the improvement of muscle strength. During the next 4 months until discharge, PSL dose was tapered and pyridostigmine was discontinued, without worsening of muscle strength. Thereafter, he showed no exacerbation during the following 17 years.

3) Thymomatous IM patient without MG

Patient 11

This 51-year-old woman presented with progressive limb muscles weakness and dysphagia, which started 1 month before admission. She had moderate to severe weakness in her limbs and respiratory muscles. Because of respiratory failure, respiratory support with a mechanical ventilator was introduced. Laboratory data showed an elevated serum CK level (695 IU/L) and positive anti-AChR (77 nmol/L) and anti-titin Abs. Needle EMG showed myopathic changes with spontaneous activities. RNS test was normal. Chest CT showed a tumor (1 cm in size) in anterior mediastinum. A needle biopsy of the tumor revealed necrotic tissues without thymoma cells. Histopathological study of the biceps brachii muscle showed abundant necrotic and regenerating fibers and endomysial CD8+ T-lymphocytes invading non-necrotic muscle fibers expressing MHC class I antigen, meeting the pathological criteria of definite PM. She was successfully treated with prednisolone (PSL). Her anterior mediastinum tumor gradually increased in size without neurological symptoms. The tumor was resected and pathologically confirmed as combined B2/B3 thymoma 5 years after the diagnosis of IM. Her 11-year follow-up showed no exacerbation of IM or thymoma or development of MG.

Figure e-1

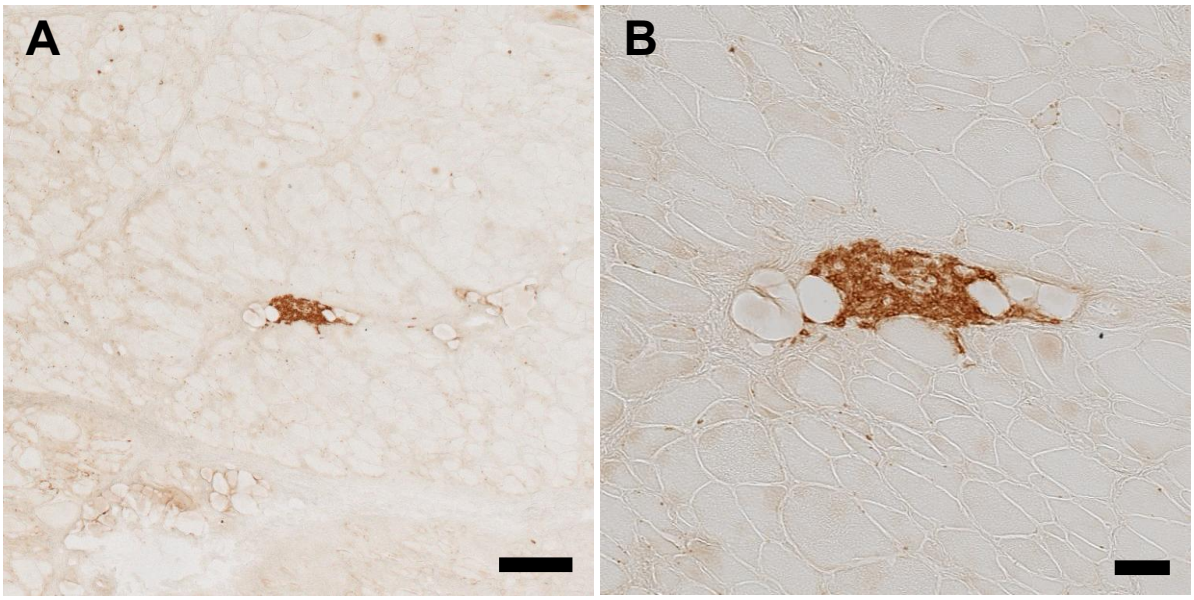

Figure e-1 Immunohistochemical staining for CD45RA in patient 8

(A) Low and (B) higher magnification view of perimysial aggregation of CD45RA-positive cells. This is a serial section of the sections shown in Figure 2C and D. Scale bars: 200 μm for A and 50 μm for B.

Figure e-2

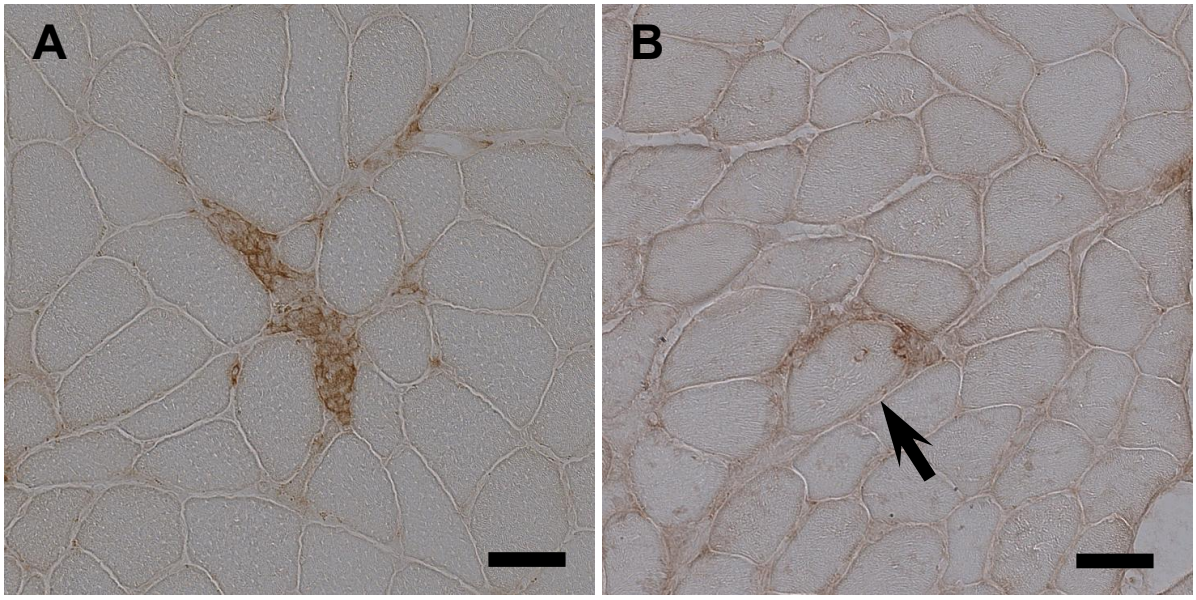

Figure e-2 Immunohistochemical staining for CTLA-4 in patients 3 and 10

Endomysial infiltration of CTLA-4-positive cells in (A) patient 3 and (B) patient 10.

Note that invading cells are positive for CTLA-4 (arrow). Scale bars: 50 μm.