Non-motor Comorbidity of Myasthenia Gravis: Myasthenia Gravis as a Systemic Immunological Disorder Involving Non-motor Systems

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Abstract:
To explore non-motor comorbidities of myasthenia gravis (MG), we present two cases of thymoma-associated MG patients. Alopecia, pure red cell aplasia, and thymoma-associated multiorgan autoimmunity were observed in Case 1, and alopecia, thrombocytopenia, hypogammaglobulinemia and nephrotic syndrome were observed in Case 2. In both cases, autoreactive T lymphocytes inappropriately stimulated by thymus tissue may have played key roles in generating the various autoimmune-associated symptoms. Consequently, systemic immunological involvement due to the thymoma-associated breakdown of immunoregulations in both motor and non-motor systems should be considered in MG patients.

Key words: myasthenia gravis, thymoma, pure red cell aplasia, alopecia, thymoma-associated multiorgan autoimmunity

Introduction
Major clinical symptoms of myasthenia gravis (MG) include motor weakness, easy fatigability, double vision, ptosis, and dysphagia, while the disease pathogenesis is characterized by autoimmune disturbances of the humoral immune system (1). The most representative antibody has been recognized as that to the muscle nicotinic acetylcholine receptor (anti-AChR antibody), which resides at the neuromuscular junction. It is well known that approximately 8%-10% of MG patients have additional autoimmune disorders, such as thyroid or collagen diseases (2). However, symptoms of non-motor complications may often be missed or underestimated, which can severely affect the activities of daily living of MG patients.

In this study, we focused on the non-motor comorbidities of MG to draw more attention to the non-motor symptoms of MG.

Case Reports

Case 1
A 51-year-old woman developed double vision, dysphagia and generalized muscle weakness at 40 years of age. Laboratory findings demonstrated elevated serum anti-AChR antibody (71 nmol/L), and repetitive stimulation testing showed waning of limb muscles. Computed tomography (CT) revealed invasive thymoma, which was resected after chemotherapy and irradiation (Fig. 1A). The postoperative Masaoka stage was estimated to be IIB, and the pathological diagnosis was determined to be World Health Organization pathological subtype B2. Because of the diagnosis of thymoma-associated MG (Class IIIa in Myasthenia Gravis Foundation of America [MGFA] scale), the patient had been treated with oral prednisolone for 3 years, which resulted in decreased anti-AChR antibody titers (0.2 nmol/L) and significant improvement in her clinical symptoms. Unfortunately, the patient stopped treatment for personal reasons. At 45 years of age, the patient revisited the hospital be-
cause of muscle weakness. At that time, she was not under any medication against MG, and a neurological examination revealed generalized muscle weakness. The anti-AChR antibody titer was again elevated (120 nmol/L), indicating the recurrence of MG. A mediastinal CT examination demonstrated recurrent multiple thymoma with vertebral and spinal invasions (Fig. 1B and C). In addition, broad alopecia was apparent (Fig. 1D). After chemotherapy, the thymoma was resected. Subsequently, the clinical symptoms of MG were controlled well with prednisolone and tacrolimus; however, alopecia remained.

At 46 years of age, the patient developed pure red cell aplasia, which was treated by frequent blood transfusions. Her red blood cell count and hemoglobin concentration were 251×10^7/μL and 7.1 g/dL, respectively. Bone marrow cytology demonstrated a depletion of erythroblasts. The use of several medications had stabilized her clinical condition for three years. At 49 years of age, she developed frequent diarrhea and multiple skin eruptions over the entire body (Fig. 1E and F). Pathological skin examinations indicated hypertrophic changes and inflammatory cell infiltration (Fig. 2A). Immunostaining results demonstrated that most infiltrating cells were T lymphocytes. In addition, CD4-positive cells were observed in the upper dermis (Fig. 2C), and CD8-positive cells were seen in the epidermis (Fig. 2D). CD20-positive B lymphocytes were scarcely seen (Fig. 2B). Immunofluorescence microscopic examinations demonstrated immunoglobulin, complement, and immune complex deposition in the skin tissue (Fig. 2E-I). These observations indicated graft-versus-host disease (GVHD)-like disturbances of the immune system, so thymoma-associated multiple organ autoimmunity was suspected. The skin lesions were treated with narrow-band ultraviolet B phototherapy, and the muscle weakness was controlled well with medications. Her clinical course is summarized in Fig. 3.

Case 2

A 46-year-old woman developed double vision, dysphagia, and general muscle weakness at 36 years of age. Laboratory examinations revealed elevated anti-AChR antibody titers (82 nmol/L), and repetitive stimulation testing demonstrated waning of the limb muscles. Thymoma was detected by mediastinal CT examinations (Fig. 4A). After being diagnosed with thymoma-associated MG (MGFA Class IVb), she received prednisolone administration followed by thymectomy and immunoabsorption therapy. The postoperative Masaoka stage was estimated to be IIA, and the pathological diagnosis was determined to be World Health Organization pathological subtype A. Prednisolone and cyclosporine administrations stabilized her clinical condition for two years. During that time, she developed alopecia (Fig. 4C).

At 39 years of age, the symptoms of MG, particularly facial muscle weakness and dysphagia, worsened, accompa-
edema resolved, and laboratory data showed significant improvement. Her alopecia was less prominent at that time (Fig. 4D). Despite several comorbidities, her muscle weakness remained stable. Her clinical course is summarized in Fig. 5.

A: A biopsy specimen showing atrophic changes and inflammatory cell infiltration. (Hematoxylin and Eosin staining, ×40). B: Infiltrating lymphocytes were negative for anti-CD20 antibodies. (anti-CD20 antibody immunostaining, ×200). C: CD4-positive T cells were mainly observed in the upper dermis (anti-CD4 antibody immunostaining, ×200). D: CD8-positive T cells were mainly observed in the epidermis. (anti-CD8 antibody immunostaining, ×100). E: Direct immunostaining for IgA was strongly positive in the keratin layer. (anti-IgA antibody immunofluorescence, ×100). F: Direct immunostaining for IgM was positive in the keratin layer and dermis. (anti-IgM antibody immunofluorescence, ×100). G: Direct immunostaining for IgG was positive in the epidermis and keratin layer (anti-IgG antibody immunofluorescence, ×100). H: Direct immunostaining for C3 was diffusely positive in both the epidermis and dermis (anti-C3 antibody immunofluorescence, ×200). I: Direct immunostaining for C1q showed positive reactions in the dermis, epidermis and keratin layers (anti-C1q antibody immunofluorescence, ×200).

Figure 2. Histopathological observations of the biopsied skin in Case 1. A: A biopsy specimen showing atrophic changes and inflammatory cell infiltration. (Hematoxylin and Eosin staining, ×40). B: Infiltrating lymphocytes were negative for anti-CD20 antibodies. (anti-CD20 antibody immunostaining, ×200). C: CD4-positive T cells were mainly observed in the upper dermis (anti-CD4 antibody immunostaining, ×200). D: CD8-positive T cells were mainly observed in the epidermis. (anti-CD8 antibody immunostaining, ×100). E: Direct immunostaining for IgA was strongly positive in the keratin layer. (anti-IgA antibody immunofluorescence, ×100). F: Direct immunostaining for IgM was positive in the keratin layer and dermis. (anti-IgM antibody immunofluorescence, ×100). G: Direct immunostaining for IgG was positive in the epidermis and keratin layer (anti-IgG antibody immunofluorescence, ×100). H: Direct immunostaining for C3 was diffusely positive in both the epidermis and dermis (anti-C3 antibody immunofluorescence, ×200). I: Direct immunostaining for C1q showed positive reactions in the dermis, epidermis and keratin layers (anti-C1q antibody immunofluorescence, ×200).

At 44 years of age, she developed thrombocytopenia (platelet count, 36,000/uL) due to Helicobacter pylori infection, which was successfully treated with antibiotics. Simultaneously, she developed hypogammaglobulinemia (IgG, 336 mg/dL). At 45 years of age, she developed an abrupt-onset peripheral edema, vomiting, and diarrhea after experiencing flu-like symptoms. Her serum albumin concentration was 1.4 g/dL, and her serum low density lipoprotein (LDL) level was 474 mg/dL with marked proteinuria (4.2 g/day). She was diagnosed with minimal change nephrotic syndrome and treated with 50 mg of prednisolone. Subsequently, her edema resolved, and laboratory data showed significant improvement. Her alopecia was less prominent at that time (Fig. 4D). Despite several comorbidities, her muscle weakness remained stable. Her clinical course is summarized in Fig. 5.

**Discussion**

The major clinical symptoms of MG include motor weakness and easy fatigability. MG is subdivided into three major clinical subtypes: early-onset, late-onset, and thymoma-associated. In patients with thymoma-associated MG, which accounts for 12%-25% of all cases, immunological disturbances possibly induced by abnormal thymus tissue may sometimes provoke various clinical symptoms, but not neuro-muscular dysfunctions (1-3). Among them, limbic encephalitis, pure red cell aplasia, alopecia, hypogammaglobulinemia, myocarditis, and taste disorders are well-known non-motor comorbidities (4-10). In addition to antibodies to
the neuromuscular junction, anti-neuronal antibodies may also provoke depression, anxiety, dementia, and autonomic failure.

Suzuki et al. have reported the frequencies of various comorbidities occurring with thymoma-associated MG. According to their data, pure red cell aplasia occurs in 5%, alopecia in 12%, and taste disorder in 10% of all cases of thymoma-associated MG (11, 12). Although the pathogenesis of these conditions remains unknown, autoreactive T lymphocytes, which are primarily stimulated in the thymus, may exhibit immunoreactivity to various organs. For example, abnormally stimulated CD8-positive cytotoxic T lymphocytes may attack premature bone marrow cells in pure red cell aplasia patients and hair follicles in alopecia pa-
Anti-striational antibodies, such as anti-titin, anti-ryanodine receptor, and anti-Kv1.4, are often detected in some MG patients. Among them, anti-Kv1.4 antibody is considered a potential marker of cardiac involvement in MG (13). In anti-Kv1.4-positive MG patients, ventricular tachycardia, complete atrial ventricular block, and severe heart failure have been reported. These symptoms may often be lethal in MG patients. The presence of auto-antibodies to taste buds is considered a possible cause of taste disorders (10-12). In Case 1, the presence of alopecia and pure red cell aplasia was compatible with the T lymphocyte-mediated theory and thus was also considered the cause of alopecia in Case 2. In addition, the recent diarrhea and broad skin eruptions in Case 1 might imply the presence of thymoma-associated multorgan autoimmunity (TAMA), which is a GVHD-like disease that can occur with thymoma (14, 15). In these patients, the thymus produces self-reactive T cells when the donor tissue acts as a source of pathogenic T cells in the recipient body in GVHD, as the abnormal thymus is incapable of appropriately teaching developing thymocytes to eliminate self-reactive T cells. Subsequently, these abnormal mechanisms due to self-reactive T lymphocytes can induce a disease that is clinically indistinguishable from GVHD, such as medication-resistant diarrhea and skin reactions. It is quite significant that MG unassociated with organ transplantation can provoke TAMA likely due to a remnant thymoma or abnormally taught T cells that attack host tissues.

In Case 2, in addition to alopecia, thrombocytopenia and hypogammaglobulinemia were also observed. The possibility that these symptoms might be due to the use of steroids and cyclosporine cannot be eliminated, although immunological disturbances associated with MG might influence the development of such symptoms (6). In addition, the presence of nephrotic syndrome in Case 2 was quite significant. T cell dysfunction is considered a major cause of nephrotic syndrome associated with post-thymectomy MG. In most cases, patients underwent thymectomy after several years, and the median period until the appearance of nephrotic syndrome is reported to be 100 months. In Case 2, the latent period to the development of nephrotic syndrome was estimated to be 9 years. Therefore, thymoma-associated T cell dysfunction is thought to persist for a long time, and secreted cytokines may increase the permeability of the glomerular basement membrane, which subsequently (after a long latent period) induces nephrotic syndrome (16-18). In our case, oral steroid administration provoked a good clinical response without the exacerbation of MG. However, responses to steroid treatment reportedly vary among patients.

The present findings may increase attentions on the non-motor comorbidities of MG. In particular, the subtype thymoma-associated MG can generate various immunological disturbances, including effects on non-motor systems due to T cell malfunctions, which are highly influenced by thymoma. It is important to understand and appropriately treat non-motor complications of MG because these are common clinical immunological disturbances associated with MG. We also summarized previously reported MG cases showing similar non-motor comorbidities in Table (5-7, 16-24). Our present cases demonstrated multiple non-motor symptoms, making them significant cases in the consideration of immunological disturbances among MG patients.

The authors state that they have no Conflict of Interest (COI).

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Table. Summary of Similar Previous MG Cases.

| Case | Age | Sex | Alopecia | PRCA | ITP | hypogammaglobulinemia | nephrotic syndrome | TANA | thymus pathology | Reference |
|------|-----|-----|----------|------|-----|-----------------------|-------------------|------|-----------------|----------|
| 1    | 45  | F   | +        |      |     | +                     | B2                |      | our present cases |
| 2    | 36  | F   | +        | +    |     | +                    | +                 |      |                 |          |
| 3    | 46  | M   | +        |      |     | n.d.                 | B                |      |                 |          |
| 4    | 46  | M   | +        |      |     | n.d.                 | 7                 |      |                 |          |
| 5    | 58  | M   | +        |      |     | B                    | 9                 |      |                 |          |
| 6    | 73  | M   | +        |      |     | AB                   | 5                 |      |                 |          |
| 7    | 57  | F   | +        | +    |     | +                    | A                |      |                 |          |
| 8    | 62  | F   | +        | +    |     | +                    | B1               |      |                 |          |
| 9    | 65  | F   | +        | +    |     | +                    | 20                |      |                 |          |
| 10   | 69  | F   | +        |      |     | +                    | A                | 21   |                 |          |
| 11   | 53  | F   | +        | +    |     | A                    | 6                 |      |                 |          |
| 12   | 58  | M   | +        |      |     | AB                   | 22                |      |                 |          |
| 13   | 26  | F   | +        |      |     | invasive thymoma     | 16                |      |                 |          |
| 14   | 46  | F   | +        |      |     | hyperplasia          | 17                |      |                 |          |
| 15   | 56  | M   | +        |      |     | hyperplasia          | 19                |      |                 |          |
| 16   | 58  | F   | +        |      |     | AB                   | 23                |      |                 |          |
| 17   | 28  | M   | +        |      |     | AB                   | 24                |      |                 |          |

n.d. not described

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