A possible role of low regulatory T cells in anti-acetylcholine receptor antibody positive myasthenia gravis after bone marrow transplantation

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Abstract

Background: Chronic graft-versus-host disease (GVHD) appears several months following allogenic hematopoietic stem cell transplantation (HSCT) and is clinically analogous to autoimmune disorder. Polymyositis is a common neuromuscular disorder in chronic GVHD, but myasthenia gravis (MG) is extremely rare. Hence, its pathophysiology and treatment have not been elucidated.

Case presentation: A 63-year-old man with a history of chronic GVHD presented with ptosis, dropped head, and dyspnea on exertion, which had worsened over the previous several months. He showed progressive decrement of compound muscle action potential in the deltoid muscle evoked by 3-Hz repetitive nerve stimulation, a positive edrophonium test, and elevated levels of serum anti-acetylcholine receptor antibodies, which suggested a diagnosis of generalized MG. No thymoma was found. Flow cytometric analysis revealed a remarkable depletion of peripheral Tregs (CD4+CD25highFOXP3+ cells, 0.24% of the total lymphocytes). Administration of prednisolone and tacrolimus was insufficient to alleviate his symptoms; however, the use of rituximab successfully improved his condition.

Conclusions: Myasthenic symptoms appeared in the process of tapering prednisolone for the treatment of chronic GVHD, supporting the diagnosis of MG associated with chronic GVHD. The present case proposes a possibility that reduction of Tregs might contribute to the pathogenesis of MG underlying chronic GVHD. Immunotherapy with rituximab is beneficial for treatment of refractory MG and GVHD.

Keywords: Myasthenia gravis, Hematopoietic cell transplantation, Graft-versus-host disease, Regulatory T cells, Rituximab, Anti-acetylcholine receptor antibody

Background

Myasthenia gravis (MG) is a neuromuscular disorder characterized by muscle weakness and pathological fatigability of skeletal muscles. The pathophysiology of MG is defined as the production of autoantibodies blocking acetylcholine receptors at the neuromuscular junction [1]. Chronic graft-versus-host disease (GVHD) is mediated by the reactivation of donor T cells against recipient tissues and appears several months after allogenic hematopoietic stem cell transplantation (HSCT) in most cases [2]. The major organs involved in GVHD include the skin, gastrointestinal tract, and liver, whereas chronic GVHD features a wide variety of autoimmune disorders, including Sjögren syndrome, scleroderma, bronchiolitis obliterans, and immune cytopenias [3]. Chronic GVHD also affects neuromuscular system. In fact, polymyositis is a common neuromuscular disease present in chronic GVHD; however, MG is extremely rare. Although the 2015
National Institutes of Health Consensus Conference categorized MG under “other features or unclassified entities” of the signs and symptoms for diagnosis and staging of chronic GVHD, there have been a limited number of MG cases following allogeneic HSCT [4], and its pathophysiology and treatment approach have not yet been well established.

We present a case of chronic GVHD developing generalized MG that was successfully treated with advanced immunotherapy. The current case revealed a marked reduction of regulatory T cells (Tregs), suggesting the possible pathogenesis of MG in patients with chronic GVHD.

Case presentation
A 63-year-old man without familial history of MG was diagnosed with secondary acute myeloid leukemia that originated from myelodysplastic/myeoproliferative neoplasms, unclassifiable 2 years prior to the current presentation. He was then treated with intensive chemotherapy, and underwent allogeneic HSCT from a human leukocyte antigen (HLA)-matched unrelated donor in the following year. Prophylaxis against GVHD consisted of tacrolimus and short-term methotrexate. He achieved remission of acute GVHD, and tacrolimus was discontinued on day 86. He then developed mild chronic GVHD of the skin and liver at 7 and 12 months after the transplantation, respectively. Fourteen months after the transplantation, he was admitted to our hospital due to progressive bilateral ptosis, dropped head, and mild bilateral weakness in-
Discussion and conclusions

MG is a very rare complication following HSCT, with an occurrence of less than 1%. Myasthenic symptoms typically develop between 22 and 60 months after transplantation [8] and most reported cases are associated with the existence of other symptoms of chronic GVHD, as MG is rarely the sole manifestation [9]. Myasthenic symptoms appear after the discontinuation or tapering of immunosuppressive agents [10], as in the present case. Patients with aplastic anemia as a background would have an increased risk of developing MG after transplantation [11]. Patients with specific HLAs (HLA Cw1, Cw7 or DR2) and a family history of MG are also at an increased risk of developing MG after HSCT [12, 13]. However, none of these risk factors was identified in the present case.

Most patients with MG associated with chronic GVHD test positive for anti-AChR antibodies. Approximately 20% of patients with chronic GVHD show positive anti-AChR antibody without myasthenic symptoms, indicating the existence of higher rates of subclinical MG [14]. In the present case, an elevated titer of anti-AChR antibody supported the diagnosis of MG, and its titer decreased in parallel with the improvement of myasthenic symptoms after immunotherapies. A few reported patients with MG after transplantation revealed...
other antibodies toward non-AChR components of the postsynaptic muscle endplate, e.g. anti-MuSK and anti-striated muscle antibodies [15, 16]. These findings suggest that pathogenic autoantibodies toward the neuromuscular junctions may be produced when discontinuing or tapering immunosuppressive treatments after transplantation.

A unique point in the present case was lower population of Tregs in the peripheral blood. Tregs are a subset of T lymphocytes that modulate the immune system and maintain tolerance to self-antigens [17, 18]. The frequency of Tregs in the peripheral blood has been reported to negatively correlate with GVHD severity [6]. Absence of Tregs coupled with donor-derived T cells leads to development of GVHD [19]. The number of Tregs in the peripheral blood decreases in untreated MG and is normalized by immunotherapy [20]. A functional impairment of thymic Tregs has been reported in the thymus of patients with MG and may play a role in triggering the autoimmune process [21]. Recent studies have reported Treg dysfunction as well as its downstream signal transducer and activator of transcription pathway is associated with immunopathology of MG, and a Treg-based immunotherapeutic approach has been suggested for experimental models of MG [22–24]. We suspect that suppression of Tregs in the present case may have partly contributed to the pathogenesis of MG as a manifestation of chronic GVHD.

Recently, several reports have shown that rituximab, a monoclonal antibody against B cell surface antigen CD20, could be an effective treatment for patients with refractory MG [25, 26]. Rituximab therapy also reduces the incidence of GVHD following allogeneic HSCT and exhibits a beneficial effect on steroid-refractory GVHD [27]. Although rituximab itself directly interacts with B cells, B cell depletion subsequently leads to expansion of Tregs and suppression of autoreactive T cells [28]. Additionally, treatment with rituximab was shown to be successful in two cases of MG associated with chronic GVHD [27, 29]. This evidence supports the utility of rituximab for refractory GVHD and MG against conventional immunotherapies in the present case.

In conclusion, we reported a rare case of chronic GVHD developing systemic MG after allogenic HSCT. Appearance of myasthenic symptoms during the process of tapering prednisolone for the treatment of chronic GVHD is a key feature of MG associated with chronic GVHD. We hypothesize that the reduced number of Tregs might play a possible role in the pathogenesis of MG and chronic GVHD. Immunotherapy with rituximab led to improvements of the patient’s myasthenic symptoms, supporting the utility of rituximab for refractory MG and GVHD.

Abbreviations
AChR: Anti-acetylcholine receptor; anti-MuSK: Anti-muscle specific kinase; CMAP: Compound muscle action potentials; GVHD: Graft-versus-host disease; HLA: Human leukocyte antigen; HSCT: Hematopoietic stem cell transplantation; MG: Myasthenia gravis; mPSL: Methylprednisolone; Tregs: Regulatory T cells; VC: Vital capacity

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Authors’ contributions
MF, TM, HO: research project conception, organization, execution and writing of the first draft. SS, KI, SK, IS, MS, TS, AH, HT, ASN, KO, YS, HO, and YT: research execution, manuscript review and critique. TI and YU: research project conception, organization, execution and writing. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Written informed consent was obtained from the wife of the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the editor of this journal.

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Not applicable.

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