Case report

Memory B cell resurgence requires repeated rituximab in myasthenia gravis

Kohei Muto a, Naoko Matsui a,*, Yuki Una a, Waka Sakai a, Shotaro Haji a, Kengo Udaka b, Hirokazu Miki c, Takahiro Furukawa a, Masahiro Abe b, Ryuji Kaji a

a Department of Clinical Neuroscience, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan
b Department of Hematology, Endocrinology and Metabolism, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan
c Division of Transfusion Medicine and Cell Therapy, Tokushima University Hospital, Tokushima, Japan

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Abstract

The immunologic effects of rituximab (RTX) in myasthenia gravis (MG) remain to be explored. We aimed to clarify immunologic reactions and their association with response to RTX in MG. Regulatory T cell and B cell profiles of MG patients were monitored. Two patients presenting with generalized MG with anti-acetylcholine receptor antibodies were treated with RTX. The treatment led to sustained clinical improvement, discontinuation of intravenous immunoglobulin or plasma exchange, and reduction of prednisolone and other drugs. One patient was in remission for more than one year, whereas the other patient exhibited deterioration of symptoms within one year. Disease activity was associated with the repopulation of IgD−CD27− and IgD−CD27+ memory B cells. Clinicians should be aware of the possibility that MG ranges in the duration of B cell depletion and additional RTX should be prescribed upon resurgence of memory B cells.

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1. Introduction

Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction that is characterized by fluctuating skeletal muscle weakness [1]. Most cases are due to circulating antibodies to acetylcholine receptor (AChR) or, in cases without anti-AChR antibodies, antibodies to muscle-specific tyrosine kinase and low-density lipoprotein receptor-related protein 4 [2]. In addition to anticholinesterase drugs, thymectomy, immunosuppressive drugs, plasma exchange (PE), and intravenous immunoglobulin (IVIg) have been used for most patients with generalized MG [3,4]. When conventional immunomodulatory regimens are ineffective for moderate or severe generalized MG, rituximab (RTX) is considered [4,5]. RTX is a monoclonal antibody directed against the CD20 antigen of B cells and their precursors. RTX is a well-tolerated therapeutic option currently explored in lymphoma and several autoimmune-mediated diseases [6,7]. Recent studies showed response rates between 78% and 100%, concomitant reduction of corticosteroid or immunosuppressive drugs for MG treated with RTX [8–11]. It is assumed that RTX functions to remove B cells rather than autoantibodies. However, autoantibodies are produced by plasma cells that rarely express CD20, and are not eliminated by RTX. To explore the immunologic mechanism of RTX in MG, we examined the clinical response to treatment and monitored T and B cell profiles during the course of therapy.

2. Patients and methods

We administered RTX to two patients diagnosed with anti-AChR antibody positive MG. To assess response to RTX, we examined disease severity, anti-AChR antibody titer, treatment, and lymphocyte populations. Disease severity was graded according to the myasthenia gravis activities of daily living (MG-ADL) score and the Quantitative Myasthenia Gravis (QMG) score before and after RTX administration (at 1, 3, 6, 9, and 12 months). Peripheral blood was also sampled before and after RTX administration (at 1, 3, 6, 9, and 12 months). Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation of blood samples on a Ficoll-Hypaque (Axis-Shield PoC AS, Norway) after blood sampling. Stained samples were acquired on a FACSVerse flow cytometer (Becton Dickinson)

* Corresponding author. Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan.
E-mail address: naoko@tokushima-u.ac.jp (N. Matsui).
and data were analyzed using FlowJo software (Tree Star). Cells were analyzed after staining using forward-scatter and side-scatter signals to establish the lymphocyte gate, exclude propidium iodide (PI) + dead cells, and define T cells or B cells. All cells were also stained with labeled monoclonal antibodies. Regulatory T (Treg) cells were defined by CD25+CD127-low on CD4+ cells. B cells were stained with CD3 and CD20 or CD3, CD19, CD27, and IgD. The documentation of CD20+B cells or double negative (DN) memory B cells and switched memory (SM) B cells was determined by the complex expression (CD19+CD27−IgD− and CD19−IgD−CD27+) after gating out CD3+ cells. Informed consent was obtained from the patients in accordance with local ethics committee procedures.

3. Case reports

3.1. Case 1

A 40-year-old woman noticed left ptosis and chewing difficulty that persisted for two months prior to her first visit to our hospital. Neurological findings showed bilateral ptosis, diplopia, and fluctuating weakness of the neck and limbs. She also complained of difficulty in swallowing, chewing, and speaking. Neurophysiological examination showed a 24% decrease of compound muscle action potential amplitude on repetitive nerve stimulation (RNS) of abductor digiti minimi at 3 Hz. The edrophonium chloride test gave a markedly positive result. Serum anti-AChR antibody titer was 12 nmol/l (cutoff value <0.2). Chest computed tomography (CT) showed an abnormality in the mediastinum. The patient was diagnosed with generalized MG with thymoma. She was started on a daily dose of 15 mg ambenonium chloride. Histological analysis of the surgically resected thymoma revealed type B1. Prednisolone was started at a low dose and raised slowly to 25 mg daily. Immunosuppressive therapy was also initiated with 3 mg of tacrolimus daily. Her symptoms had been well controlled by oral corticosteroid and tacrolimus for approximately 20 years. She complained of stomach discomfort at age 59 and was diagnosed with gastric diffuse large B cell lymphoma by upper gastrointestinal endoscopy. Tacrolimus was discontinued and RTX was initiated at 375 mg/m2 weekly for four weeks. This was followed by three cycles of cyclophosphamide, vincristine, and prednisone combination chemotherapy. Thereafter, she underwent focal radiation and complete remission was achieved at age 60. Consequently, her MG symptoms stabilized for approximately two years. However, she developed dropped head, easy fatigability of the proximal extremities, and speaking difficulty at age 62. Anti-AChR antibody titer increased to 69 nmol/l. Repeated IVIg (0.4 g/kg/day for five days) was partially effective for a while. Gastric lymphoma had remained in remission, but because of failure of control of MG symptoms, RTX was administered weekly at 375 mg/m2 for four weeks followed by monthly at the same dose. After three months of RTX therapy (month 3), mild clinical benefits were observed. The patient reported less diplopia and improved chewing. In addition, the anti-AChR antibody titer decreased. After six months of RTX (month 6), easy fatigability was resolved. The dose of corticosteroids was returned to 20 mg every other day (Fig. 1). No symptom deterioration was observed for more than 18 months (data not shown).

The serum anti-AChR antibody titers showed a downward tendency associated with improved MG symptoms (Fig. 1). Following RTX therapy, Treg showed a mild upward trend. The number of circulating CD20+B cells was rapidly decreased and remained nearly undetectable during the entire course. CD20+B cell depletion could decrease anti-AChR antibody production. DN memory B cell population became dominant following RTX administration, but began to decrease at three months. SM B cells showed a long-lasting decrease after RTX administration (Fig. 2).

3.2. Case 2

A 46-year-old woman presented with a three-year history of fluctuating bilateral ptosis, diplopia, and easy fatigability of the proximal extremities. She consulted our hospital because of swallowing, chewing, and speaking difficulty. Physical examination showed bilateral ptosis, diplopia, mild dysarthria, swallowing and chewing difficulty, mild weakness of extremities, and respiratory symptoms. All symptoms were exacerbated during the day and on physical exercise. RNS showed no overt decreasing pattern, but single-fiber electromyography revealed...
Fig. 2. Time-series data of T and B cell profiles. (A) [left] Representative CD25$^+$CD127$^{\text{low}}$ (Treg) gated on CD4$^+$ cells, CD20$^+$CD3$^-$ B cells, and IgD$^-$CD27$^+$ switched memory (SM) B cells or IgD$^-$CD27$^-$ double negative (DN) B cells gated on CD3$^-$CD19$^+$ cells. All dot plot data were obtained from Case 1. (B) [right] Line plots represent time courses of Treg, CD20$^+$ B, DNB, and SMB cells.
blockage of extensor digitorum communis. The edrophonium chloride test gave positive results. A high anti-AChR antibody titer was detected (2700 nmol/l). Chest CT did not show any abnormalities. The patient was diagnosed with generalized MG without thymoma.

She was prescribed a daily dose of 180 mg pyridostigmine. Prednisolone was initiated and the dose was gradually increased to 40 mg/day. After three months, her symptoms did not improve, so she was prescribed a five-day course of PE followed by thymectomy, the histology of which showed normal thymic tissue. Because generalized weakness and bulbar symptoms in particular were exacerbated after the thymectomy, PE was started following IVIg (0.4 g/kg/day for five days). One week after IVIg, her symptoms gradually improved. Immunosuppressive therapy was initiated with 250 mg cyclosporine daily. Improvement after PE or IVIg lasted only for 3–4 months, and generalized weakness worsened again. She was prescribed PE and 3–4 courses of IVIg every year, but was unable to tolerate the medication because of headache or gastrointestinal symptoms. RTX was administered intravenously on a weekly schedule for four weeks at the dose of 375 mg/m², followed by a monthly schedule for two months at the same dose. No side effects were detected during or after RTX infusion. After one month of RTX therapy, she reported less dyspnea. Cyclosporine was reduced from 250 mg/day to 200 mg/day. After three months of RTX therapy, fatigue was improved, but the AChR antibody titer did not show obvious decrease. Nevertheless, the patient no longer required PE or IVIg. MG symptoms improved slightly but worsened again after nine months of RTX therapy. After 12 months of RTX therapy, because of exacerbation of MG symptoms, PE was administered. The anti-AChR antibody titers mildly decreased after three months, but gradually increased after nine months. Following RTX therapy, the proportion of Treg cells was unchanged. Circulating CD20⁺ B cells were nearly undetectable after treatment, but returned to the level before treatment after 12 months. Interestingly, DN memory B cell population increased after RTX therapy and remained high compared to that before the therapy during the entire course. SM B cell population gradually decreased following RTX therapy, but tended to increase after six months. MG symptoms improved after PE rapidly. However, additional RTX (a monthly schedule for three months at the same dose) was administrated again. After that, circulating CD20⁺ B cells were undetectable and memory B cells decreased again (data not shown).

4. Discussion

We report two anti-AChR antibody positive MG patients who were prescribed RTX in combination with standard therapy. One patient showed excellent and long-term response to RTX therapy. The other patient showed partial response to RTX with substantial reduction of immunosuppressive drug and discontinuation of IVIg, but clinical improvement did not continue for 12 months. We found that clinical improvement corresponded to CD20⁺ B cell depletion and memory B cell recovery was associated with exacerbation of symptoms.

Prior to RTX therapy, both patients had been taking prednisolone and immunosuppressants, and undergoing repeated PE or IVIg therapy. Similar to previous reports, sustained clinical improvement was observed in both patients, and conventional immunotherapies could be reduced [10].

RTX dose varied among studies. The standard dosage regimen is (1) 1 g infusion in two divided doses, two weeks apart; (2) 375 mg/m² weekly infusion for four weeks; and (3) 375 mg/m² weekly infusion for four weeks, followed by monthly infusion for two months [8–10]. We adopted standard dosage regimen (3), but the dosage in Case 1 was 375 mg/m² weekly infusion for four weeks, followed by once a month infusion because of the past history of chemotherapy combined with RTX. There is the possibility that a difference in the dosing in both cases might influence the overall clinical course. Both patients did not show common adverse events directly related to the infusions (flushing, pruritus, chills, or rigors) [4]. Clinical improvement was reported 4–18 months following RTX therapy [12]. Case 1 patient showed remission for more than 18 months (data not shown), whereas Case 2 patient presented with exacerbated MG symptoms within 12 months.

B cell targeted therapy has been recently considered a therapeutic option for autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis [13,14]. CD20 antigen expression is restricted to the late pre-B cell stage and is maintained until B cell differentiation into plasma cells. Thus, RTX is assumed to act by depleting the precursors of plasma cells, although the exact mechanism remains a question [6]. It is well known that the effect of immunosuppressive therapy in MG is partly related to the increase in circulating Treg cell population [15]. Case 1 patient showed a little increase of Treg cell population after 6 months of RTX. This was in line with the previous study showing Treg cell population was increased in MG after immunotherapy.

Previous studies have used peripheral B cell count as a marker for RTX retreatment and the reduction of potential side effects [16,17]. In this series, we monitored the circulation of CD20⁺ B cells as well as other B cell subsets. Human IgD CD27⁻ DN memory B cells typically account for less than 5% of the CD19⁺ population in healthy subjects, but both cases showed increased pretreatment DN memory B cell population that could become IgD CD27⁺ SM B cells potentially [18,19]. Human SM B cells contain precursors of antibody-producing cells, such as plasmablasts or plasma cells [20–22]. In all human disease states treated with RTX, rapid depletion of circulating B cell population occurs and B cell depletion lasts for 12 months on average in peripheral blood, after which a new ontogeny repopulates the B cell pool [6].

Although there was virtually complete elimination of CD20⁺ B cells in both cases, the decrease of the anti-AChR antibody titer and clinical improvement were limited in Case 2 patient. This limitation might be influenced by the difficulty of increase of immunosuppressive therapy by various side effects (headache, nausea, and glaucoma). One of the reasons for the deterioration of symptoms within 12 months was that the DN memory B cell population increased compared to that before treatment. Another reason was that the SM B cell population
slightly increased six months after RTX initiation. Taken together, our results are consistent with the previous observation that a rapid resurgence of memory B cells portends a poor outcome in autoimmune diseases [23,24]. Case 1 patient presented with a prolonged reduction of anti-AChR antibody, but the decrement of anti-AChR antibody in Case 2 patient was limited to the first month after treatment. The increment of CD20+ and memory B cells in Case 2 patient can be related to a worsening of symptoms. There is no generally accepted RTX schedule in MG, but we should consider repeated RTX by monitoring memory B cells to prevent deterioration of symptoms.

In conclusion, clinical improvement was correlated with the reduction of anti-AChR antibody titer. Repeated RTX therapy should be considered in cases with resurgence of memory B cells as remission does not last.

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References
