LETTERS TO THE EDITOR

PROLONGED B-CELL DEPLETION IN MuSK MYASTHENIA GRAVIS FOLLOWING RITUXIMAB TREATMENT

Rituximab (RTX) therapy is used increasingly to manage myasthenia gravis (MG) patients, and case series have shown particular benefit for those MG patients who have antibodies to muscle-specific kinase (MuSK-MG).[1] We report a MuSK-MG patient with persistent and severe B-cell depletion 3 years after receiving RTX.

A 62-year-old woman developed ptosis, bulbar symptoms, and weight loss in 2000. In 2005, she developed hypercarbic respiratory failure and was eventually diagnosed with MuSK-MG. She had 3 additional hospitalizations for exacerbations, including 1 for crisis, and required bilevel positive airway pressure (BiPAP) at night. An initial excellent response to therapeutic plasma exchange waned over time. Intravenous immunoglobulin, azathioprine, and prednisone did not provide significant improvement. In 2010, she was treated with 6 doses of 1000 mg RTX over approximately 8 weeks (578 mg/m^2 per infusion) and remained on prednisone and azathioprine. Following the RTX infusions she had a tenuous course and later developed progressive respiratory insufficiency and required nocturnal BiPAP. Chest computed tomography (CT) showed no evidence of thymoma.

Blood biomarker samples were drawn 34 months after RTX treatment, at which time she required supplemental oxygen. Clinical evaluation demonstrated persistent oculobulbar and facial weakness and disability (MG-composite: 23; MG-Manual Muscle Testing: 23; MG-Quality of Life-15: 30).[2–5] We measured B-cells by flow cytometry after staining for CD19 and identified helper and cytotoxic T cells by CD4 and CD8 expression, respectively.

Polychromatic flow cytometry demonstrated 49.5% T-helper and 44.7% cytotoxic T-cells. Only 0.06% of lymphocytes were $CD19^+$ B-cells; naive, memory, and plasma cell subpopulations were undetectable (Fig. 1). Repeat B-cell markers performed over 36 months after her initial RTX treatment continued to show profound B-cell depletion with $<1\%$ $CD19^+$ B-cells. Creatine kinase and thyroid profile were normal. She had experienced no serious infections.

After depletion with RTX, B-cell populations typically recover within 12 months.[6] This MuSK-MG patient had profound, prolonged B-cell depletion 3 years after receiving RTX. It has been observed that recovery of B-cell populations begins with naive B-cells, and memory B-cell regeneration may be delayed.[7] However, in patients with autoimmune disease, such prolonged B-cell depletion after RTX has only been reported in 2 systemic lupus erythematosus patients, both of whom were given RTX in combination with cyclophosphamide.[8] In these cases, B-cells remained low 5–7 years after RTX therapy. The underlying mechanism and the effect of concomitant cyclophosphamide therapy on the risk of developing prolonged B-cell depletion with RTX are uncertain. In our case, it is also unclear how the unconventional RTX dosing regimen, combined with other immunosuppressive drugs, may have affected B-cell recovery. Due to poor disease control, our patient continued to receive azathioprine and varying doses of prednisone, raising the risk for serious infections.[9] As the use of RTX for neurologic diseases increases, clinicians need to be aware of the possibility of prolonged B-cell depletion, particularly when combined with other immunosuppressive agents.

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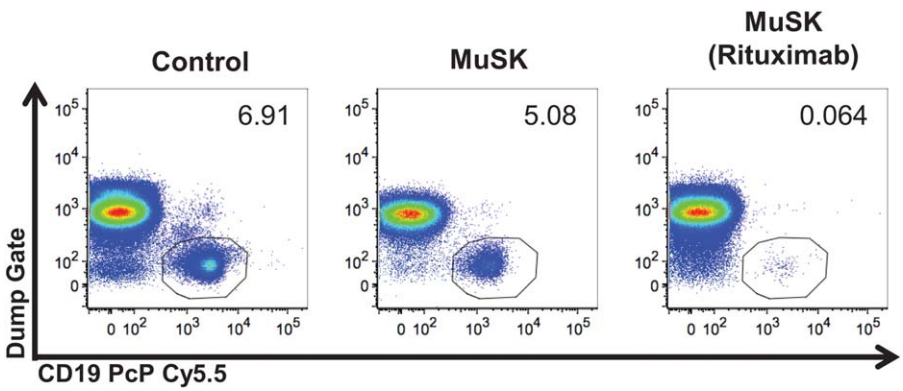

FIGURE 1. Prolonged B-cell depletion 34 months after rituximab treatment. Peripheral blood mononuclear cells from a healthy control, a patient with MuSK-MG, and our patient with MuSK-MG treated with rituximab were surface stained with CD19 PcP Cy5.5 conjugate. To isolate lymphocytes, a dump channel stained for CD3, CD14, and CD16 Pacific Blue along with LIVE/DEAD dye conjugated to the same fluorophore was used to gate out macrophages, neutrophils, natural killer cells, dendritic cells, and dead cells. Circles indicate B-cell populations. The logarithmic scales on the X- and Y-axes represent the fluorescence intensity, and the numbers in the flow plot represent the frequency of $CD19^+$ B-cells among all lymphocytes in the peripheral blood. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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R521C MUTATION IN THE *FUS/TLS* GENE PRESENTING AS JUVENILE ONSET FLAIL LEG SYNDROME

A 24-year-old man presented with painless rapidly progressive lower limb weakness, more severe on the right and more severe distally, resulting in paraplegia within 8 months. His weight dropped from 80 to 70 kg in the absence of bulbar symptoms (e.g., dysphagia or choking episodes). He had no back pain, cramps, dyspnea, or sphincter symptoms. There was no family history suggestive of neuromuscular disease. Examination showed almost complete paraplegia with severe diffuse lower limb atrophy, but without fasciculations. Deep tendon reflexes in the legs were absent and plantar reflexes were downgoing. Strength and deep tendon reflexes in the upper limbs were normal. Cranial nerve and sensory examination findings were normal. There was no cognitive impairment.

Elevated creatine kinase (CK) levels (up to 1408 IU/L) before needle electromyography (EMG) were present and were confirmed 1 month later. Motor nerve conduction studies showed absent tibial and fibular motor compound muscle action potentials bilaterally and were normal in the upper limbs. Sensory nerve studies were normal in all 4 limbs. EMG showed abundant fibrillation potentials at rest and decreased recruitment in L3 to S1 myotomes bilaterally. EMG was normal in the upper limbs and in the rectus abdominis, thoracic paraspinal, sternocleidomastoid, genioglossus, and masseter muscles. Brain and total spine MRI were normal. Cerebrospinal fluid analysis was normal. Right quadriceps muscle biopsy revealed small angulated fibers and fiber type grouping, consistent with a neurogenic process. No mutations were found in *SMN1*, *SMN2*, or androgen receptor genes. At 10 months after onset, deep tendon reflexes were brisk in the upper limbs, and there was no other change. EMG showed the previous findings plus acute denervation in the left deltoid muscle. Screening for ALS genes, including *C9orf72*, *SOD1*, *TDP43*, *FUS*, *Ataxin 2*, *Optineurin*, and *Ubiquitin*, identified a R521C missense mutation in the *FUS* gene. The patient's relatives declined genetic testing.

Mutations in the *FUS* gene cause autosomal dominantly inherited ALS. *FUS* mutations account for approximately 4% of familial ALS cases and less than 1% of sporadic ALS cases, making them the fourth most common cause of familial ALS after *C9orf72*, *SOD1*, and *TDP43* mutations.[1] *FUS* mutations are associated with earlier symptom onset (mean age 44 years) and shorter disease survival (mean, 3.4 years) than is typical for ALS.[2] *FUS* mutations are also found in aggressive forms of ALS that begin before age 25 years, termed juvenile-onset ALS. The R521C missense mutation is the most frequent *FUS* mutation. It is often associated with