AB010. OS02.04. Characterization of autoantibody in thymoma with myasthenia gravis by single-cell sequencing of B-cells

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Abstract: Thymomas are frequently associated with paraneoplastic autoimmune syndromes, with the most common being myasthenia gravis (MG). MG is characterized by autoantibodies against muscle antigens, most frequently the acetylcholine receptor (AChR). Patients with thymoma also present with autoantibodies against striational muscle proteins (STR-Abs), such as the sarcomeric protein titin and the ryanodine receptor. These autoantibodies have been primarily regarded as diagnostic or prognostic markers, but little is known about their pathological mechanisms. Comprehensive mechanistic studies have been hindered by the lack of patient-derived monoclonal antibodies (mAbs). Such mAbs could help to define immunogenic epitopes in known or novel autoantigens, and would be useful for deciphering pathological mechanisms *in vitro* or in animal models. Therefore, we studied mAbs derived from a patient with thymoma and MG, with the patient's written informed consent and under a Stanford IRB approved protocol. The patient had Masaoka-Koga stage II type B2 thymoma, with multiple recurrences over a period of 8 years. The patient's MG symptoms included fatigable muscle weakness, the presence of anti-AChR antibodies, and high titer STR-Abs. The patient also had myositis with muscle-related symptoms worsening after thymectomy. We sequenced the repertoire of the patient's plasmablasts, which are antibody-producing cells derived from the activated B-cell clones, using a barcode-based method for sequencing single-cell immunoglobulin genes developed in our lab. We then expressed 26 mAbs from clonally expanded families of antibodies from two different timepoints that are 6 months apart. The first timepoint was 2 years post-Rituximab, coinciding with a tumor recurrence and slow progression of muscle weakness. The second timepoint was a month after radiotherapy when the patient was admitted with severe muscle weakness and pain. Treatment included plasmapheresis/intravenous immunoglobulin (IVIG) and Rituximab, with limited improvement over the weeks following hospitalization. The patient was on steroids at both timepoints. Anti-Titin serum antibody titers increased by 60% between these two timepoints. Two of the mAbs that were expressed reacted with the main immunogenic region of titin in enzyme-linked immunosorbent assay (ELISA), and one of the clones was present at both of the timepoints investigated. These clones were detected despite B-cell depletion by treatment with Rituximab. Our results suggest that (I) sequencing single-cell immunoglobulins is a powerful technique for isolating and functionally characterizing mAbs against autoantigens in thymoma and that (II) persisting or recurring autoreactive clones in patients with thymoma, such as anti-titin clones, may be associated with refractory paraneoplastic syndromes (PNS) despite use of immunosuppressive therapies.

Keywords: Paraneoplastic syndromes (PNS); autoimmunity; myositis; myasthenia gravis (MG)

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