Autoantibodies in Thymoma-Associated Myasthenia Gravis With Myositis or Neuromyotonia

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Background: About 50% of patients with thymoma have paraneoplastic myasthenia gravis (MG). Myositis and myocarditis (NMT) will also develop in some. Patients with thymoma-associated MG produce autoantibodies to a variety of neuromuscular antigens, particularly acetylcholine receptor (AChR), titin, skeletal muscle calcium release channel (ryanodine receptor [RyR]), and voltage-gated potassium channels (VGKC).

Objective: To examine whether neuromuscular autoantibodies in patients with thymoma correlate with specific clinical syndromes.

Methods: Serum and plasma samples from 19 patients with thymoma-associated MG, of whom 5 had myositis and 6 had NMT, underwent testing for antibodies to AChR, titin, RyR, and VGKC.

Results: Antibodies to AChR and titin were found in 19 and 17 patients, respectively. Antibodies to RyR correlated with the presence of myositis ($P = .03$); they were found in all 5 patients with myositis and in only 1 patient with NMT, but also in 4 of 8 patients with neither disease. Antibodies to VGKC were found in 4 patients with NMT, 1 of 3 patients undergoing testing for myositis, and 2 of 7 patients undergoing testing with neither comorbidity. Presence of RyR antibodies correlated with high levels of titin antibodies.

Conclusions: The results appear to distinguish partially between 3 groups of patients with thymoma-associated MG: the first with RyR antibodies and myositis or myocarditis, the second with NMT without RyR antibodies, and the third without RyR antibodies, myositis, or NMT. Differences in the thymoma may underlie these pathologic associations.

Arch Neurol. 2000;57:527-531

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**PATIENTS AND METHODS**

**PATIENTS**

Serum or plasma samples from 19 patients with thymoma and combinations of MG and other neuromuscular disorders were available. Ten patients were from Norway; 5 patients, England; 2 patients, Germany; and 2 patients, the United States. Thymomas were classified histologically according to Kirchner and Muller-Hermelink. Clinical features of the patients are shown in Table 1. Five patients had MG and myositis and/or myocarditis. One of these patients has been described previously as having rippling muscle disease with electrically silent muscle cramps, in addition to myositis and MG. The myositis diagnosis was based on clinical symptoms, elevated serum creatine kinase levels, EMG findings, and results of muscle biopsy. The myocarditis diagnosis was based on cardiac symptoms without any other cause and typical electrocardiography findings. The diagnosis was verified by postmortem examination in 2 of 3 patients with myocarditis. Six patients had NMT as well as MG. The NMT diagnosis was based on clinical criteria and EMG findings. Eight patients with MG, but no signs of other neuromuscular disorders, were selected randomly from the serum bank of thymoma-associated MG at the University of Bergen, Bergen, Norway. Serum and plasma samples were stored at −60°C until use.

**ANTIBODY ASSAYS**

Each assay was performed on all the serum samples together. Antibodies to AChR were determined by immunoprecipitation of iodine 125 (125I)-α-bungarotoxin–labeled human AChR, and values were given as nanomoles of 125I-α-bungarotoxin–binding sites precipitated per liter of serum as previously described.

Antibodies to VGKC were determined as previously described by immunoprecipitation of 125I-α-dendrotoxin–labeled extracts of human frontal cortex, and values were given as picomoles per liter. The upper limit of normal was 100 pmol/L (mean + 3 SD among 6 laboratory control samples, 6 samples with Lambert-Eaton myasthenic syndrome [LEMS], and 3 samples with MG without apparent NMT; none of them were positive for the presence of VGKC antibodies).

Antibodies to titin were measured by means of enzyme-linked immunosorbent assay using a recombinant titin antigen (MG130) as previously described. Results are given as optical density (OD) values referring to the absorbance of test serum samples diluted 1:200, the serum dilution giving the best discrimination between samples with a selection of positive and negative findings. The upper limit of normal was OD value 60 (the mean + 3 SD among 20 serum samples from blood donors).

Antibodies to RyR were measured by means of Western blotting with purified RyR. Terminal cisternae of sarcoplasmic reticulum from rabbit skeletal muscle were purified by means of sucrose gradient centrifugation. The terminal cisternae fractions were electrophoresed on sodium dodecyl sulfate polyacrylamide gels and transblotted onto nitrocellulose. The blots were blocked for 60 minutes with 5% dry milk in phosphate-buffered saline solution; incubated for 120 minutes with MG serum samples diluted to 1:50; incubated for 60 minutes with peroxidase-conjugated rabbit antibodies to human IgG diluted 1:500 (Dako, Copenhagen, Denmark); and color developed with 4-chloro-1-naphotol as previously described. Serum samples staining the RyR protein band in the terminal cisternae fraction were considered positive for anti–RyR antibodies.

**STATISTICS**

Correlations between presence of antibodies and clinical syndromes were assessed by means of Fisher exact test. The t test was used to compare means. All analyses were performed using commercially available software (SPSS 8.0 for Windows; SPSS Inc, Chicago, Ill). P<.05 was considered significant.

**RESULTS**

Patients with thymoma-associated MG produce autoantibodies to a variety of neuromuscular antigens in addition to the AChR and VGKC antibodies, ie, antibodies to the skeletal muscle calcium (Ca²⁺) release channel (ryanodine receptor [RyR]) of sarcoplasmic reticulum that transmits signals from sarcolemma to contractile filaments, and antibodies to cytoplasmic filamentous proteins, particularly titin, or neurofilaments. Thyroid epithelial cells express epitopes shared by the target antigens for some of these antibodies. It is assumed that autoreactive T lymphocytes are generated in the thymic tumor and that they subsequently stimulate antibody production against various muscle antigens.

The incidence of these antibodies in the different clinical subgroups of thymoma-associated neurologic disorders has not been determined previously. We measured antibodies to AChR, VGKC, titin, and RyR in 19 patients with thymoma-associated MG, 11 of whom had associated myositis or NMT. The results suggest at least 3 subgroups of thymoma-associated MG with different spectrums of autoantibodies.

All 19 patients with thymoma-associated MG had antibodies to AChR, and 17 had antibodies to titin. The titers of AChR and titin antibodies did not correlate significantly with any of the clinical syndromes (Figure), but the titer of titin antibodies was significantly higher in patients with RyR antibodies than in those without such antibodies (Table 2).

Antibodies to RyR were detected in 10 patients: 5 patients had myositis; 1 patient, NMT; and 4 patients, no associated disorders (Figure). Patients with RyR antibodies had significantly higher frequency of myositis than patients without RyR antibodies (Table 2). One of the patients with myositis and RyR antibodies in whom VGKC antibodies were not detected also had electrically silent muscle cramps (patient 9, Table 1).

Antibodies to VGKC were detected in 7 of 16 patients with thymoma-associated MG undergoing testing: 4 of them had NMT, 1 of them had myositis, and 2
Thymoma-Associated MG and Other Associated Disorders

To determine whether antibodies to RyR or VGKC may be useful in distinguishing thymoma-associated MG from other MG syndromes, we studied sera from 19 patients with MG with thymoma and from 17 controls. The patients had MG for 1 to 20 years (mean, 10 years) at the time of diagnosis of the thymoma and had no previous thymectomy. The mean ± SD age at onset of neuromuscular symptoms was similar in the 3 syndromes: 52 ± 15 years in NMT, 46 ± 17 years in myositis, and 60 ± 10 years in MG.

Eleven of the thymomas were classified as cortical, 3 as invasive carcinomas, 2 as mixed thymomas, and 1 as an atypical carcinoid. Thymic histologic characteristics did not correlate significantly with the antibodies or clinical syndromes. One patient who had RyR and VGKC antibodies had a thymic carcinoma and MG without clinical evidence of NMT or myositis, but EMG and muscle biopsy were not performed to exclude these disorders (patient 1, Table 1). The atypical carcinoid was found in a patient with NMT who had VGKC, AChR, and titin antibodies, but no RyR antibodies (patient 17, Table 1).

On the basis of these results, 3 groups of thymoma-associated MG can be tentatively distinguished. The first is characterized by the presence of serum RyR antibodies and by association with myositis and/or myocarditis, and the second by the absence of RyR antibodies and by association with NMT. The third has no RyR antibodies, myositis, or neuromyotonia. Although numbers are small, it was striking that RyR and VGKC antibodies coexisted in only 2 patients.

Myasthenia gravis with myositis tends to be severe, with a poor response to resection of the thymoma. The association between RyR antibodies and inflammatory myopathy may, therefore, account for the previously observed relationship between RyR antibodies and severity of MG. These patients can deteriorate rapidly with life-threatening muscular weakness and cardiac failure shortly after thymectomy, and RyR antibodies may be useful as markers for such patients who may need to start immunosuppressive treatment before thymectomy.

It is not clear whether antibodies to RyR are pathogenic, but 1 patient with high titers of RyR antibodies (as judged by very strong staining in Western blots) and no detectable VGKC antibodies had rippling muscle disease. He complained of muscle spasms in the forearms, legs, back, and neck, especially when the muscles were stretched after rest. Spontaneous muscle contractions could be activated by percussion and were seen to spread over the muscle surface. The EMG showed the muscle contractions to be electrically silent (distinguishing the condition from NMT), indicating contraction of the myofilaments without depolarization of the myofiber surface membrane. Spontaneous release of calcium from sarcoplasmic reticulum into myoplasm is a possible mechanism for induction of electrically silent muscle cramps. Since the RyR forms the Ca2+ release channel of sarcoplasmic reticulum, it is possible that the rippling muscle disease in our patient was caused by RyR antibody–mediated intracellular Ca2+ release. It has been shown that RyR antibodies from patients with MG affect RyR channel opening in vitro. To have functional effect on intracellular Ca2+ release in vivo, RyR antibodies must get access into a sufficient number of viable muscle cells that are not yet damaged by inflammation. This could explain why the rippling phenomenon is so rare among patients with positive findings for RyR antibodies.

Titin antibodies are to a large extent synonymous with so-called striational antibodies. These antibodies, which were present in 17 of 19 patients, are sensitive markers for the presence of a thymoma. When titers of titin antibodies are especially high, it may indicate presence of RyR antibodies and myositis.

The presence of VGKC antibodies in 4 of 6 patients with NMT is consistent with the evidence that thymoma-associated NMT results from an antibody-mediated defect in peripheral motor nerve function, as previously reported in 6 patients without thymoma. Absence of detectable VGKC antibodies, as in 2 of the patients with NMT, was also reported in those without thymoma when measured by immunoprecipitation. However, VGKC antibodies were detected in all patients with NMT (including those with thymoma) using a molecular immunohistochemical approach. Antibodies to VGKC have not been detected in healthy controls, but they were found in 2 of 7 of our patients with thymoma-associated MG without clinical symptoms of NMT, suggesting that NMT may be latent in some of these patients.

The lack of overlap between RyR and VGKC antibodies in our patients suggests that the thymomas are of

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*MG indicates myasthenia gravis; ellipses, no data; and NMT, neuromyotonia. The male predominance in patients with neuromyotonia and myositis was not statistically significant.
†The mean ± SD age at onset of neuromuscular symptoms was similar in the 3 syndromes: 52 ± 15 years in NMT, 46 ± 17 years in myositis, and 60 ± 10 years in MG.
the following 3 different types: those with a tendency to induce autoimmunity against striated muscle proteins in addition to the neuromuscular synapse, those that are more apt to induce additional autoimmunity against nervous tissue antigens, and those with autoimmunity confined to the AChR. This is supported by different reports of patients with thymoma-associated myasthenia gravis (MG) with myositis or myocarditis, neuromyotonia (NMT), or no associated disorders (NAD). Dashed lines indicate upper normal values (0.3 nmol/L for AChR antibodies, 100 pmol/L for VGKC antibodies, and optical density [OD] value of 60 for titin antibodies).

The best characterized paraneoplastic disorder is LEMS, which is caused by antibodies to voltage-gated Ca²⁺ channels, and elimination of the antigen by tumor resection can lead to clinical improvement, the immune response in LEMS almost certainly is provoked by the tumor antigens. By contrast, thymomas do not express intact AChR, and the thymoma epitopes shared by neuromuscular antigens do not bind patients’ antibodies. Furthermore, thymoma-associated MG often responds poorly to resection of the tumor. The primary event(s) that lead to AChR antibodies in thymoma-associated MG might also trigger the production of titin antibodies, since some titin epitopes are expressed in thymomas; subsequent events or other factors presumably underlie the spreading of the immune response to involve RyRs with associated myositis, or neuronal antigens with associated NMT. It will be interesting to see whether the response to treatment differs between these subgroups.

Accepted for publication November 8, 1999.

We thank R. W. C. Janzen, MD, for contributing with clinical information on the German patients.

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REFERENCES


