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Myasthenia gravis: clinical, immunological, and therapeutic advances

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We give an update on clinical, immunological, and therapeutic advances in the field of myasthenia gravis, including a summary of suggested therapeutic recommendations.

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Clinical characteristics

Myasthenia gravis (MG) is an autoimmune disease characterized by a fluctuating pathological weakness with remissions and exacerbations involving one or several skeletal muscle groups, mainly caused by antibodies to the acetylcholine receptor (AChR) at the post-synaptic site of the neuromuscular junction. MG has a prevalence of 85–125 per million, and an annual incidence of 2–4 per million. The disease has two peaks: one between 20 and 40 years dominated by women; and the other between 60 and 80 years equally shared by men and women (1).

Ocular symptoms with diplopia and ptosis occur early in the majority of patients, and can be the only manifestation of the disease. In 85% of the patients, MG becomes generalized, usually within 3 years, affecting limbs – especially the proximal parts, axial muscular groups such as the neck muscles, and facial and bulbar musculature causing loss of facial expression, speech difficulties, and chewing and swallowing problems. Typically, the muscular weakness increases during exercise. When the respiratory muscles are affected, MG becomes life threatening and the patient may enter an MG crisis, requiring intensive care including mechanical ventilation (2). Death due to respiratory failure or cardiopulmonary complications can be the end result of such crisis, but is very rare today (3). The prognosis of MG is generally good: in a Danish population-based survival study, the overall 3, 5, 10, and 20 years survival was 85, 81, 69, and 63%, respectively (4). MG-related crisis occurs with an annual incidence of 2.5% among MG patients (5). This development is caused by improved pharmacological treatment, plasmapheresis, thymectomy, and a dramatically improved critical illness management.

Subgroups of MG

MG is a heterogeneous disease, and is classified into different subgroups (6–9).

The ocular MG subgroup includes AChR antibody positive, non-thymoma MG with purely ocular (non-generalized) symptoms. Ocular MG occurs at any age but is more common in children and in late-onset males (1). HLA-DQ6 has shown some association with ocular MG in Asian infants (10).

The early onset MG subgroup includes AChR antibody positive, non-thymoma, generalized MG with onset of MG before the age of 50 years. Thymus hyperplasia is predominant in this subgroup. This is the largest subgroup consisting of about 65% of all MG patients. These patients are predominantly females (male/female ratio: 1:4) in their second or third decade of life (11, 12). The serum concentration of AChR antibodies is generally high, and titin and ryanodine receptor (RyR) muscle antibodies occur only very rarely (8, 13, 14). These patients have a high frequency of concomitant autoimmune diseases (1, 15). There is a strong positive correlation with several HLA genotypes: in

Caucasians HLA A1, B8, DQB1, DR3, DR52a (16–20); in Japanese HLA DPB1, DQB1, DR9 (21, 22).

The late-onset MG subgroup includes AChR antibody positive, non-thymoma, generalized MG with onset of MG at 50 years or later. Thymus atrophy is predominant in this subgroup (23). The occurrence of late-onset MG is equal in men and women and with a peak between 70 and 80 years (1, 12). The concentration of AChR antibodies is usually lower than in the early onset subgroup (8). One half of the late-onset MG patients have titin and RyR antibodies (8). There is a weak association with HLA-A3, B7 (6), DR2, HLA-DR4 (24), and in titin antibody positive patients HLA-DR7 (19).

The thymoma MG subgroup contains MG patients with thymoma regardless of the extent of muscular involvement. These patients usually have AChR antibodies. Thymoma occurs in about 15% of MG patients, and it is predominantly of the cortical type. Thymoma MG occurs at any age with a peak of onset around 50 years, and this subgroup occurs equally frequently in males and females. In addition to AChR antibodies, there is a frequent occurrence of titin and RyR antibodies in thymoma MG patients (8, 14, 25). The presence of a thymoma does not give a more severe MG. Thymoma and non-thymoma MG patients have similar MG long-term prognosis (26–28). HLA DR2 has been found to be associated with thymoma mostly in women (24).

Seronegative MG patients are AChR antibody negative with no evidence of thymoma, regardless of the extent of muscular involvement and age at MG onset. There are reports indicating a difference in pathogenesis between AChR antibody positive and negative MG, including the occurrence of muscle specific kinase (MuSK) antibodies in 10–40% of AChR antibody negative MG patients (29–31). Seronegative MG patients lacking MuSK antibodies appear to have less severe MG than seropositive MG patients (32). Seronegative MG patients lacking MuSK antibodies may have circulating pathogenic antibodies against muscle antigens, but these antibodies remain to be identified.

Immunological aspects

Autoimmunity and pathogenesis

In 1960, John Simpson proposed that MG is an autoimmune disease caused by an autoimmune attack on the motor endplate. Patrick and Lindstrom (33) demonstrated that this autoimmune attack was caused by antibodies to the AChR at the post-synaptic site of the motor endplate. This was confirmed in MG patients by Almon et al. (34), who found that the majority of MG patients had in their sera AChR antibodies. The AChR antibodies are found in about 85% of the patients and with very high specificity for MG (35). The AChR is undoubtedly the main target for the autoimmune reaction in MG. The cellular autoimmune response is the initial step in the pathophysiology of MG, underlying a subsequent humoral autoimmune response (36, 37). Humoral autoimmunity in MG involves both AChR and non-AChR antibodies. Inflammatory myopathy may occur in some MG patients, and it has been assumed that this could be the result of polymyositis as an expression of a more widespread defect in immune regulation (38). EMG-confirmed myopathy in MG patients has been found to correlate with the occurrence of titin and RyR antibodies (39).

Antigens and antibodies in MG

The AChR is a transmembrane glycoprotein located at the postsynaptic site of the neuromuscular junction, consisting of five subunits (α–ε) (40). The main immunogenic region (MIR) for the AChR antibodies is located on the α-subunit (35). AChR antibodies impair the neuromuscular transmission by complement-mediated focal muscle membrane damage, accelerated degradation of AChR, and also direct blockade of AChR ligand binding (35, 41). The serum concentration of complement components C3 and C4 is low in MG patients with high AChR antibody concentration, further supporting the involvement of the complement system in the *in vivo* pathophysiology of AChR antibody positive MG (42). AChR antibodies are present in the sera of more than 85% of MG patients with generalized MG, and in 70% of patients with ocular MG, and are found only very infrequently in healthy individuals (35). AChR antibodies are polyclonal, mainly IgG, and of the IgG1 and IgG3 subclasses (43). The AChR antibody concentration does not correlate with MG severity in an MG population (7, 8).

Strauss et al. (44) detected antibodies that bind in a cross-striational pattern (striational antibodies) to skeletal and heart muscle tissue sections in sera from 30% of MG patients. van der Geld and Strauss (45) found that these antibodies are mainly present in sera from MG patients with thymoma and late-onset MG. Aarli et al. (46) showed that nearly all MG patients with thymoma have antibodies to an antigen extracted by citric acid from skeletal muscle, and these antibodies have been found to react not only with skeletal muscle but also with the neoplastic epithelial cells

of lymphoepithelial thymomas, indicating a role in the pathogenesis of MG (47). Striational antibodies and citric acid extract antibodies were later found to include antibodies against several muscle antigens, the best defined being titin and RyR.

Titin is a giant protein (3000 kDa) and is the third most abundant protein in the skeletal and cardiac sarcomere (48). The MIR of titin is called myasthenia gravis titin-30 (MGT-30) and is situated near the A/I band junction (13).

The RyR is a calcium release channel located in the sarcoplasmic reticulum. There are two forms of RyR, skeletal (RyR1) and cardiac (RyR2). RyR antibodies from MG patients react with both. The MIR is included in the pc2 Ry1 (peptide chain 2 and RyR type 1) fusion protein and is located close to the N-terminus of the RyR (49). Some sera include antibodies also against a more centrally located RyR region called peptide chain 25 (pc25). Titin and RyR antibodies occur more often in severe MG (50), and are capable of complement activation *in vitro* (42).

MuSK is expressed at the neuromuscular junction (29). Up to 41% of AChR antibody negative patients with generalized MG have serum autoantibodies against MuSK. Whether MuSK antibodies are involved in the pathogenesis of AChR antibody negative MG, or their presence is merely an epiphenomina is unclear yet (29, 31). The frequency of MuSK-associated MG seems to show variation between different populations, and being especially low in Scandinavia. MuSK antibodies may correlate with MG severity in AChR antibody negative MG (30). MuSK antibodies have been reported in AChR antibody positive thymoma MG in one study (51). The clinical role of MuSK antibodies in MG is still to be established.

Cultured, dissociated thymic lymphocytes from MG patients secrete monoclonal striational antibodies that bind to skeletal muscle myosin, α-actinin, and actin (52). Thymoma MG patients have higher titers of anti-myosin and anti-actomyosin antibodies than MG patients without thymoma (53, 54).

Antibodies against rapsyn (a 43-kDa postsynaptic protein essential for anchoring and clustering AChR) have been identified in MG, but are also found in sera from patients with lupus and chronic procainamide-associated myopathy (9).

Thymus and thymoma in MG

In thymoma MG, the initial steps in the triggering of humoral immunity take place inside the thymmoma (16, 55). In 50–60% of MG patients, especially in those with MG debut before 40–50 years of age, the thymus is hyperplastic. The colonization of the perivascular space of the thymic medulla with B-cells and germinal centers is similar to the structure found in lymph nodes (16). Older patients usually have atrophic thymuses. Few germinal centers are present in some of them (23).

Thymoma is a neoplasm derived from thymic epithelial cells and characterized by the presence of non-neoplastic T cells (56). Thymomas, especially those of cortical type, are associated with MG in about 50% of the cases (57). The presence of muscle-like epitopes on thymoma cells has been demonstrated (47). MG-associated thymomas are enriched in AChR-like epitopes (55, 57, 58) and AChR-specific T cells (18, 55). In addition, titin and RyR epitopes have been identified in the thymoma (14, 59), and they occur along with co-stimulatory molecules on antigen-presenting cells (60). There is therefore good reason to believe that autosensitization in thymoma-associated MG takes place inside the thymoma, and that thymoma-associated MG is a true paraneoplastic disease (42, 59, 61). T cells are autosensitized to muscle-like epitopes presented on the neoplastic epithelial cells of the thymoma (47, 55, 58). Autosensitized T cells are normally eliminated or functionally depressed by clonal deletion and anergy (62), but an altered microenvironment inside the thymus provoked by the neoplasm causes a failure of the elimination mechanisms, resulting in a subsequent autoantibody production.

Etiology

There is a genetic predisposition for MG. Although MG is not only maternally inherited, 30% of MG patients have one maternal relative with MG or another autoimmune disorder, and the occurrence of other autoimmune diseases in MG patients is markedly elevated (1, 58). The genetic predisposition for MG involves the MHC class I and II regions (HLA associations with the different MG subgroups are discussed later) (11), the AChR-α-subunit (63), IgG heavy and light chain (64), FcγRII (65, 66), and TCR genes (67).

Although infection is postulated in the initiation of most autoimmune diseases through tissue damage, exposure of self antigen, and activation of self-reactive T cells that recognize homologous sequences of a microorganism through molecular mimicry, there is so far no clear evidence that microbial infections can cause chronic autoimmune disease (41, 68). However, antibodies cross-reactive with bacteria and herpes simplex virus have been demonstrated in the sera of MG patients (69). In

experimental autoimmune MG, antibodies to herpes simplex virus antigen cross-react with the AChR and are capable to induce MG (70).

The midsize neurofilament NF-M, which contains an AChR-like epitope and is present in thymoma-associated MG, is recognized by MG patient antibodies to the AChR-α subunit, suggesting that this neurofilament has a possible role in the etiology of MG. There is an increase in the number of NF-M receptors on T cells in MG patients with thymoma (58).

The diagnosis of MG

The diagnosis of MG is based on the history and typical clinical findings. It can be confirmed pharmacologically by the edrophonium (Tensilon®; Cambridge Laboratories, Newcastle, UK) test, where this acetylcholine esterase-inhibiting drug gives an immediate and reproducible improvement of the MG signs after intravenous administration, in 90% of the MG patients. However, positive tests have been reported in some patients with other nerve and muscle diseases (71). Failure of neuromuscular transmission in MG leads to a decremental response to repetitive nerve stimulation by electromyographical (EMG) examination in two-thirds of the MG patients (72). Increased jitter on single-fiber EMG (SFEMG) is even more sensitive than repetitive nerve stimulation, showing positive results in virtually all MG patients if the muscles most likely to be involved are examined (72). The jitter phenomenon can partly be explained by that the fibers contributing to the decrement are continuously blocked during voluntary contraction, and partly because smaller motor units explored by SFEMG are probably more abnormal in MG than the larger motor units contributing to the decrement (73). The diagnosis is also supported by the detection of AChR antibodies in the serum. This test is positive in about 85% of the patients and with very high specificity for MG (34). The absence of AChR antibodies in a patient with myasthenic symptoms may offer diagnostic challenges (74). Such patients should be examined for MuSK antibodies.

Grading the severity of MG is important when investigating and diagnosing MG. A number of grading systems has been developed to quantify the severity of MG symptoms and signs, useful in both clinical and research settings (1, 75, 76).

The final diagnosis of a thymoma in MG is made by histopathological examination post-surgery. However, the presence of titin/RyR antibodies in a young MG patient strongly suggests the presence of a thymoma. These antibodies and CT scan of the anterior mediastinum share a similar sensitivity for thymoma MG (8). MRI is an accurate instrument in detecting invasive thymoma both preoperatively and in postoperative follow-up (77).

Therapeutic aspects

Pharmacological treatment

Double-blinded placebo-controlled studies on the treatment of MG are rare, and most experience in this field is based on generally accepted clinical experience. Acetylcholinesterase inhibitors are the first pharmacological choice in the treatment of MG. Acetylcholinesterase is an acetylcholine-hydrolyzing enzyme which binds the overflowing acetylcholine in the neuromuscular junction, keeping the junction clean from excessive transmitter. Acetylcholinesterase inhibitors bind to the acetylcholinesterase, inhibiting its action (78). Physiostigmine, an acetylcholinesterase inhibitor, was introduced in 1934, and then the synthetic neostigmine was introduced. Pyridostigmine is a more recent long-acting reversible acetylcholinesterase inhibitor. Acetylcholinesterase inhibitors increase the amount of available acetylcholine in the neuromuscular junction. This leads to enhanced binding of acetylcholine to the diminished number of AChRs on the myasthenic muscle cell membrane, causing contractility improvement. All known acetylcholinesterase inhibitors are primarily targeted towards an active site shared by all acetylcholinesterases (78). Their side effects are therefore related to enhanced cholinergic activity in other tissues and organs than the skeletal muscles, causing bronchospasm, bradycardia, salivation, hidrosis, miosis, nausea, and diarrhea.

When additional pharmacological treatment is needed, immunosuppressive drugs are the second choice. These are corticosteroids, azathioprine, cyclophosphamide, cyclosporine, and methotrexate. While the steroid effect appears rapidly, the clinical effect of other immunosuppressants may take a few weeks and up to several months to develop. Immunosuppressive drugs act at many levels of the immune system, inhibiting both cellular and humoral mechanisms and reducing the damage caused by autoimmunity in MG. Immunosuppressive therapy, but no other therapy in MG and not thymectomy, causes significant reduction in $CD4^+$ and $CD2^+/CD4^+$ T cells in the peripheral blood (79). These drugs can be associated with several serious side effects, and the treatment should be monitored carefully (80, 81). The need for immunosuppressive drugs is highest

in late-onset MG and thymoma-associated MG (50).

Mycophenolate mofetil is a new immunosuppressant in the treatment of MG, selectively blocking B- and T-lymphocyte proliferation. It has shown promising results in the treatment of progressive MG (82). Tacrolimus is another new immunosuppressant that has demonstrated favorable effects in the treatment of MG, both as monotherapy or add-on to prednisolone (83). Tacrolimus, which is an enhancer of RyR-related sarcoplasmic calcium release, is especially beneficial in MG patients with RyR antibodies (84). Rituximab, a chimeric monoclonal antibody directed against the B-cell membrane antigen CD-20 inducing depletion of B cells and subsequent reduction in antibody production, has also been reported to have a favorable effect on MG (85).

Systemic tolerance and immune modulation in experimental MG has been induced both by oral and nasal administration of AChR antigen fragments, nasal administration suggested to be more effective (86). The mechanism for such tolerance is not clear, but an immune suppression caused by antigen-specific $CD4^+$ Th3 cells producing TGF-β has been suggested (86). In experimental autoimmune MG, feeding mice with immunodominant T-cell epitope was found to inhibit Th1/Th2 cytokines and reduce the concentration of AChR antibodies, relieving the MG symptoms (87). Systemic tolerance is so far restricted to experimental MG.

Plasmapheresis and immunoglobulin

Plasmapheresis removes circulating antibodies, resulting in an improvement of the MG severity within a few days (88). The concentration of AChR antibody may fall to 50% of its initial level (89). This treatment can cause overload and strain on the cardiovascular and pulmonary systems, and the treatment should be used with caution in patients who are at high risk of developing such complications.

The intravenous infusion of immunoglobulin modulates the immune response and is almost as fast and effective as the plasmapheresis, but with lower rate of cardiovascular and pulmonary complications (90).

Plasmapheresis and immunoglobulin treatment are indicated in severe cases of MG, such as MG crisis, and in severe MG cases with poor response to standard pharmacological treatment (81, 91). Such treatment is also used as pre-thymectomy and presurgery prophylaxis to prevent postoperative MG crisis.

Thymectomy

Already in 1949, Keynes observed that the effect of thymectomy seemed to be less in older-age MG patients. Simpson suggested in 1958 that the earlier the onset of the disease, the better was the response to thymectomy. The outcome after thymectomy is less favorable in patients older than 45 years (92). In most studies showing positive associations between thymectomy and MG remission, there are confounding differences in baseline characteristics of prognostic importance between thymectomy and non-thymectomy patients. These studies have been reviewed and examined (93). The exact benefit of thymectomy for MG patients remains to be established. However, when thymectomy is studied in subgroups of MG, the benefit from thymectomy seems to be highest in early onset AChR antibody positive MG, and lowest in late-onset titin/RyR antibody positive MG (32, 50, 94).

Thymectomy can be performed transternally or through a video-assisted thoracoscopic approach (VATET). VATET is performed with the aid of a special device with two sternal retractors elevating the sternum from above and below and facilitating access to the mediastinum. The mediastinal pleura is incised bilaterally under the control of a video camera inserted through the thoracoscopic ports (95). The post-thymectomy results in MG patients for both methods have been reported to be equal (96).

Therapeutic recommendations

It is not easy to give specific therapeutic guidelines in MG as MG is a heterogeneous disease and not all the patients can be treated following a single recommendation. However, the following recommendations may facilitate the therapeutic approach to an MG patient.

After the diagnosis of MG is established, an acetylcholinesterase inhibitor should be introduced, such as pyridostigmine 60 mg three times a day. At this early stage, the patient should be investigated for thymoma, and if proven the patient should undergo surgery. If a thymoma is not found and the patient belongs to the early onset MG subgroup and is AChR antibody positive with generalized MG, thymectomy should be considered within 1 year after MG diagnosis, especially if the response to pyridostigmine therapy is poor and the disease is progressive. Immunosuppressive drug treatment should be considered as an add-on to pyridostigmine in thymectomized and non-thymectomized patients with progressive MG symptoms. In this case,

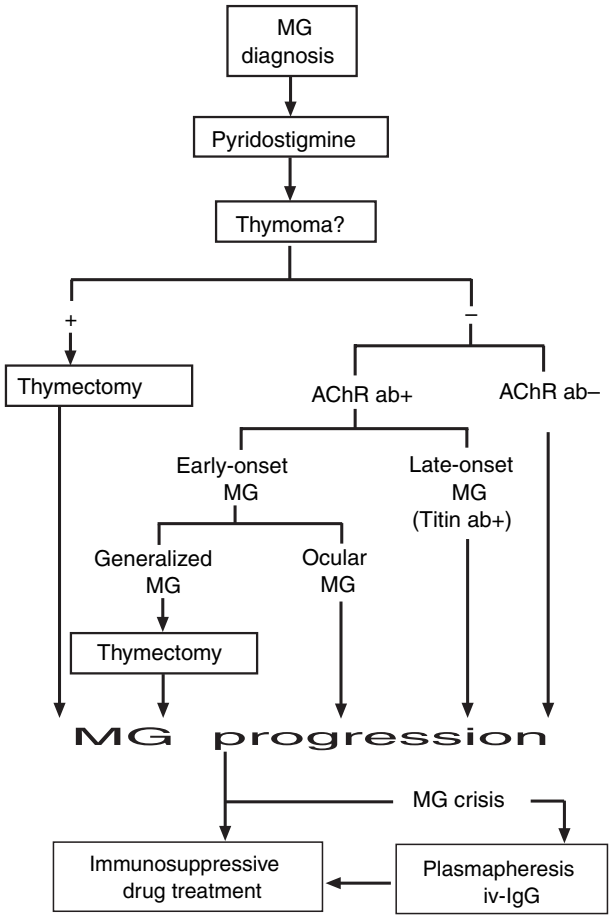

Figure 1. Therapeutic recommendations in MG.

steroids such as prednisolone should be chosen and given on alternate days, usually by increasing the dose to 60–80 mg initially and then with a gradual and slow reduction to 20 mg or lower. Non-steroid immunosuppressants, such as azathioprine, should be introduced (usually 100–150 mg/day) in addition if long-term treatment with steroids is regarded necessary (Fig. 1).

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