Intercommunication between the Neuroendocrine and Immune Systems: Focus on Myasthenia Gravis

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Abstract
Crosstalk exists between the nervous, endocrine, and immune systems, and perturbations in these interactions have been associated with disease. This includes production of neuroendocrine factors that alter immune system activity and increase susceptibility to or severity of immune-related conditions, such as myasthenia gravis (MG) – a T-cell-dependent, B-cell-mediated autoimmune disorder. MG results from impairment of transmission to the neuromuscular junction and involves the thymus – especially in early-onset disease, but the exact mechanism by which the thymus impacts disease is unclear. MG afflicts millions of individuals worldwide each year, and both men and women can develop symptoms. However, prevalence and age of onset differ between men and women. Women exhibit higher incidence and earlier age of onset compared to men, and disease fluctuates during pregnancy. This suggests that sex hormones play a role in influencing disease outcome. In this review, we will consider what is known about the manifestation of MG, theories on how different forms of MG are influenced or alleviated by steroid hormones, current treatment options, and what measures could be important to consider in the future.

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Introduction
Myasthenia gravis (MG) is an autoimmune disorder that results in muscle weakness due to T-cell-dependent, antibody-mediated deterioration of the neuromuscular junction (NMJ). Antibodies generated during MG can be directed against muscle acetylcholine receptors (AChR), muscle-specific receptor tyrosine kinases (MuSK), or the muscles themselves. Women tend to have greater incidence and exhibit symptoms at a younger age; however, incidence is higher in men when MG is diagnosed much later in life [1]. Although biologically heterogeneous, disease generally categorized as paraneoplastic or non-paraneoplastic [2] (table 1). In this review, we will discuss what is known about triggering events that initiate MG,
involvement of the thymus, and what future strategies should be considered for therapies aimed at alleviating this debilitating condition.

**Forms of Disease**
Symptoms of MG include dropping eyelids and double vision from weakness of eye muscles (class I); difficulties chewing, swallowing, speech, and nasal tone and problems with muscles around the mouth; inability to hold objects, write, walk, stand, or climb stairs from problems with arm and leg muscles; and difficulty breathing due to problems with respiratory muscles (classes II–V, depending on severity). Muscles of the heart and intestine are generally not severely impacted. Symptoms vary between patients and tend to fluctuate under different circumstances (i.e., better with pregnancy, worse after delivery), although there are distinguishable features between the 2 major forms of MG.

Most patients with paraneoplastic MG are female and have early-onset MG (prior to age 40) that is characterized by anti-AChR antibodies and thymomas with lymphocytic infiltrates and germinal centers containing T cells, B cells, and plasma cells (reviewed in [3]). It is believed that thymomas develop from dysregulation of lymphocyte selection and presentation of self-antigens by neoplastic epithelial cells. In MG, thymomas usually express AChR-like epitopes and have many autoreactive T cells, which are thought to be positively selected for survival and exported to the periphery where they activate B cells that produce antibodies – including those against AChR. Negative selection and regulation of autoreactive T cells could be impaired in thymomas due to a deficiency in expression of the autoimmune regulator gene (AIRE) as well as loss of regulatory T cells (T_{reg}) [4]. Typical non-paraneoplastic MG patients are male and exhibit symptoms later in life (>40 years) [5]. These individuals differ from paraneoplastic MG patients because they usually have a normal or atrophic thymus and rarely develop thymomas. They also have a wider variety of autoantibody repertoire, which includes antibodies against AChR, MuSK, and the striated muscle proteins titin and ryanodine [6].

**Risk Factors**
Several factors have been correlated with disease onset including hormonal status, as evidenced by disease alteration during pregnancy and postparturition [7–9], and genetic predisposition [6]. Whatever the initiating events, they lead to dysregulated T cell activity and subsequent autoantibody production [10]. Polymorphisms in some immune-related and nonimmune-related genes are associated with increased susceptibility to MG. These include genes for the major histocompatibility complex (MHC) class II molecule human leukocyte antigen DR (HLA-DR), the α-subunit of AChR (CHRNA1), and the master autoimmune regulator (AIRE). MHC expression on antigen-presenting cells – such as monocytes, B cells, and dendritic cells – provides antigen that is necessary to stimulate T lymphocytes. Upon activation, T cells proliferate, secrete cytokines, and target cells expressing their cognate antigen for elimination. Other MHC genes have also been associated with increased susceptibility to MG, such as the class II gene HLA-DQ [11, 12] and class I gene HLA-A [13]. Non-HLA genes have also been associated with increased risk. One of the most frequently identified is CHRNA1, which is the gene that encodes the α-subunit of the AChR. Certain polymorphisms of CHRNA1 have been linked to altered expression in the thymus and subsequent breakdown of tolerance to AChR [14]. It is not clear whether dysregulated AChR expression on a specific cell type in the thymus – epithelial, thymocyte, or myoid cell – is critical for inducing disease. Another gene associated with increased susceptibility to MG is AIRE. Under normal conditions, T cells undergo negative selection in the thymic medulla to delete autoreactive T cells prior to their release into the periphery. Medullary thymic epithelial cells express several hundred different self-antigens (MHC, tissue-restricted antigens, etc.) under the

### Table 1. Clinical manifestations of MG

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Paraneoplastic</th>
<th>Nonparaneoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset</td>
<td>&lt;40 years of age</td>
<td>&gt;40 years of age</td>
</tr>
<tr>
<td>Gender differences</td>
<td>Incidence higher in females</td>
<td>Incidence higher in males</td>
</tr>
<tr>
<td>Status of thymus</td>
<td>Hyperplasia, neoplasia</td>
<td>Normal, atrophic</td>
</tr>
<tr>
<td>Autoantibodies involved</td>
<td>Primarily AChR but also MuSK</td>
<td>AChR, MuSK, striated muscle proteins (titin, ryanodine)</td>
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control of AIRE, which facilitates induction of apoptosis in potentially autoreactive T cells [15]. In addition, it promotes apoptosis of terminally mature medullary thymic epithelial cells to drive tolerogenic cross-priming by thymic dendritic cells. Expression of AIRE is absent in nearly 95% of thymomas, including those associated with MG [16], and it is clear that defective expression of AIRE favors thymic development of self-reactive T cell clones.

Another risk factor involved in MG onset is gender. Women exhibit greater incidence of MG than men, and pregnancy influences manifestation of disease [17, 18]. This indicates a role for sex hormones, which is likely a direct effect since many immune cell populations – including B and T lymphocytes – express receptors for sex and other steroid hormones [19]. A number of studies implicate estrogen as a primary mediator of MG disease [20], although progesterone could also be involved [21]. Estrogen is known to modify both innate and adaptive immunity and can promote production of antibodies by B lymphocytes as well as influence T_{reg} function [22]. At elevated concentrations, estrogen can enhance humoral immunity by inducing helper T cell (T_{H}) responses. In an animal model of MG, estrogen was shown to be important for driving AChR-specific T_{H} and subsequent production of anti-AChR antibodies – resulting in increased disease severity [23]. In addition, a separate study showed that factors produced in the thymic environment altered estrogen receptor expression, and expression was increased on thymocytes and peripheral blood T cells of MG patients [24].

**Biology of Disease**

The exact triggering sequence that leads to MG is unknown, although the involvement of the thymus and T cells is certain (fig. 1). The balance between CD4+ T_{H} lymphocyte subsets (T_{H1}, T_{H2}, T_{H17}, T_{reg}) is key to effective immune responses and protection from disease [25], and dysregulated T_{H1} activity has been demonstrated in the pathogenesis of MG [26]. T_{H1} responses are important for clearing viral and intracellular bacterial infec-
The Neuromuscular Junction

The NMJ serves as the point of communication between nerves and muscles. It functions to convert an electrical impulse to a chemical signal that initiates muscle contraction [31]. The NMJ is located between an autonomic nerve terminal that releases the neurotransmitter acetylcholine (ACh) and stimulates AChR on surfaces of muscle fibers – the neuron motor endplate: (a) the presynaptic region consists of the unmyelinated motor nerve ending where ACh is synthesized in the cytoplasm and stored in vesicles until an action potential arrives and initiates vesicle fusion and ACh release into the synaptic cleft – the space between the nerve terminus and muscle cell; (b) the postsynaptic membrane contains AChR on its surface to bind the ACh released by the nerve ending after it diffuses across the postsynaptic cleft. Transmembrane MuSK is expressed selectively by skeletal muscle cells and is colocalized with AChR in the postsynaptic membrane [32], and (c) Schwann cells cap the NMJ and basal lamina and are critical for junction maintenance [32]. The release of vesicular ACh is a calcium-dependent process, and calcium is obtained through voltage-gated Ca^{2+} channels on the depolarized nerve. Once ACh diffuses across the synaptic cleft, it interacts with AChR on the muscle membrane, and ion channels on the membrane open – initiating a local depolarization process called the endplate potential. In healthy NMJs, the endplate potential is significantly larger than the threshold for generating an action potential in a muscle fiber. The difference between the 2 activation requirements is the safety factor of neuromuscular transmission, which is significantly affected in MG [33]. The postsynaptic survival time of ACh is typically short since ACh is quickly hydrolyzed by acetylcholinesterase (AChE) at the postsynaptic membrane – releasing both acetate and choline.

In MG, physical changes occur at the NMJ. The synaptic cleft widens, there is a reduced number of AChR available, and the postsynaptic membrane is compromised with repeated targeting by the immune system [34]. Although the amount of ACh released with each nerve stimulus is quantitatively normal, the postsynaptic membrane is often severely altered. Autoantibodies targeted against AChR are a common characteristic of MG and can disrupt signaling at the NMJ using 3 distinct mechanisms, with the type of destruction depending on the specific autoantibodies involved. The first and most important of these is complement-mediated lysis of the postsynaptic membrane at the motor endplate [2]. Membranes become permanently altered with distortion and weakening of the sarcolemma. A change in membrane structure alters the number of available AChR on the membrane surface and reduces the number of voltage-gated sodium channels. This, consequently, increases the action potential threshold of the muscle fiber. Secondly, when divalent anti-AChR antibodies are present, there is accelerated internalization and degradation of AChR caused by the cross-linking of AChR by IgG. Lastly, the binding site of AChR may be directly blocked by autoantibodies specific for the binding site or for another site that sterically alters the accessibility of the AChR binding site.

Autoantibodies in MG

The mammalian AChR consists of 4 subunits (α, β, γ, δ), and the α-subunit has 2 isoforms. In adults, the pentameric receptor is comprised of 2 α-subunits and 1 each of the β-, γ- and δ-subunits. Each subunit contains 4 α-helices (M1–M4) that span the membrane; however, the N-terminal and C-terminal regions remain in the extracellular space [31]. One binding site for ACh is located on each α-subunit, and the sequential binding of 2 ACh molecules controls the opening of the AChR cation-selective channel [31]. The primary immunogenic region of AChR has been identified by serum antibody binding in samples from MG subjects [31, 35, 36]. It is located on the N-terminal segment of the α-subunit of AChR at positions that correspond to the extracellular domain of AChR, an area prominently displayed and accessible for antibody binding [31]. Importantly, the immunogenic region of each α-subunit is close to the ACh binding site. In the majority of MG patients (approx. 80%), serum antibodies directed against AChR at the NMJ are present [2]. The remaining patients may also have anti-AChR antibodies but the affinity is too low to be detected [37, 38]. Leite et al. [37] reported that in a group of MG patients who were seronegative for anti-AChR antibodies by conventional diagnostic assays, 66% had low-affinity antibodies that were able to bind AChR.
Other autoantibodies have also been identified in MG patients. Approximately 10% of patients develop antibodies against MuSK, which interferes with the activity of AChR by blocking action potential in muscles and depleting the synapse of MuSK [38, 39]; however, the exact mechanisms of action of anti-MuSK antibodies are not well defined. When Cole et al. [40] injected anti-MuSK-positive human IgG from MG patients into mice, MuSK was depleted from the postsynaptic membrane, which led to disassembly of postsynaptic AChR clusters, retraction of the nerve terminal and fatiguing muscle weakness. In patients with paraneoplastic MG, serum antibodies directed against the intracellular striated muscle proteins titin and ryanodine as well as ryanodine receptor (RyR) are often detectable [6]. Antibodies to titin and RyR are found in up to 95% of MG patients with thymoma and are often used to diagnose disease subtype [41]. In MG thymomas, genetic transcripts and immunogenic epitopes from both titin and ryanodine have been detected, and patients with antibodies against RyR are reported to have poorer MG outcomes and more aggressive thymomas [41).

**Treatment Strategies**

Therapy for MG patients focuses on long-term management of the disease, such as thymectomy and the use of steroids and nonsteroidal agents for chronic immunosuppression but also short-term management of acute exacerbations (MG crisis) with plasma exchange and intravenous immunoglobulin (IVIg). Targeted therapy for MG management includes AChE inhibitors that reduce degradation of ACh at the neurological synapse, thereby increasing the duration of ACh signaling. With the exception of cholinesterase inhibitors, traditional treatments for MG are nonspecific for the mechanisms of disease. Although therapeutically effective in many patients, responses to treatments vary widely and only alleviate some symptoms but with significant side effects.

**Thymectomy**

Surgical removal of the thymus is commonly recommended for MG patients that develop thymomas but is also performed in some patients within 2 years of diagnosis [41]. Because of the strong linkage between the thymus and disease, thymectomy would seem a reasonable treatment but is not always effective [42]. A wide range of outcomes has been observed following thymectomy; some patients (30–60%) who do not develop thymomas are reported to have complete remission in the 2 years following thymectomy, while patients with thymomas and MG have fewer rates of complete remission (10–20%) [41]. Those who develop thymomas are also more likely to experience more exacerbated disease [2, 41].

**Anti-Inflammatory Therapies**

Glucocorticoids (GCs) are produced in the adrenal glands following activation of the hypothalamic-pituitary-adrenal axis during a stress response, and GCs are commonly used in the treatment of many autoimmune/inflammatory conditions. They bind to intracellular receptors to inhibit cytokine release and other activities of a variety of immune cells, including dendritic cells, granulocytes, and monocyte/macrophage populations as well as B and T lymphocytes. There have been several reports of the effects of GCs on B cell development, and they can induce T lymphocyte apoptosis and a shift in the cytokine pattern by $T_{H1}$ to reduce inflammation [19]. The use of GCs was among the first treatment regimens for MG and remains the most common immunotherapy for reducing symptoms [2]. GCs, such as prednisone, are a long-term therapeutic approach for MG and have been shown to reduce the number of circulating lymphocytes in serum to achieve improvement or remission of disease in the majority (approx. 75%) of patients [43–45]. In ocular MG, evidence suggests that GC treatment may delay or stop the progression to other classes of MG. Unfortunately, GC treatment has a number of side effects, including hypertension, impaired glucose tolerance, osteoporosis, psychosis, cataracts and steroid myopathy [2]. This illustrates the need for other treatments to replace or augment chronic use of GCs.

Steroid treatments as an immunosuppressant are not always effective in some MG patients and warrant the use of agents. Results have been achieved using azathioprine (purine antimetabolite), which interferes with T and B lymphocyte proliferation. In retrospective studies, azathioprine was shown to be effective in 70–90% of MG patients [2, 46, 47]; however, it can take several months (up to a year) for efficacy and is often used in combination with prednisone [47, 48]. A similar nonsteroidal agent used in the treatment of MG is mycophenolate mofetil, which selectively blocks purine synthesis to suppress T and B cell proliferation. Cyclosporin and tacrolimus are also useful treatments because they inhibit T cell proliferation through disruption of calcineurin signaling and, thereby, block the synthesis of IL-2 and other cytokines important for CD4+ T cell function [2]. Some MG patients have high levels of serum antibodies directed
against the RyR [6], and tacrolimus can act as both an immunosuppressive agent as well as enhance RyR-related sarcoplasmic calcium release. Studies have reported favorable effects when tacrolimus is used as a monotherapy and when used in combination with prednisolone [49–51]. One study reported reduced levels of proinflammatory cytokines (IFN-γ, IL-2, IL-10, and IL-13) and lower numbers of activated B cells in patients undergoing combination therapy with tacrolimus for 24 weeks, in addition to reduction of steroid dosage (often used as a mark of success in combination therapy trials) in 74% of patients [51].

Other Therapies

AChE terminates the actions of ACh; therefore, treatments aimed at blocking AChE activity increase the likelihood that ACh will interact with AChR and induce muscular activity. The AChE inhibitor pyridostigmine bromide inhibits hydrolytic cleavage of ACh and is the most common AChE inhibitor drug used for MG treatment. Excess doses of AChE inhibitors can block depolarization of the synapse and neuromuscular transmission, resulting in cholinergic overdose and increased muscle weakness. The use of these drugs also has side effects, including stomach cramps, diarrhea, vomiting, sweating, bradycardia, atrophicventricular block, diaphoresis, and increased bronchial and nasal secretions [5, 33]. Furthermore, patients with anti-MuSK antibodies may have poor tolerance to AChE inhibitor drugs; and if antibodies directed against AChE block ACh access to the receptor, AChE inhibitors have little impact. Although successful in some patients, AChE inhibitors typically do not provide sustained symptom relief nor slow disease progression [35].

Myasthenic crisis (short-term exacerbated disease) is a complication of MG defined as worsening of muscle weakness that results in respiratory failure and requires intubation and mechanical ventilation [52]. Management is similar for the different forms of MG and involves reducing the level of autoantibodies. Two common treatment strategies are used in conjunction with high doses of GCs for the management of myasthenic crisis. Plasma exchange is also used to deplete anti-AChR antibodies as well as other serum antibodies [52]. This can be an effective strategy, but repeated treatments reduce coagulation factors, can precipitate cardiac events and stroke, and are only effective for a few weeks [53]. IVIg treatment is similar to plasma exchange as a treatment for acute myasthenic crisis and has less severe side effects than plasma exchange – generally limited to fever, nausea, and headache [53–55]. IVIg neutralizes the blocking effects of antibodies to AChR and is also used to treat other autoimmune/inflammatory disorders and neuromuscular pathogenesis, including Guillain-Barré syndrome and multifocal motor neuropathy [37, 53–56].

Conclusions/Future Directions

Certain individuals are more susceptible to the breakdown of tolerance against endogenous proteins that leads to excessive inflammation and tissue damage following immune system activation – as occurs with the autoimmune disorder MG. Therefore, it is important to understand host factors that can contribute to increased risk. This includes deciphering the interplay between the neuroendocrine and immune systems to help in the development of strategies to reduce susceptibility and severity of disease. In this review, we discussed how disease develops in the different forms of MG; however, more studies are necessary to identify the specific mechanisms by which host factors modify immunity and break tolerance to disrupt transmission in the NMJ. Several lines of evidence indicate additional strategies that could be useful in ameliorating disease. This could include the use of soluble receptors to ACh, which could serve as decoys in MG patients that have circulating anti-AChR antibodies. Other strategies might include treatments aimed at modifying thymus activity to re-establish tolerance against AChR, MuSK, the striated muscle proteins titin andryanodine, or other NMJ targets in MG patients. These and other treatment modalities could be considered in combination with current therapies in an effort to ensure that patients with MG are able to control their disease and improve their quality of life.

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References


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