Teaching Course 16

Autoantibodies in neurological disorders - Level 2

What’s new in myasthenia gravis?

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Myasthenia gravis (MG) is one of the best-characterized antibody-mediated diseases, thanks to the accessibility of the neuromuscular junction and the availability of effective animal models.

In MG, pathogenic auto-antibodies (Abs) cause morphological and functional alterations of the postsynaptic membrane resulting in impaired nerve-muscle transmission and fatigable muscle weakness. The result of the Ab attack is a reduction of acetylcholine receptor (AChR) clustering, which is crucial to the synapse efficiency and is regulated by a complex network of core proteins, such as neural agrin, the muscle-specific tyrosine kinase receptor (MuSK), and the low-density lipoprotein receptor-related protein 4 (LRP4) (1). Both AChR and the MuSK-LRP4-agrin complex are targets of the autoimmune response in MG, with differences in specific IgG isotypes and pathogenic mechanisms.

MG is increasingly recognized as a syndrome more than a single disease, as it includes several subtypes which differ in terms of clinical characteristics, prognosis and response to therapies. Abs against AChR and MuSK cover together 90% of patients with MG, as AChR Abs are detected in 80-85% and MuSK Abs in 5-8% of the whole MG population (2). In these disease subtypes, genetic predisposition, mechanisms of tolerance failure and Ab effects have extensively been studied. As a rule, AChR and MuSK Abs are mutually exclusive (their coexistence in the same patient is exceedingly rare) and are very specific.
for MG. Both are detected by radioimmunoassay (RIA) which is sensitive, quantitative and largely available. Therefore, when MG is suspected on the basis of clinical history and signs, AChR Abs are the first to be tested, and MuSK Abs should be assayed in all AChR-negative patients.

In double seronegative (dSN) MG (i.e. MG with neither AChR nor MuSK Abs on standard RIA), other Abs have been detected with different assays, namely cell-based assays (CBAs). CBAs, which employ live transfected cells, have not gained widespread use as they require specific skills and equipment (3). Limited availability of serological testing can complicate the diagnosis in dSN patients. On the other hand, methodological differences make it difficult to establish the real prevalence of these new Abs.

**MG associated with AChR Abs**

MG with Abs to AChR (AChR-MG) is characterized by a broad clinical spectrum from isolated ocular symptoms to severe life-threatening weakness, a bi-modal age at onset with female predominance in the early onset population, and a high frequency of thymus alterations as thymic follicular hyperplasia (TFH) and thymoma. AChR Ab positivity rate varies according to the age at onset, from 60-70% in childhood to 97% in patients older than 70 years, and to weakness extension, from 50% in ocular myasthenia to 90% in generalized disease, and it approaches 100% in thymoma patients (4-5).

AChR Abs are IgG1/IgG3 and induce MG through two main mechanisms: complement-mediated focal lysis of postsynaptic membrane and increased AChR internalization through cross-linking by bivalent IgG molecules (antigenic modulation) (5).
The muscle AChR is a pentameric glycoprotein made up of four subunits (2a,1b,1g/e,1d); the fetal AChR (2a,b,g,d) is replaced by the adult isoform (2a,b,e,d) by 33 weeks of gestation (6). A high proportion of AChR Abs in patients' serum target the a extracellular region and, particularly, a conformational set of epitopes (the so-called main immunogenic region - MIR) which includes the sequence 66-76 (7). Abs to MIR effectively induce experimental autoimmune MG (EAMG) and show a closer correlation with disease severity than the whole AChR Ab titer (7). Both in immunized animals and in patients with MG, Abs against AChR cytoplasmic domains and subunits other than the a subunit are commonly found, suggesting that AChR released from the disrupted muscle membrane fosters epitope spreading and provides a continuous source of antigen. While Abs to cytoplasmic domains are not considered pathogenic, the presence of extracellular epitopes, of other AChR regions, can enhance Ab-mediated complement activation (8). In addition, Abs against the AChR fetal isoform are potentially pathogenic during pregnancy. In fact, the presence in myasthenic mothers, of Abs against the g-subunit, which disrupt the function of embryonic AChR, can be responsible for the so-called “fetal AChR inactivation syndrome” (FARIS). FARIS clinical features range from lethal arthrogryposis multiplex congenita to mild myopathy predominantly involving facial and bulbar muscles (9). Diagnosis can be difficult (the mothers can be completely asymptomatic) but is crucial, as in the absence of adequate treatment the fetal disease recurs in subsequent pregnancies (10).

AChR Ab effector mechanisms and specificities are targets of new therapeutic options. Complement inhibition has proved effective in generalized refractory AChR MG (11), in preliminary studies antigen-specific immunoadsorption effectively depleted pathogenic Abs (12), a
A phase I trial of a therapeutic vaccine including peptides complementary to the MIR has recently been completed (ClinicalTrials.gov identifier: NCT02609022), and immunization with AChR cytoplasmic domains prevented EAMG induction and suppressed the ongoing disease in mice (8). AChR MG includes three main patient populations: early onset (age of onset <50 years), late onset, and thymoma-associated MG. Since these groups have different HLA associations and thymus pathologies, the autoimmune response to AChR may be elicited by distinct mechanisms.

Compartmental enrichment of B cells is a common finding in organ-specific autoimmunity. In early onset AChR-MG the inflamed organ is the thymus, though the site of tissue injury is the motor end-plate. TFH is characterized by ectopic germinal centers and AChR Ab production, and inflammatory changes in the thymic environment are thought to play a crucial role in sensitization against AChR (13). Whether the activation of autoreactive T and B cells inside the thymus is triggered by immune response to pathogens (14) is a matter of debate (15). Despite uncertainty about the driving mechanisms, the pathogenic role of TFH in AChR-MG is generally accepted and provides the rationale for therapeutic thymectomy in these patients. The MG thymectomy (MGTX) trial has recently provided class I evidence that thymectomy has favorable impact on generalized non-thymomatous AChR-MG (16).

Ten to 15% of AChR-MG patients have a thymoma, a tumor originating from epithelial cells and harboring a variable proportion of non-neoplastic thymocytes. MG-associated thymomas are mostly of B (cortical) types with sparse medullary areas and active thymopoiesis. Defective negative selection with the export of autoreactive CD4+ T cells, and reduced generation of T regulatory (Treg) cells appear to be the key features associated with the occurrence of MG (17).
Poly-autoimmunity is frequent in AChR-MG. The early-onset subtype holds the highest association rate with other autoimmune diseases, with endocrinopathies (namely thyroiditis) among the most common. On the other hand, thymoma-MG is more typically associated with cutaneous, hematologic and neurologic disorders. Among the latter, neuromyotonia and autoimmune encephalitis are the most frequent (18).

AChR Ab production requires antigen-specific CD4⁺ T helper (Th) cells. Contemporary studies have highlighted the role of Th17 cells in the loss of tolerance to AChR (19), have shown an increased rate of circulating follicular Th cells in generalized MG (20), and have reported defects of both Treg (21), and B regulatory (Breg) cells (22). Longitudinal studies, evaluating changes in these cell subsets in different MG phases and in response to treatment, will clarify their role as markers of disease activity.

It is well known that AChR Ab titer does not correlate with MG severity and can still be positive many years after thymectomy and pharmacologic treatment, in asymptomatic patients. AChR Abs are produced by plasma cells (PCs), very likely long-lived PCs that are protected by their survival niche against most immunosuppressants (23). While chronic Ab production by long-lived PCs is thought to be involved in refractory MG development, antigenic specificity and IgG isotype can contribute to Ab pathogenicity and, ultimately, to symptom persistence and severity.

**MG associated with MuSK Abs**

MG with MuSK Abs (MuSK-MG) is characterized by a striking prevalence in women and a focal weakness pattern with predominant involvement of bulbar, neck and respiratory muscles. Ocular symptoms are milder and
less asymmetrical than usually observed in MG patients and can be overlooked. Lack of daily fluctuations, focal atrophy, unresponsiveness to cholinesterase inhibitors and negative results of electrodiagnostic testing in limb muscles can further complicate diagnosis. Thymus histology is mostly normal-for-age with sparse lymphoid infiltrates and thymectomy does not appear to improve the course of the disease. MuSK-MG responds to immunosuppressive therapy (particularly to steroids) and most patients experience sustained improvement with rituximab (24).

MuSK Abs are mostly IgG4, with smaller proportions of IgG1-3. Given to their ability to exchange Fab-arms, IgG4 behave as bi-specific Abs and can neither cross-link membrane-bound antigens nor activate complement. Although MuSK IgG4 undergo Fab-arm exchange in vivo, these hybrid Abs are pathogenic likely through direct interference with the protein function (25).

MuSK extracellular region consists of three immunoglobulin-like (Ig) domains and a cysteine-rich domain (CRD) (26). The MIR of MuSK is in the Ig-1 domain (27), though Abs against Ig-2 and CRD have also been described (28-29). As Ig-1 domain is crucial to MuSK-LRP4 interaction, epitope-mapping studies support the view that MuSK Abs reduce MuSK activation by interfering with LRP4 binding (30). Presynaptic defects and Ab-mediated block of MuSK binding to ColQ (the collagen tail of acetylcholinesterase) (31), can contribute to the disease pathogenesis.

In MuSK MG, epitope spreading is uncommon (27), and MuSK Ab titer correlates with disease severity better than AChR Abs (32). Such a correlation is even closer for MuSK-Ig1 Abs (27). The sustained decline of MuSK Ab titer after B cell depletion suggests that short-lived rather than long-lived PCs are the main Ab producers. A recent study has confirmed
this view, showing that plasma blasts were indeed the main contributors to MuSK Ab production (33). In addition, an increased frequency of CD4+ T cells producing inflammatory cytokines (34), and a decreased rate of Breg cells have been reported (35) in these patients, mirroring the changes observed in AChR-MG.

The association of MuSK-MG with IgG4-related disease has been reported in a single case (36). The co-occurrence of other IgG4 Abs has not been systematically investigated.

AChR and MuSK Ab detection by CBA

In CBA, AChRs are expressed on the surface of human embryonic kidney cells, and clustered by co-expression with rapsyn as they are in vivo (37). Serum Abs to “clustered AChR” have been detected in 16%-45.8% of dSN-MG (38-40) patients often in association with juvenile and mild disease (40). These Abs are mostly complement activating IgG1 and, in passive transfer studies, reduced miniature end-plate potentials and decreased AChR expression at mouse end-plates (37).

Recently, 8% of dSN-MG patients were found positive for MuSK Abs with a highly specific CBA. These patients were younger and less severely affected than RIA-positive MuSK-MG patients (41). Collectively, these studies show that AChR and MuSK Ab detection by CBA is specific and can improve the diagnosis of MG, particularly in young patients with limited forms of the disease.
Abs to LRPA, agrin and intracellular antigens

Abs against LRP4 have been reported at varying rates, from 18.7% in a multicenter analysis from Europe (42) to 1% in a recent survey from China (43).

LRP4-immunized animals developed myasthenic weakness (44-45) and LRP4 Abs (mostly IgG1 and IgG2) interfered with agrin binding and reduced AChR clustering in vitro (46). While such effector mechanisms would predict a severe phenotype, patients with Abs only to Lrp4 are predominantly affected by mild disease (42-43, 47) with little evidence of neuromuscular transmission impairment (48). Lrp4 Abs can co-occur with AChR or MuSK Abs, more commonly with the latter (42-43, 46); these double positive cases tend to have more severe symptoms (42).

Abs to agrin have been reported in 34 MG patients in different studies, often in association with other disease-specific Abs, mostly AChR Abs (49-51). Clinical features are not yet characterized. Sera from agrin-positive patients inhibited MuSK phosphorylation and AChR clustering in myotubes (49) and active immunization with N-agrin induced EAMG in mice (52).

Interestingly, LRP4 and agrin Abs have been found in patients with amyotrophic lateral sclerosis at higher rates than in MG (53-54). These reports need confirmation.

Abs to intracellular targets can be found in MG. Titin and ryanodine receptor Abs (also called striational Abs) are detected in AChR-MG, are strongly associated with thymoma and, to a lesser extent, with late-onset MG and are markers of thymoma in patients with early-onset disease (55). Cortactin is a tyrosine kinase substrate that acts downstream of agrin/MuSK in promoting AChR clustering (56). Abs to cortactin were
reported in 23% of dSN-MG and 9% of AChR-MG patients, mostly in association with mild disease (57). Their role as biomarker is uncertain.

In summary:

• AChR and MuSK Abs are specific and well-characterized. These Abs identify two disease entities with distinct pathogenic mechanisms, clinical features and treatment responses

• CBAs have improved the serological diagnosis of MG. Multicenter studies and assay standardization are crucial to define the prevalence and clinical relevance of “new” Abs

• Abs to intracellular proteins are likely not pathogenic, are not diagnostic of MG but can provide clinically useful information.
References


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