Myasthenia research over the last 50 years – a personal perspective

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ABSTRACT

Myasthenia gravis (MG) research has, in many respects, been a trail blazer for the growing number of autoantibody-mediated disorders that affect the nervous system. The breakthroughs in MG understanding were made in the 1970s and even 50 years later, MG still remains a topic which scientists, clinicians and, most recently Pharma, return to as the most common and well-studied disorder. Here, some of the main discoveries will be reviewed very briefly focusing on how the knowledge of the disease evolved during the first decades after the discovery of acetylcholine receptor antibodies. It should be noted that this is a personal perspective and not a systematic or fully referenced review.

Keywords: History of myasthenia gravis, acetylcholine receptor, muscle specific kinase, autoantibodies, thymus

Earliest Times

MG was a topic of interest to neuroscientists and neurologists for three centuries before the discoveries of the 1970s. Table 1 lists the most important contributors to the history of MG research starting with Thomas Willis[1] in the 17th century. The clinical and physiological characterization began to move forward with Erb[2] and Goldflam[3] who described the fluctuating fatigue, and Jolly[4] helped explain fatigue by demonstrated the decreasing muscle contraction during repetitive nerve stimulations. By 1901, Campbell and Bramwell[5] had published a detailed description of myasthenia gravis. Meanwhile, Weigert[6] noticed collections of lymphocytes in MG patient muscle and later Buzzard[7] hypothesized that there might be an "autotoxic" agent. The description in 1934 by Walker of how, as in curare poisoning, the symptoms of MG were rapidly reversed by the cholinesterase inhibitor, physostigmine, led to the first systematic treatment for the disease.[8] All these observations helped demonstrate that MG was a disease of the neuromuscular junction and was likely due to some sort of inhibitory circulating substance. The history of MG research is covered briefly in a 2002 review,[9] and in a more detailed and beautifully illustrated book by Keesey.[10]

By 1960, several groups, including neurologists Straus and Nastuk, examined the role of the immune system on muscle fibers, finding cytotoxic damage caused by MG sera, and immunoglobulins and complement bound; importantly, however, these signs of autoimmunity were not at the neuromuscular junction itself but very evident on the muscle fiber striations.[11,12] In retrospect, the patients whose sera were positive in these experiments almost invariably had thymomas; these antibodies later became known as anti-striated muscle antibodies, strongly associated initially with the tumors. At this time, tissue specific antibodies were beginning to be recognized more widely, particularly those involved in thyroid disease.[13] In 1960, Simpson published a hypothesis,[14] reviewing the clinical associations of MG, including the often-enlarged hyperplastic thymus, the fluctuating disease course, the associations with a number of other autoimmune conditions (including thyroid disorders), and the transfer of disease to neonates. He proposed, with some prescience, that MG was a condition caused by an antibody to an "endplate" protein.

In 1952, Fatt and Katz[15] had identified miniature endplate potentials as the postsynaptic depolarization resulting from the release of single packets or quanta of ACh. Elmqvist and colleagues in Sweden[16] found that the miniature endplate potentials were reduced in amplitude in MG muscle. They concluded from their studies, somewhat tentatively, that the defect lay in the release of acetylcholine rather than in the response of the postsynaptic muscle.

Until that point, there was no way of identifying the postsynaptic "receptor" for ACh. It took the work of Taiwanese scientists, Chang and Lee,[17] whose main interest was snake toxin envenomation, to identify a component of venom from Bungarus multicinctus, the banded krait, that paralyzed rodent neuromuscular preparations. Conveniently, the toxin, α-bungarotoxin, was a polypeptide and could be easily radio-iodinated. They found that ^{125}I-α-bungarotoxin bound essentially irreversibly to the postsynaptic muscle membrane, exclusively at the NMJ, suggesting that it was binding to the elusive "receptor" for ACh.

The question was how to purify this large membrane protein. First, there was a much easier source than mammalian tissue. It had been known for years that the electric organs of electric eel or Torpedo were innervated somewhat similarly to muscle and responded strongly to acetylcholine (reviewed in detail by Keesey[18]). Second, in 1968, a group at the Weizmann Institute led by Cuatrecasas[19] had shown that it was possible to purify a protein to high specificity if you could immobilize its ligand on an insoluble matrix, apply the protein soup, wash and then "elute" the specific protein by introducing a ligand that competed with the matrix-attached ligand. This seminal discovery eventually led to the use of cobratoxin-columns to purify the toxin-binding protein from the electric organs of electric eel or torpedo (and subsequently human muscle).[20,21] By eluting with high concentrations of carbachol or d-tubocurarine, a number of groups achieved relatively pure ACh receptor (AChR) proteins and began to study its subunit structure.

Quinquennial meeting, New York 1975

All these findings came together in the early 1970s, and the results were presented at the MGFA conference on MG in 1975. I was lucky enough to be there, having been asked to write a conference report for Nature News and Views,[22] an opportunity that, although approached with considerable timidity at the time, turned out to be a wonderful stepping stone for my future career.

Firstly, Fambrough, Drachmann and Satyamurti had answered the pre- or post-synaptic question – to a large extent – by showing that there were less ^{125}I-α-bungarotoxin binding sites at the MG NMJ compared with control NMJs.[23] In the same year, Patrick and Lindstrom found that rabbits immunized against the purified AChRs from electric eel developed an MG-like syndrome, reversible by cholinesterase inhibitor, that could be transferred to healthy rabbits by the serum that contained antibodies to the immunizing AChR.[24] Lindstrom had devised a radio-immunoprecipitation method for measuring these antibodies that relied on incubating the serum with ^{125}I-α-bungarotoxin bound to solubilized electric eel AChR, and then immunoprecipitating with an antibody specific for rabbit IgG. The precipitate formed with the rabbit serum IgG contained the ^{125}I-α-bungarotoxin-AChR. [24] This led Almon and others to demonstrate that MG patients also had antibodies that interfered with binding of a-bungarotoxin to the AChR.[25] Meanwhile, Lindstrom together with Seybold, Lennon and others, used solubilized human muscle in the radio-immunoprecipitation assay, with precipitation by antibodies specific for human IgG, and found that 85% of patients were positive for AChR antibodies compared with a variety of controls. This test has formed the basis for an assay which, despite the radioactivity (which in fact is minimal), is still used widely.[26] Control sera are very rarely positive and the levels in patients vary but are often orders of magnitude higher than the controls.

Role of the antibodies

The question then became were these antibodies the cause of MG or could they be an epiphenomenon with no pathogenic role? Toyka, Drachmann and colleagues reported at the 1975 meeting that when MG IgG antibodies were injected into mice daily, the mice developed weakness and their endplate had very small miniature endplate potentials – reproducing well the neurophysiological hallmark of the disease.[27]

This was strong evidence that the serum IgG was causative; the reverse was to remove or reduce the AChR antibodies from patients and see if they improved. It was reasonable to suspect that antibodies were being made in the thymus or lymph nodes draining the thoracic cavity. Already before the antibodies were discovered, Matell and others[28] had found improvement in patients treated with adrenocorticotropic hormone and begun to use azathioprine as an immunosuppressive treatment. Impressively, they also found that thoracic duct drainage achieved clinical improvement, and that injection of the drainage fluid back into one patient caused deterioration – the perfect human experiment.

In the UK, plasma exchange was beginning to be used regularly for Goodpasture's disease (autoimmune glomerulonephritis) and the procedure was tried in MG by Pinching et al.[29] They found dramatic clinical improvement within days and, on further investigation, AChR antibody levels showed a striking inverse relationship with strength during the five day procedure and in the following weeks as the AChR antibody levels recovered and the patient's symptoms returned.[30] It should be noted that to get these results, each MG serum had to be titrated to find the optimal serum concentration for measuring that individual's antibodies over time, and this concentration varied considerably between different patients; this is seldom done nowadays and routine AChR antibody titers are seldom helpful in assessing treatment responses.

Since those seminal findings (reviewed in 1980[31]), MG research has expanded in many directions. Figure 1 uses a heatmap to illustrate the main topics and how interest in them has waxed and waned over time. The following sections will cover the topics asterisked.

Levels and characteristics of antibodies to the AChR in MG

The antibodies were found to be polyclonal IgG, predominantly IgG1 with some IgG3, and they appeared to react differently with AChR from normal muscle, denervated muscle and extraocular muscles.[32] They were very high affinity for the native AChR – as identified by binding to AChR in the solubilised muscle extracts – and did not bind well to denatured protein on western blots. However, monoclonal antibodies (mAbs), raised against purified eel AChR could bind to human AChR and one in particularly bound to a well-defined epitope on the surface of each of the two alpha subunits.[33] Since this monoclonal antibody (mAb 35) inhibited a variable but often large proportion of MG patients' antibodies, the two binding sites were termed the main immunogenic regions or MIR.[34] Similar results were obtained with mAbs raised against the human AChR, one of which, mAb M3D6, competed with mAB 35 and showed similar ability to compete with patient AChR antibodies.[35] In addition, other AChR mAbs bound to the beta or delta subunits, and four bound only to the fetal isoform in which the gamma subunit replaces the adult epsilon subunit[36] (see Figure 2). In fact, studies on the human antibodies binding to human AChRs (mostly identified by competition with subunit defined mAbs) showed considerable heterogeneity both in the levels and in their specificities, raising questions regarding which antibodies might be most pathogenic, and whether some are non-pathogenic and potentially protective; these questions have still not been clearly addressed.

Figure 1. A heat-map displaying some of the main topics of interest from International Conferences on Myasthenia Gravis over the last 50 years. Note that publications until 2008 included short papers from submitted abstracts as well as the contributions from invited speakers. For 2022, in order to include here some of the newer topics, all invited and submitted abstracts were searched.

Fig 1.

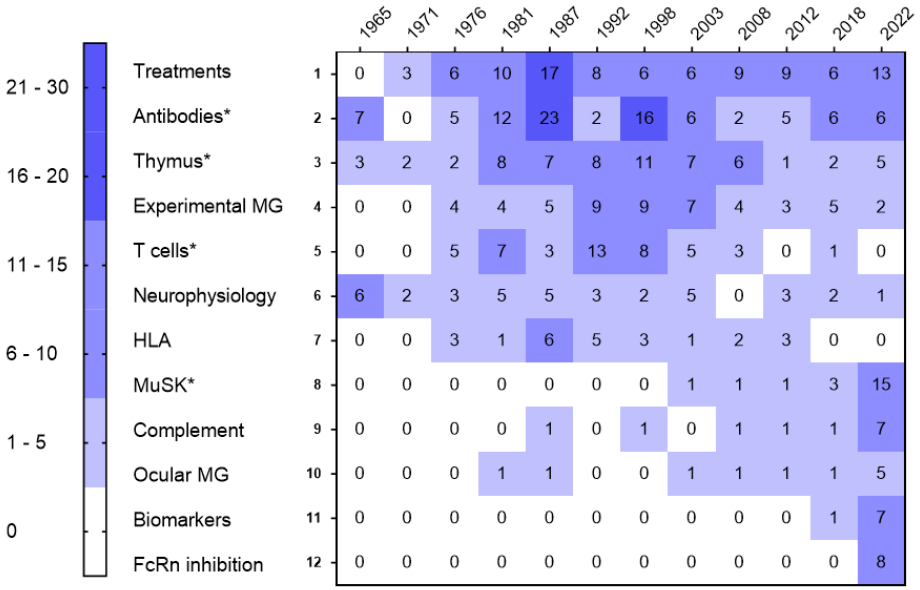
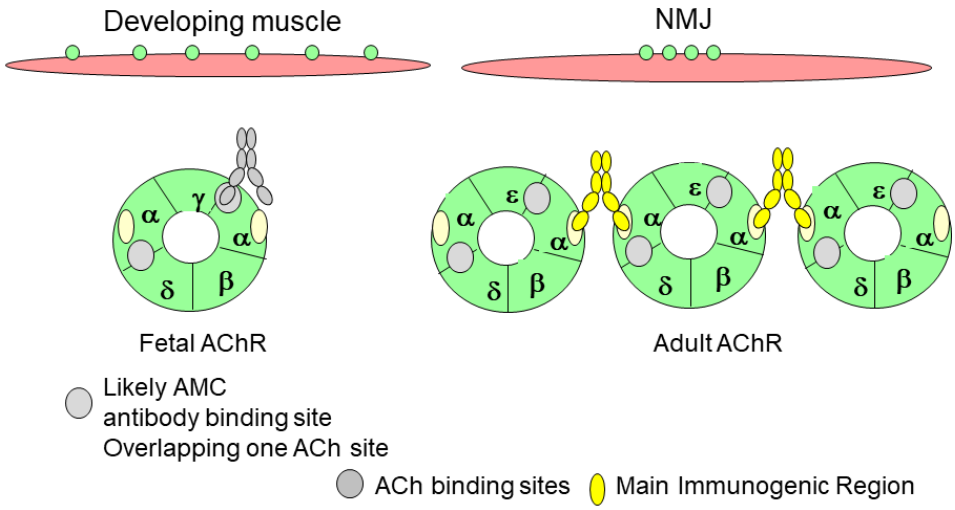


Figure 2. Simple diagrams of the adult and fetal AChRs and the most important binding sites for antibodies.
A. In humans, the fetal AChR can still be detected up to 31 weeks gestation[96] and it is likely that adult AChRs are present for some time before that. In mothers whose children develop AMC (arthrogryposis multiplex congenital), the antibodies block the AChR ion channel function and are assumed to bind to a fetal-specific site overlapping the ACh binding site. This is less clear in maternal antibodies of children with the recently described FARAD (fetal acetylcholine receptor antibody-associated disorder). Note also that because the fetal AChR shares the other three subunits with the adult form, antibodies to any of these subunits will bind both forms. Nevertheless, many of the FARAD mothers' antibodies are highly selective for binding to fetal AChRs (on the gamma subunit); but these may not necessarily inhibit fetal AChR function.

B. The adult AChR and how MIR antibodies can easily cross-link the receptors. Note that additional antibodies can help build up complexes that stimulate complement activity.[46]

Fig 2.



Mechanisms of action

The pathogenic mechanisms of the antibodies were identified in the late 1970s and 1980s. Engel's electron-microscopic studies demonstrated clearly that the NMJs were damaged with reduced numbers and depths of the secondary folds and widened synaptic gap.[37] Within the synapse, he and his colleagues found IgG bound and complement factors including C3 and the membrane attack complex.[38] The reduced binding of peroxidase labelled α-bungarotoxin confirmed relationships between IgG bound complement activation and AChRs lost. Curiously, despite the evident involvement of complement-mediated damage in MG, it is only over the last few years that attention has begun to focus on complement-mediated activity in MG. In the first of several trials, anti-complement therapy was effective in refractory MG[39] and a recent publication describes a method for assessing the complement-activating ability of individual patients' AChR antibodies that should help stratify patients who will respond to this type of therapy.[40]

Another mechanism discovered early was that of internalisation of the AChR.[41] This is particularly likely to occur with antibodies binding the MIR because, as illustrated in Figure 2, they can easily cross-link AChRs. It should be noted that the creation of complexes of this kind will also increase the likelihood of complement activation; however, using human-derived monoclonal antibodies bound to epitopes on different AChR subunits, complement activation was much more effective using combinations of the antibodies rather than antibodies to single subunits;[42] this suggests a role for the heterogeneous antibodies to other subunits that are found in MG.

It was disappointing that the antibodies did not often show direct inhibitory effects on the AChRs. This would likely need antibodies that bind to at least one of the two ACh binding sites, which are distinct from the MIR and at the interfaces with the two adjacent subunits (Figure 2). Those antibodies appear to be rare, and the mechanisms are more likely dominated by complement-mediated damage and internalisation. One exception, however, is fetal specific antibodies as described below.

Maternal MG and fetal AChRs

In the 1990s, a small number of women, mostly with MG, had babies who had stopped moving in utero and were born with severe, often fatal, arthrogryposis multiple congenita (AMC) rather than the well-known transient neonatal myasthenia. AMC is due to lack of fetal movement of any cause, including many genetic disorders, but the presence of AChR antibodies in the mothers, and the fact that consecutive pregnancies were affected, strongly implicated a maternal cause. IgG antibodies from two of the mothers, unusually, rapidly blocked fetal AChR currents while having no effect on adult AChR currents.[43] This suggested that they bound to the fetal gamma subunit in such a way as to block the binding of ACh to the adjacent alpha subunit (Figure 2); moreover, passive transfer of the mothers' antibodies to pregnant mice resulted in pups born with deformities and respiratory failure.[44] The numbers of reported cases with this condition is small, but it is now recognized that some children have milder symptoms in utero and survive, but have long-term consequences, a syndrome initially termed fetal acetylcholine receptor inactivating syndrome (FARIS).[45] The antibodies often bind preferentially to the fetal AChR but since the functional studies have not yet been performed, fetal acetylcholine receptor antibody associated disorder (FARAD) is more appropriate. The features in 40 children, all of whose mothers had AChR antibodies, include polyhydramnios and mild contractures in utero as well as hypotonia, feeding and respiratory difficulties at birth and dysmorphism, feeding difficulties, and speech impairment long term; only 50% of the mothers had diagnosed MG raising the possibility that FARAD could be a, previously undiagnosed, cause of neuromuscular developmental disorder in some neonates.[46]

Subgroups of MG

There were early hints in the 1970s of interesting associations between MG, gender, age of onset, and specific HLA (human leucocyte antigen) polymorphisms. Over the next decade many groups enlarged on these findings.[47] As the number of MG patients increased (partly the result of having diagnostic antibody tests available), three different subgroups of MG began to emerge: early onset (before 40 years), late onset (after 40 years) and those with thymoma.[48] Only when separated into these three groups was it clear that there were different gender ratios and HLA polymorphisms. Although the genetic analysis has since become much more complex, these distinctions remain; moreover, as the population ages the number of patients developing MG after the age of 50 years, predominantly males, now far exceeds those, mainly female, who develop MG as children or younger adults. However, there is still little understanding of how these genetic polymorphisms, and the more recent GWAS studies contribute to the aetiology of MG.

The role of the thymus.

Involvement of the thymus in the pathology of MG was seen in autopsies from earlier times but possibility of thymectomy for MG was serendipitous. Removal of the thymus by Sauerbruch[48] when performing thyroidectomy for a woman with thyroid disease led to marked improvement in her MG, and Blalock noted improvement in a woman when he removed her thymomatous gland.[50]

Since then, thymectomy, mainly for early onset MG, has been the source of much research material. Surprisingly, lymphocytes derived from the thymus could be shown to make AChR antibody spontaneously in culture.[51] In fact, the thymus contains B and T cells, some of which have been shown to be specific to AChR, which are surrounded by muscle-like cells that express AChRs on their surface.[52]

It is not surprising, therefore, that the levels of AChR antibody often decreases after removal of the thymus.[53,54] In most cases, the clinical response to thymectomy is slow, and given the success and quicker effect of immunotherapies, particularly steroids, it was questioned whether thymectomy was necessary. As Gronseth and Barohn reported in their retrospective review of controlled, non-randomized studies,[55] thymectomy conferred only moderate benefits. This was the basis for the multicentre international trial of thymectomy, first established in 2003 by John Newsom-Davis, which was eventually reported in 2016 led by Wolfe and colleagues;[56] this showed that thymectomy plus steroids conferred significant clinical improvement with less requirement for steroids, compared to steroids alone.

Thymic tumours are found in about 10% of MG patients, usually between the ages of 30 and 60, and they are mainly lymphoepithelial.[57] Thymoma patients seldom improve after removal of the tumour (unlike Blalock's patient) and may even get worse. They are always AChR-Ab positive but also often have antibodies to striated muscle proteins, specifically titin and ryanodine receptor.[58,59] These bind to intracellular proteins and are unlikely to be causative, but their presence in MG patients can be helpful as a biomarker for thymoma, especially in younger individuals. Antibodies to cytokines IFNa and IL12 can also help predict thymoma recurrence[60] but are seldom measured. The thymoma itself does not express native AChRs, but the epithelial cells express individual subunits of the AChR[61] which are thought to sensitise T cells which then migrate to the periphery.[62] Finally, in late-onset MG, the thymus is usually atrophic (ie. normal for age), yet these patients, whose numbers are growing owing to the increasing life expectancy of the general population, often have antibodies that are specific for titin and ryanodine receptor, despite no evident thymoma.

T cells in MG

As soon as it became clear that MG was a high affinity IgG antibody mediated disorder, it was assumed that the B cell antibody response was dependent on AChR-specific T cells, and that the epitopes recognized by the T cells would likely be more restricted than the B cells that produced the heterogeneous antibodies. The hope was that, if a specific T cell receptor response could be identified, the responding T cells could be selectively deleted. From the 1980s, the individual subunits of the AChR from Torpedo electroplax and then human muscle, were sequenced and cloned for expression studies.[63,64] Several groups produced recombinant AChR subunits by E. coli expression, and looked for proliferative T cell responses to the purified subunits, then epitope mapping the responses with overlapping synthetic peptides sequences, either in peripheral blood mononuclear cells or thymic lymphocytes. Hohlfeld and colleagues first found peripheral-blood lymphocytes responding to purified *Torpedo* AChR[65] and, when the human AChR subunits were sequenced, he and others went on to clone T cells specific for responding to human AChR.[66-68]

Disappointingly, there was diversity of responses to AChR peptides between MG patients, and sometimes control cells also responded. T cell responses could be restricted by the appropriate MG-associated HLA but often they were restricted by a less MG associated HLA.[66] Pools of overlapping peptide sequences frequently stimulated T cell responses, but it was not clear whether these cells would have responded to the native AChR as presented to B cells in vivo. When recombinant proteins were used as antigen, some of the responses were shown to be to E. coli contaminants rather than the AChR itself.[69] More encouraging, a small number of patient T cells responded, surprisingly, to the AChR epsilon subunit (adult receptor), and the response could be mapped to one specific epitope.[70] It was possible to cause apoptosis in responding T cells cloned from one patient by means of a tetrameric class II peptide complex in vitro[71] but, unfortunately, the hope of a specific T cell epitope that could be the target for such a therapy in a high proportion of patients has not yet been realised.

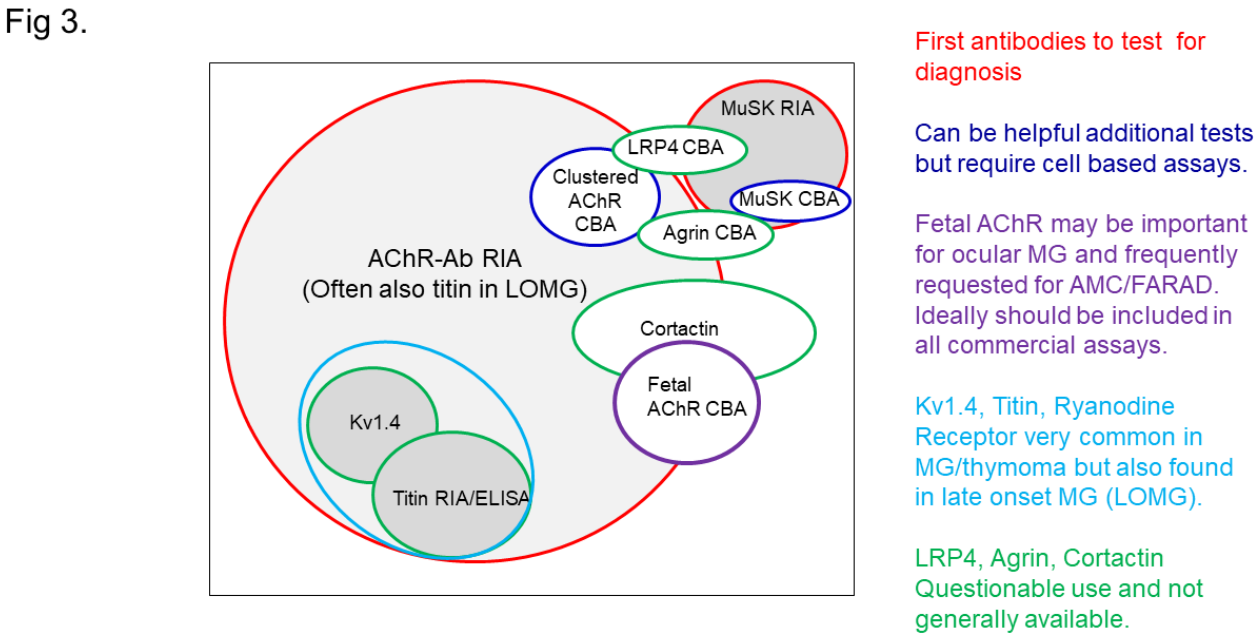
Origin of the immune response

Could the autoimmunity in MG be secondary to an infection? In the 1980s, there was considerable interest in the work of Jerne[72] who described antibody idiotypes and how their networks could control immune responses. A few publications appeared to show that AChR specific antibodies arose as a result of dysregulation of an "idiotypic" network, perhaps initiated by a microbial antigen[73] or by cross-reaction with epitopes shared on microbial antigens,[74,75] although the ELISA techniques used were questioned.[76] Moreover, the absence of an infectious history in most patients, the very high affinity of the AChR antibodies, and their clear preference for binding to the native protein rather than isolated subunits or synthetic peptides, strongly implied that the B cells are stimulated by the native human antigen. It is still possible, however, that low affinity antibodies to the AChR, possibly induced by cross-reaction with a microbial antigen, precedes the production of high-affinity pathogenic antibodies. Nevertheless, two attempts to demonstrate the presence of viruses in myasthenia gravis patients, including in the thymus itself, were unsuccessful.[77,78] A review in 1998 discussed these issues in more detail.[79]

Seronegative MG

In 1976, when reporting the AChR antibody assay results, Lindstrom[26] drew attention to the presence of some patients who appeared completely negative, and this "seronegative" MG group has been a focus of interest ever since. Importantly, these patients usually responded very well to plasma exchange, confirming that they probably did have an antibody-mediated condition, and passive transfer of their IgG to mice resulted in some changes in NMJ function, but not as clear-cut as transfer of those with AChR antibody positive IgG;[80] moreover, clinically the patients

Figure 3. Antibodies in myasthenia gravis patients. Note that a number of antibodies have been reported in MG, but not all of them are tested widely, and there are still around 5% of patients with generalised MG who have no detectable MG-related antibody and a higher proportion of those with ocular MG.



were somewhat different, often with more bulbar features.[81] One improvement was the much later introduction of the cell-based "clustered AChR' antibody test which detected antibodies in a proportion of those who were otherwise seronegative.[82] More exciting, was the discovery in 1994 by DeChiara et al. of a new potential antigen at the NMJ, muscle specific kinase (MuSK),[83] and the subsequent identification of MuSK's interaction partner low density lipoprotein-related protein 4 (LRP4).[84] Antibodies to MuSK[85,86] and LRP4 are now detected routinely in many labs, by radio-immunoprecipitation or cell-based assays. These patients can be severely affected with weakness and long-term muscle atrophy often predominant in the facial, bulbar and respiratory muscles,[87] and they have been difficult to treat effectively. The thymus is seldom hyperplastic, and thymectomy is not usually undertaken.[88] Intriguingly, however, they respond well to rituximab, and indeed better than the patients with AChR antibodies.[89] Nevertheless, some patients relapse which has provided an opportunity to explore the characteristics of the emerging B cells ($CD27^{high}CD38^{high}$ plasmablasts) and to identify the affinity-matured MuSK antibodies they produce.[90]

MuSK antibodies are different from AChR antibodies since they are mainly IgG4, not IgG1, they are monovalent, and they inhibit the interaction between LRP4 and MuSK that initiates MuSK phosphorylation and AChR clustering during development, and maintains AChR clusters in mature muscle.[91,92] In MuSK-MG, monovalent cloned human IgG4 antibodies had more pathogenic potential than the same antibodies when made divalent.[93] On the other hand, IgG1,2 and 3 MuSK antibodies exist in most patients and they also reduce AChR clusters in vitro.[92] However, instead of inhibiting MuSK phosphorylation as IgG4 antibodies do, they either have no effect (Cao et al. in preparation) or enhance MuSK phosphorylation.[94] IgG4 antibodies are proving to be of particular interest in a number of antibody-mediated diseases, including several that affect the central nervous system[95], but in most conditions co-existing divalent IgG1-3 antibodies exist and the mechanisms need to be explored comprehensively.

Since the discovery of MuSK antibodies, LRP4, agrin and other neuromuscular junction proteins have been tested for antibody binding (see Figure 3). Although antibodies to these proteins can be found in a minority of patients, they are not widely tested in routine laboratories, and despite many attempts by a number of research centres, there remain some patients (perhaps 5%), usually with relatively mild symptoms, who are persistently negative.

Final comments

There is a long history of research into the neuromuscular junction and the diseases that affect it; myasthenia gravis remains one of the best studied neurological diseases, and has provided a model, although with some obvious limitations, for understanding and treatment of the now well-defined antibody-mediated disorders of the central nervous system.

There are new approaches to study of myasthenia gravis that have flourished over the last 20 years, particularly in genetics, human derived monoclonal antibodies, biomarkers such as miRNAs, and trials of better targeted immunotherapies. Nevertheless, there are still many aspects that are

unexplained and deserve further research, some are now being investigated more intensively as was clear in the 2022 meeting (Figure 1), particularly ocular MG, novel biomarkers and the roles of complement and fetal FCR.

Conflicts of interest

I received a proportion of royalties for MuSK antibody assays until 2021. No other disclosures.

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Table. Important developments in the early research into myasthenia gravis

Year	Author	Observations
1672	Willis (1)	A woman with long-standing paralysis that affected her limbs and her tongue. "She speaks freely and readily enough for a while, but after a long period of speech ... she is not able to speak a word and is as mute as a fish. Her voice does not return for one or two hours". Hypothesis: a failure of some circulating substance to reach the muscles.
1895	Jolly (4)	Repetitive stimulation of the nerve that innervates a muscle produces a decreasing muscle contraction in MG patients, which explains their weakness and fatigue.
1901	Weigert (6)	Collections of lymphocytes ('lymphorrhages') in muscle and other tissues (but not brain) from MG patients.
1905	Buzzard (7)	Hypothesis: a circulating toxin, possibly an 'autotoxic' agent, was the cause of the disorder.
1934	Walker (8)	Mary Walker, recognizing the similarities between MG and curare poisoning, tried the curare antidote, physostigmine, with success in an MG patient.
1952	Fatt and Katz (14)	First demonstration of miniature end-plate potentials using fine glass electrodes inserted into muscle fibres. Acetylcholine is released in small quanta that cause small depolarisations of the muscle membrane.
1960	Nastuk (10)	Cytolytic effect of MG sera on frog muscle fibres in vitro and MG sera contain a complement-activating substance.
1960	Strauss (11)	Complement-fixing antibodies specific for muscle fibres in MG. IgG and complement are involved in MG.
1960	Simpson (13)	The female bias, fluctuating course, other autoimmune disorders, thymic abnormalities, and transfer of myasthenia to neonates indicated a circulating immunoglobulin was responsible for MG. Hypothesis: MG caused by an antibody to an "endplate (NMJ)" protein
1962	Chang and Lee (16)	Demonstrated that bungarotoxin from Bungarus multicinctus bound to postsynaptic membrane blocked neuromuscular transmission. Hyp: it binds to the muscle acetylcholine receptor.
1964	Elmqvist et al. (15)	First description of reduced miniature end-plate potentials at NMJs of MG patient. Could be pre- or post-synaptic; but they concluded that a reduction in acetylcholine release was more likely than a reduction in the postsynaptic response.

1968	Cuatrecasas (18)	Showed how a ligand bound to an insoluble substance (such as bead polymers) could be used to purify the receptor for that ligand.
1970 – 1972	Changeux and Miledi (17,18)	Cuatrecasas method employed cobra-toxins to purify AChRs from torpedo and eel electric organs. The AChR is a membrane, detergent-soluble protein that retains bungarotoxin binding in solution.
1973	Patrick and Lindstrom (20)	Rabbits immunized against purified electric eel AChR developed weakness, that responded to anti-cholinesterase. Hyp: an experimental model of MG.
1973	Fambrough, Drachmann and Satyamurti (21)	Used radioactive bungarotoxin to measure AChRs and found reduced AChRs in MG muscle.
1974	Almon et al. (22)	MG sera inhibit binding of ^{125}I-α-bungarotoxin binding to rat denervated muscle AChR. First demonstration of effect of MG antibodies on AChR.
1976	Lindstrom et al. (23)	Radio-immunoprecipitation by patient IgG antibodies of ^{125}I-α-bungarotoxin human AChR demonstrated in 85% of patients.
1975, 1977	Toyka et al. (24)	Injection of immunoglobulin G from MG patients into mice produced weakness and a reduction in the number of AChRs at the NMJ.
1977 1978	Pinching et al. (26) Newsom-Davis et al. (27)	Plasma exchange, which removes circulating antibodies and other soluble factors, produced a marked clinical improvement. For an individual MG patient, the clinical benefit correlated inversely with the level of AChR specific antibody.
1980	Engel et al. (33)	Both IgG and complement present at the NMJs of MG patients and co-localize with the remaining AChRs

These landmarks are focused on early observations and the most relevant work of the 1970s.
Hypothesis = hypothesis-generating.