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Pathophysiology of Childhood-Onset Myasthenia: What is happening at the neuromuscular junction?

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ABSTRACT

Myasthenia is caused by abnormalities in signal transduction at the neuromuscular junction. Its pathophysiology which is broadly classified into acquired myasthenia gravis and congenital myasthenic syndrome has been elucidated and its treatment has progressed over the past half century. Childhood-onset myasthenia gravis is less common than adult-onset myasthenia gravis, and therefore the pathophysiology has not been well studied, and treatment has continued to be based on research in the adult setting. However, treatment of children should be based on an understanding of their pathophysiology, and research on pathophysiology and treatment methods that take into account their unique growth and development is desired. In recent years, studies on myasthenia have been reported from around the world, confirming that the pattern of the number of patients by age of onset differs between East Asia and Western Europe, and that the Japanese characteristic of a high incidence in childhood is widely seen in East Asia. Furthermore, differences in the incidence of congenital myasthenic syndrome between East Asia and Western Europe are also evident. Thus, there are racial differences in the pathophysiology of myasthenia based on genetic background, and their pathophysiology and relevance are gradually becoming clear.

Congenital myasthenic syndrome is caused by genetic defects in various proteins involved in the assembly of AChRs at the neuromuscular junction, resulting in impaired neuromuscular signaling and the appearance of myasthenic symptoms. On the other hand, myasthenia gravis which is a T cell-dependent, antibody-producing autoimmune disease, is caused by autoantibodies against several proteins, mainly AChR antibodies. These proteins, like congenital myasthenic syndrome, are involved in the assembly of AChRs at the neuromuscular junction. Autoantibodies to these proteins prevent the assembly of AChRs, resulting in myasthenic symptoms due to the inability of neuromuscular signaling.

The ocular muscle type, which is common not only in Japan but also in East Asia, has low antibody titers and seronegative MG is relatively common. In this review, I would like to summarize and discuss what is happening at the neuromuscular junction in myasthenia, especially the pathophysiology of myasthenia gravis with low antibody titers from various viewpoints, such as the presence of antibodies to neuromuscular junction proteins, the inability to measure antibody titers due to measurement sensitivity issues, diversity by muscle site or type, or involvement of cellular immunity.

Introduction

Myasthenia gravis (MG) is a neuromuscular disorder caused by an immune disturbance at the neuromuscular junction, resulting in symptoms such as muscle weakness and fatigue. In 1964, Elmqvist et al. reported abnormalities in miniature endplate potential, and demonstrated that MG is a neuromuscular junction disease [1], and in 1973, Patrick et al. demonstrated that immunization of rabbits with the acetylcholine receptor (AChR) could induce a myasthenic state similar to that in humans [2]. This demonstrated that the pathogenesis of this disease is an autoimmune disease of the neuromuscular junction. Subsequently, studies of the pathogenesis of MG have progressed in Europe and the United States, and myasthenic conditions caused by genetic mechanisms in addition to autoimmune diseases have also been investigated.

Currently, myasthenia, which is caused by impaired signal transduction at the neuromuscular junction, can be broadly divided into acquired MG and congenital myasthenic syndrome (CMS). The condition commonly referred to as MG is an acquired MG, which is a T cell-dependent, antibody-producing condition that results in the production of autoantibodies, which has been shown to decrease AChR at the neuromuscular junction [3]. Most of this pathology is caused by autoantibodies, represented by AChR antibodies, but it has been established that complement is also involved at this time, with morphological destruction of the neuromuscular junction. A series of antibodies, including AChR antibodies, have been shown to be antibodies against proteins involved in the assembly of AChR at the neuromuscular junction [4-6]. On the other hand, congenital myasthenic syndrome is a condition caused by a genetic defect in a protein involved in signal transduction at the neuromuscular junction that results in the failure to produce the protein [4-5].

While much has been revealed in Western research on whether the signaling disorder at the neuromuscular junction is caused by an immunological mechanism or a genetic abnormality, various studies have been conducted in Japan, mainly by the Intractable Disease Study Group under the jurisdiction of the Ministry of Health, Labor and Welfare, and the results have been accumulating. In the 21st century, studies in this field have been reported from various regions and countries around the world, as well as studies in the pediatric field, and it has become clear that this disease has subtly different pathophysiology in each region and that medical care is practiced in the medical culture fostered in that region [4-6].

When people travel around the world and live in different medical cultures, they will receive medical care in the region in which they reside. When a person who has moved from a different region develops MG, we as medical professionals must adequately estimate the pathophysiology of the patient in front of us. As a pediatrician who has been practicing clinically in Japan for many years, I would like to offer a glimpse into the heterogeneity of myasthenia, a pathophysiology that has been developed and clarified over the past half century.

(1) FORMATION OF NEUROMUSCULAR JUNCTION

The neuromuscular junction must be well formed for rapid and reliable neuromuscular signaling. Denervation of rat leg muscles promotes the synthesis and distribution of AChRs in the postsynaptic membrane, which can be divided into two types: the fatal type and the adult type. The adult type AChR consists of α, β, γ, δ, and ε -subunit, and the fatal type consists of α, β, γ, δ-subunit [7]. Surgical denervation and/or presynaptic blockade of neuromuscular transmission amplifies subunits of mRNA of AChR in muscle cells, junctional and extra-junctional AChRs are increased, and are distributed in the postsynaptic membranes [8]. The extra-junctional AChRs are said to have γ-subunits rather than ε -subunits [8].

AChR proteins synthesized in muscle cells are assembled at specific sites to form neuromuscular junctions. This involves not only stimulation from nerves, but also a number of proteins. (Figure) Nerve terminals secrete agrin, which binds to LRP4 and activates the MuSK molecule. The complex forms a dimer and activates Dok-7 in muscle cells, and when AChE/ColQ protein binds to the MuSK protein molecule, the complex is further activated, bringing AChRs that were widely and thinly distributed in the vicinity of the MuSK to the MuSK neighborhood and building the neuromuscular junction [9]

A number of proteins are involved in the assembly of AChRs at the neuromuscular junction, and mutations in these proteins or the production of autoantibodies against them can impair neuromuscular signaling. Mutations that prevent the formation of normal protein molecules, resulting in impaired neurotransmission, are called CMS. On the other hand, acquired myasthenia gravis is a condition in which autoantibodies are the primary etiologic factor causing MG symptoms by disrupting signaling at the neuromuscular junction. Currently autoantibodies recognized to cause MG are AChR antibody, MuSK antibody, LRP4 antibody, and anti-agrin antibody. However, autoantibodies against various other proteins involved in neuromuscular junction formation are still under investigation.

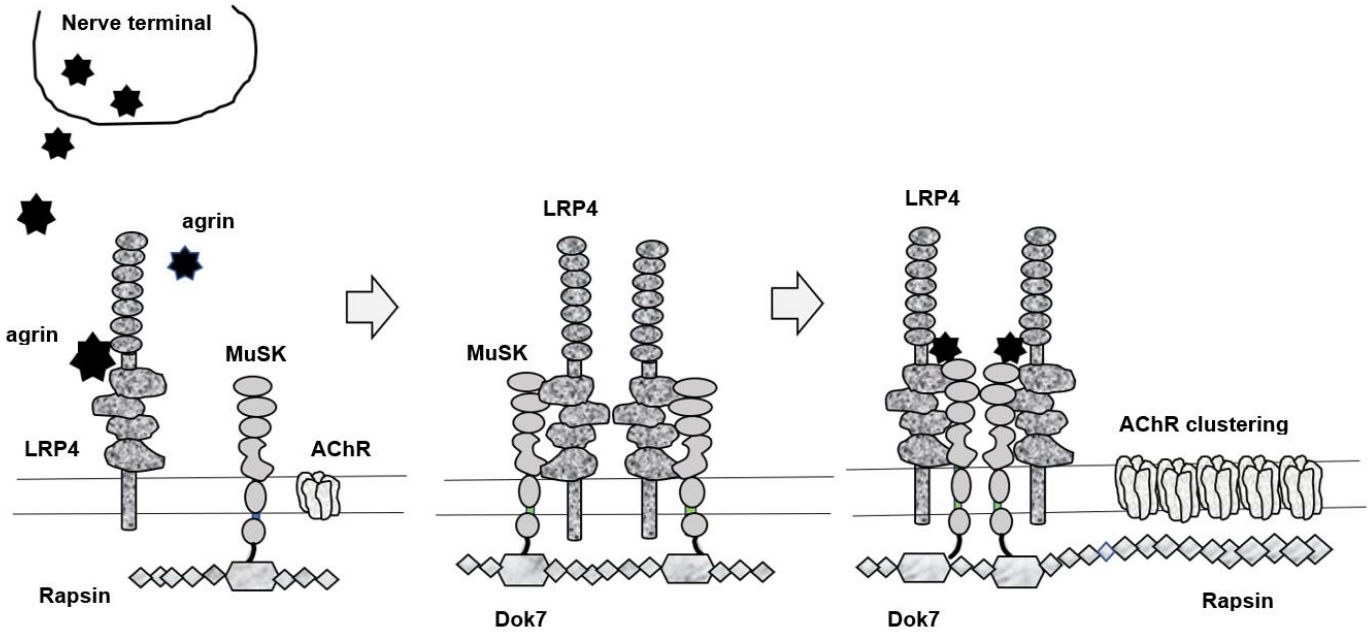

Figure: AChR clustering (Hayashi, No To Hattatsu 2022 (Ref.4), Pathophysiology 2023(Ref.5), and Clin Exp Neuroimmunol 2023(Ref.6))

(2) CHANGES AT THE NEUROMUSCULAR JUNCTION

In myasthenia gravis, there is destruction of the neuromuscular junction and a decrease in AChR. The gap between nerve endings and muscle, called the synapse at the neuromuscular junction, is inherently narrow, and degranulated acetylcholine molecules diffuse to the postsynaptic membrane on the opposite muscle side and then bind to AChRs present on the postsynaptic membrane. In 1973 Fambrough et al. performed muscle biopsies of 8 MG patients and measured AChR density at the neuromuscular junction and reported that AChR density was reduced to 11-30% in MG compared to normal subjects [3]. Also, in 1974 Almon et al. reacted AChR extracted from rat leg muscle with patient serum and reported that the binding activity of α-bungarotoxin to AChR was reduced in the serum of at least 5 of 15 MG patients [12]. Furthermore, in 1975 Bender et al. demonstrated morphologically that α-bungarotoxin bound to human muscle tissue disappears when reacted with patient serum [13]. This indicates that AChR present in the postsynaptic membrane reacts with patient serum, thereby reducing its density at the neuromuscular junction in MG patients themselves. Thus, it was established that the pathogenesis of MG is an autoimmune disease in which antibodies in the patient's blood act to disrupt signaling at the neuromuscular junction.

Engel et al. reported that normal neuromuscular junctions have a narrow synaptic gap and well-constructed synaptic folds, whereas in MG, the synaptic gap is enlarged, the synaptic folds are lost, and debris is seen in the enlarged synaptic gap [14]. This is caused by complement reacting with autoantibodies as described below.

(3) AUTOANTIBODIES TO THE NEUROMUSCULAR JUNCTION

In acquired MG, autoantibodies against the neuromuscular junction are formed, and the neuromuscular information exchange is impaired by these autoantibodies. There are three possible mechanisms: binding antibodies, blocking antibodies, and complement involvement [15].

(3-1) Involvement of AChR antibodies

First, binding antibodies often recognize the α-subunit of AChR, and the Fab moiety of the antibody binds to the AChR and bridges the two AChRs, thereby accelerating their uptake into muscle cells and increasing their decay rate [15-17]. Generally, the term AChR antibody refers to a binding antibody.

Second, the blocking antibody recognizes and binds to the ACh-binding site or its vicinity in the α-subunit of AChR [18,19]. The AChR is composed of two α-subunits and one each of β, γ (or ε), and δ. The ACh binding site exists at the contact sites of the α/γ (or α/ε) and α/δ subunits [20]. The α-subunit is a protein consisting of 437 amino acids linearly linked together, but each amino acid has various electric charges and thus assumes a three-dimensional structure and penetrates the muscle cell membrane four times. The N-terminal 210 amino acids are completely outside the membrane, and ACh with a molecular weight of 146 and antibodies with a molecular weight of approximately 150,000 react with that part of the subunit. When binding of blocking antibodies that recognize that region or its vicinity occurs, the small ACh binding site is occupied by a large antibody, or the surrounding structure is altered by the binding of the blocking antibody, making ACh binding impossible [18,19].

Third, there is a mechanism by which complement acts to disrupt the morphology of the neuromuscular junction [14,21]. The morphological changes in the neuromuscular junction caused by complement result in a wide synaptic gap and coarse distribution of AChRs, which allows little information exchange [22]. When complement repeats the reaction up to C9, it forms a membrane attack complex (MAC) and destroys the membrane [21]. When the presence of complement is confirmed at the neuromuscular junction [23] and the complement component C3 is removed using snake venom, this synaptic destruction is no longer seen and symptoms improve [24]. Recently, drugs that inhibit complement activity have been used with some success.

(3-2) Neonatal transient myasthenia gravis and fetal myasthenia gravis

Some infants born to MG mothers develop neonatal transient myasthenia gravis [25-28]. IgG antibodies are transferred to the fetus via the placenta except for subclass IgG2, but most AChR antibodies are IgG1 and IgG3. Therefore, if the mother's antibody titer is high, the antibodies are naturally transferred to the fetus and symptoms should appear. However, only 10-12% of newborns develop MG, and most are asymptomatic despite having AChR antibodies in their blood [29]. It is not well understood why newborns do not develop the disease.

The antibodies that cause this neonatal transient myasthenia gravis decrease over time because they are not due to antibodies produced by the child itself, but are transplacental transfer of maternal antibodies. Even if symptoms appear in the first few days of life, they gradually subside with continued treatment and management during the weeks of symptoms.

In rare cases, if the mother's antibodies are specific and transfer to the fetus in high concentrations and are strong enough to inhibit movement in utero, the fetus may develop a condition called congenital joint contracture (arthrogryposis congenita) [30,31].

(3-3) Antibodies against Muscle Specific Tyrosine Kinase

When AChR antibodies are negative, it is called seronegative MG; in 2001, Hoch et al. reported that antibodies against muscle specific tyrosine kinase (MuSK) are present in 70% of seronegative MG, which accounts for 20% of generalized MG [32]. In the same group, McConville et al. reported that in 66 patients with seronegative MG, 27 (41%) were positive for MuSK antibodies, 11 of whom had prominent bulbar symptoms [33]. Several subsequent reports have shown that in Caucasians, approximately 20% of seronegative MG are MuSK antibody positive, and 30-40% of generalized seronegatie MG are MuSK antibody positive [34,35], and a report from the Mayo Clinic showed that 38% of adult seronegative MG were positive for MuSK antibodies [36]. In contrast, a survey of adult MG in Asia showed that 26.4% in China [37] and 26.7% in Korea [38] were positive for MuSK antibodies. It was reported that MuSK antibodies were positive in 12 (6.7%) of 180 patients with childhood MG with onset before 14 years of age in China in whom antibodies were measured, but the details are not clear [39], and we have not been able to find any reports of pediatric MuSK-MG from Korea. From Taiwan, there is a report that only 1 of 36 AChR antibody-negative patients with juvenile MG was positive for MuSK antibody [40]. In Japan, Ohta et al. reported that 27% of 85 patients with generalized seronegative MG had MuSK-MG [41], but the epidemiology of pediatric MuSK-MG is poorly investigated and only case reports are available for pediatric cases [42,43]. Thus, subtle differences in the frequency of the disease exist by region and race.

The subclass of MuSK antibodies is mainly IgG4, which, like AChR antibodies, crosses the placenta to cause symptoms, and neonatal transient myasthenia gravis has been reported from mothers with MuSK-MG [44]. It is rare in the ocular muscle type with MuSK-MG [45,46].

MuSK is responsible for the assembly of AChRs on the postsynaptic membrane in collaboration with several protein molecules to facilitate efficient signal transduction at the neuromuscular junction. MuSK antibodies have an adverse effect on AChR assembly and reduce functional AChRs, but histopathology does not confirm the loss of AChRs [47]. AChR antibodies are predominantly of the IgG1 and IgG3 subclasses, while MuSK antibodies are predominantly of the IgG4 subclass, which crosses the placenta but does not bind complement and is an IgG monomer. Konectzny et al. examined the sera of 14 MuSK-MG patients and found that MuSK antibodies were predominantly IgG4 monovalent with some IgG1-3. MuSK antibodies do not cause intracellular uptake of MuSK and impair agrin-induced AChR aggregation, resulting in MG symptoms [48,49].

The most common sites of symptoms in MuSK-MG are the face, neck, articulatory swallowing, and respiratory muscles, where muscle weakness and atrophy occur. This may be due to differences in the structure of the neuromuscular junction in these muscles, as well as differences in response, such as lower MuSK expression in the scapulohyoid muscles [50]. MuSK-MG presents with bulbar symptoms, is easily severe, and may become more severe with the use of anticholinesterase agents, and the thymus gland is normal and therefore thymectomy is not generally performed. Thus, the pathophysiology of MuSK-MG and AChR antibody-positive MG is different.

MuSK, a protein present at the neuromuscular junction, is essential for AChRs to cluster at the neuromuscular junction, but when MuSK is deficient or when antibodies inhibit the original function of MuSK, AChRs cannot cluster, resulting in the inefficient transmission of information from nerves and the development of MG. Subsequently, it was found that several antibodies other than AChR and MuSK antibodies cause MG.

(3-4) Double or triple seronegative myasthenia gravis

When both AChR and MuSK antibodies are negative, it is called double seronegative MG, and when LRP4 antibody is also negative, it is called triple seronegative MG. Rodriguez Cruz et al. reported that of 42 MG patients considered to have double seronegative MG measured by immunoprecipitation, 16 (38.1%) were positive for AChR antibodies measured by cell-based assay [51]. In the same article, 26 patients were also negative by cell-based assay, and LRP4 antibodies were negative in all 21 patients who could be tested. However, Pevzner et al. reported that sera from 12 of 13 double seronegative MG patients showed protein deposition at the neuromuscular junction in mice, and sera from 4 of the 13 patients suppressed AChR aggregation on cultured muscle cells by more than 50% [52]. Higuchi et al. reported that 9 of 300 AChR antibody-negative MG patients had LRP4 antibody, and 3 of these 9 were also positive for MuSK antibody [53]. LRP4 antibody-positive MGs are present among double seronegative MGs, but vary from 2-45% by region [54, 55].

Recently, antibodies against agrin have also been investigated, and their antibodies have been detected in double or triple seronegative MG by measuring

antibodies against AChR, MuSK, and LRP4 [56-59]. LRP4 and agrin are also involved in AChR assembly together with MuSK. The presence of these antibodies inhibits AChR assembly and disrupts signaling at the neuromuscular junction.

In general, four conditions are required for autoantibodies to be causative of the disease [60]. (1) the antibody must be identifiable in the patient's serum, (2) passive immunization of the patient's serum must cause the characteristic pathology, (3) active immunization with the antigen must cause the disease, and (4) removal of the antibody must improve symptoms. For AChR antibodies, all of these conditions are satisfied. Looking at antibodies against MuSK, LRP4 and agrin for these conditions, Shigemoto et al. induced MG symptoms in rabbits immunized with MuSK protein and pathologically confirmed a decrease in AChR aggregation at the neuromuscular junction [61]. Viegas et al. created a MuSK-MG condition in mice by passive immunization with MuSK antibodies as well as active immunization with MuSK protein [62]. Similarly, Shen et al. actively immunized mice with the extracellular domain of LRP4 protein to induce MG symptoms, and passively immunized mice with serum from rabbits immunized with LRP4 protein to induce the same MG symptoms [55]. Ulsoy et al. [63] and Mori et al. [64] also observed MG symptoms in mice immunized with LRP4 and created LRP4-autoimmune animals. Yu et al. suppressed MuSK phosphorylation and AChR aggregation by passively immunizing mice with immunoglobulin generated from MG patient sera with LRP4/agrin antibodies [65], and Yan et al. immunized mice with agrin and caused MG symptoms [58].

The acquired MG condition is thought to be caused by the formation of autoantibodies against protein substances at the neuromuscular junction. Currently approved antibodies available are AChR antibody, MuSK antibody, LRP4 antibody, and anti-agrin antibody. There are many other proteins involved in AChR cluster formation, and several antibodies against these proteins have also been identified. It remains to be verified whether these antibodies are really involved in the pathogenesis of the disease.

(4) CONGENITAL MYASTHENIC SYNDROME

Congenital myasthenic syndrome (CMS) is characterized by pathological muscle weakness and fatigability caused by an inborn defect of a protein molecule at the neuromuscular junction. Although the onset of the syndrome is characterized by onset at age 2 years or younger, there are many cases in which muscle weakness at birth is mild and develops during childhood or adulthood, requiring a detailed medical and family history at birth. In addition, CMS is often complicated by muscle atrophy and small deformities, making it important to differentiate CMS from MG as well as from muscular dystrophy, congenital myopathy, and other disorders. Repeated nerve stimulation is essential for definitive diagnosis, and careful repeated nerve stimulation is recommended in cases of muscle weakness and atrophy without elevated CK [66].

A report from the United Kingdom showed a high prevalence of 9.2 per million for CMS compared to 1.5 per million for childhood-onset autoimmune MG in those under 18 years of age [67]. A Mayo Clinic report showed an incidence rate of 1.2 per million for autoimmune MG and 2.3 per million for CMS in childhood-onset myasthenia under 19 years of age [68]. No epidemiological reports on CMS have been seen from Asia, only sporadic reports. From Japan, Azuma et al. reported 4 patients with ColQ abnormalities and 5 of their mutations, and 5 patients with AChR abnormalities and 6 of their mutations, for a total of 9 patients and 11 of their mutations, but they are considered likely to be underdiagnosis [69]. Thus, comparing the frequency of childhood MG in Europe, the United States, and East Asia, CMS shows a contrasting pattern of onset, with a high incidence in Europe and the United States and a low incidence in East Asia, indicating a racial difference.

Ohno et al. recently reported a review summarizing 35 different genetic abnormalities [70]. It is possible that other unknown proteins are involved in this final stage of AChR assembly, and we must wait for further studies.

(5) PATHOPHYSIOLOGICAL CLASSIFICATION OF MYASTHENIA GRAVIS

(5-1) Ocular and generalized myasthenia gravis

MG can be broadly classified into ocular MG and generalized MG. It is known that the ocular type is more common in Japanese children with MG, and that the AChR antibody titer is low and the ratio of negative titers is high [71]. In an epidemiological survey in Japan in 2006, ocular MG accounted for 80.6% of cases occurring at less than 5 years of age and 61.5% of cases occurring at 5 to 10 years of age [72]. As shown in the table, the ocular MG accounts for a higher proportion of pediatric MG in East Asia than in the West [39, 40, 68, 72-77]. In addition, cases initially considered to be ocular MG are characterized by the fact that in East Asia, the clinical course of the disease does not often shift to a generalized type [39,40,73,75]. In contrast, ocular MG cases in Europe and the United States frequently develop into the generalized type, often within 2 years after onset [76,77]. Thus, there are several differences between East Asian and Western pediatric MG.

Table: Comparison of childhood-onset MG; Ocular MG is often in Asia. (Hayashi, Pathophysiology 2023 (Ref. 5))

	Author [reference]	Year	Nation	Number of patients	OMG	Onset of Age(yr)	OMG to GMG
Asia	Murai [73]	2011	Japan	268	80%	<10	
	Lee HN [73]	2016	Korea	88	97%	<18	
	Huang X [74]	2013	China	964	88%	<14	
	Gui [75]	2015	China	424	83%	<14	11.8%
	Yang L [39]	2022	China	343	93%	<14	13.4%
	Chou CC [40]	2019	Taiwan	54	83%	<20	4.8%
Western	Mansukhani [68]	2019	USA	146	23%	<19	
	Popperud [76]	2017	Norway	63	59%	<18	
	Vecchio [77]	2020	UK	74	51%	<16	

Why is the ocular MG more common and why is the ocular muscle more likely to be affected [5,49,80]? Comparing the neuromuscular junction of the limb muscles with that of the ocular muscles, there are several electrophysiological differences: the ocular muscles require rapid and complex movements of the eyeballs, and they are also required to hold still. In addition, the AChR subunits differ, and it is known that ε is replaced by γ in the AChR subunit of the eye muscle, whereas the limb muscle AChR originally consists of four types, α, β, ε, and δ [81]. The γ-subunit forms the fetal AChR and in rodents many muscles are replaced by the ε-type at about 1 week of age [82]. However, this replacement does not occur in ocular muscles. It has been reported that the neuromuscular junction of ocular muscles has a more complex morphology than that of limb muscles [83], with ε-subunits in simple innervated neuromuscular junctions such as soleus muscle and γ-subunits in multiple innervated neuromuscular junctions of the external eye muscle [82-84]. These differences in neuromuscular junctions allow for detailed and complex eye movements, and the absolute difference in muscle size and thickness creates the so-called "safety factor", which explains why the eye muscles with less margin are more prone to symptoms. It has been explained that symptoms are more likely to occur in the eye muscles with less room to spare [85,86].

Seronegative MG is common in pediatric MG with ocular type [71]. Whether or not antibodies are truly absent is a major issue, and Tsujihata et al. confirmed deposition of anti-AChR antibodies at the neuromuscular junction of limb muscles by performing limb muscle biopsies in patients with ocular MG [23]. It was also found that patients thought to have seronegative MG were able to detect antibody titers against cell-bound AChR [51,87]. In Japan, Oda reported in 1993 that antibodies could be identified in sera of ocular MG that were negative by the conventional immunoprecipitation assay method, using the cell-bound AChR assay method with human ocular muscle as antigen [88].

5-2) Latent generalized myasthenia gravis

Segawa and Nomura proposed the concept of latent generalized MG by diagnosing latent generalized MG as a condition in which systemic symptoms do not appear, and only ocular symptoms are present, although the pathophysiology extends to all muscles of the body [89]. Since the pathophysiology of MG involves autoantibodies, the latent generalized type of MG is considered to be diagnosed when there are no clinical symptoms in the whole body yet, but evoked electromyography of the limb muscles shows waning, since antibodies must be deposited in other muscles throughout the body, even if the symptoms are only in the ocular muscles. The deposition of AChR antibodies at the limb neuromuscular junctions in patients with ocular symptoms, reported by Tsujihata et al., illustrates this concept [23]. As the advent of cell-based assays has made clear, even low titers of AChR antibodies in the blood may be firmly deposited in the tissues. The treatment of a patient diagnosed with the ocular type may change markedly when the patient is diagnosed with the latent generalized type.

This subtype is not used in the MG disease classification commonly used in the world today, but it is widely used in the pediatric field in Japan [6,90]. MG often starts with ocular symptoms at the onset, and can be divided into two groups: one that develops into a systemic form and the other that remains ocularly symptomatic. It is also possible that some cases diagnosed as generalized MG during the course of the disease may be judged as waning positive in the evoked electromyography before developing into the systemic form [91], although the uncertainty of the evoked electromyography itself remains [92]. Such cases are called latent generalized MG. Early identification of the generalized type among the ocular types has the advantage of early therapeutic intervention. Therefore, when latent generalized MG is diagnosed, even if only ocular symptoms are observed, treatment is considered to be for the generalized type.

(6) CHILDHOOD THYMUS AND THYMIC SELECTION

Myasthenia gravis is often associated with thymic abnormalities such as thymoma and thymic hyperplasia. Without distinguishing between thymoma and thymic hyperplasia, Yoshikawa et al. reported thymic enlargement in 22.1% of Japanese patients [93] and 20.1% in Korean patients [94]. Murai et al. reported 32.0% for thymoma and 38.4% for thymic hyperplasia. In MG in children under 9 years of age, thymoma was 4.9% and thymic hyperplasia was 16.8% less common than in adults [72]. Similarly, in China, thymoma and thymic hyperplasia were found in 14.8% and 66.4%, respectively, while thymoma made up 2.9% and thymic hyperplasia 86.5% in pediatric MG patients aged 14 years or younger [74]. Popperud et al. reported that 50 of 63 pediatric MG patients with onset at 18 years or younger had thymectomy [76]. Of these, 13 of 21 prepubertal patients and 37 of 42 post-pubertal patients underwent thymectomy. Thymic hyperplasia occurred in 7 of 13 thymectomized prepubertal patients (54%) and 23 of 37 thymectomized postbubertal patients (62%). They reported no thymoma both before and after puberty [76]. Heckmann and others have also reported that thymoma is rare in children [95]. Thus, in both children and adults, the critical difference is whether the thymus is a tumor or hyperplasia, and if it is a thymoma, removal is the treatment of choice.

The thymus gland is an organ that all humans are born with and plays a major role in the development of immunity. If it were not present, we would have primary immunodeficiency. Blood stem cells produced in the bone marrow emerge in the peripheral blood. Lymphocytic progenitor cells are taken up by the thymus organ, where they encounter various proteins expressed on the thymic epithelial cells and pass through the steps of β-selection, positive selection, and negative selection to distinguish between self and non-self [96,97]. As a result, they emerge into the peripheral blood as selected T lymphocytes that do not respond to self. If this is not sufficient, T lymphocytes that mistakenly recognize and attack self as non-self are produced, which then attack self and cause so-called autoimmune diseases.

Thus, the thymus is essentially an important organ for the development of immunity. After the thymus has done its job of establishing immunity in the young, it atrophies, often disappearing by the age of 40 [96,98,99]. Therefore, if thymectomy is indicated in children, it should be

performed after puberty. Thymoma, which occurs more frequently in adults, has another implication. Removal of thymoma, an enlargement of the thymus gland that should have atrophied and is causing harm to the person, is considered the essential treatment.

In 1966, Perlo et al. studied the frequency by age of onset in 1355 MG patients and reported the existence of two peaks, one for the younger female and the other for the elder male, which differed according to sex [100]. Carr et al. also showed in a systematic review that there is a clear difference in age of onset [101]. Similarly, surveys in Japan have shown a pattern of different peaks of disease onset in men and women [72,93]. In 1980, Compston et al. added HLA analysis, and found that the younger female group had higher AChR antibody titers, more HLA-A1, B8 and/or DRw3, and more often presented with thymic hyperplasia. On the other hand, they reported that elder males, who are more likely to have thymoma, have lower antibody titers and more HLA-A3, B7, and/or DRw2 [102].

(7) INVOLVEMENT OF CELLULAR IMMUNITY

Several HLA differences in autoimmune MG have been reported, and Vandiedonck et al. reported a strong correlation between MG with thymic hyperplasia and the 8.1 ancestral haplotype (HLA-A1-B8-DR3) [103]. Popperud et al. studied in Norwegians [104] and found a strong correlation between this ancestral haplotype 8.1 (AH8.1; A*01-G*08-C*07-DRB1*03:01-DQB1*02:01) alleles and juvenile MG. At the same time, they reported that HLA-B*8-DRB1*04:04 was more frequent in early-onset MG in Europe at age 40 years or younger, and HLA-DRB1*15:01 alleles were more frequent in late-onset MG at age 60 years or older, especially HLA-DRB1*04:04 was predominant only in prepubertal onset and that HLA types differ between older-onset and younger-onset MG [104]. A high frequency of HLA-DRB1*03 has been reported from Sweden [105], Portugal[106] and Tunisia [107], and ancestral haplotype 8.1 is a major contributor to MG development in Europe. Looking at reports from East Asia, HLA-A*0207, HLA-B*4601, HLA-DRB1*0403, HLA-DRB1*0901, and HLA-DRB1*1602 were reported from China as HLAs with high frequency in infants [108]. From Japan, Matsuki et al. reported that the incidence of HLA-DR9 and DRw13 is high in childhood-onset MG, and even higher in DR9/DRw13 heterozygosity[109]. Shinomiya et al. reported that childhood-onset MG is highly correlated with HLA-DRB1*1302/DQA1*0102/ DQB1*0604 and HLA-DRB1*0901/DQA1*0301/DQB1 *0303 [110]. The DR13 haplotype is thought to have a close evolutionary relationship with the DR3 haplotype, which is thought to be related to Caucasian MG. Although the same East Asian populations share a common HLA type that differs from Western Europe, there may be some related immunogenetic background [110,111]. On the other hand, we examined HLA in 71 Japanese MG patients, both adults and children, and found a high frequency of HLA-DRw9 in the ocular MG and HLA-DRw8 in generalized MG [112]. In a study of the relationship between HLA and clinical course in 53 pediatric and adult MG patients, it was reported that the group with HLA-DRw8 had high AChR antibody titers and often had other autoantibodies such as antinuclear antibodies and thyroid autoantibodies. In contrast, the group with HLA-DRw9 had relatively low AChR antibody titers and no autoantibodies [113]. It seems certain that a high percentage of East Asian MGs present with ocular symptoms, mostly in children, and that these children have a high frequency of HLA-DR9 (HLA-DRB1*09;01). How these phenomena, which differ greatly between East Asia and Western Europe, are related to the onset of MG is an issue that remains to be investigated.

The human MHC-class II, HLA-DR, expresses antigen peptides bound to its pocket on the surface of antigen-presenting cells and reacts with the TCR of T cells. Berman and Patrick studied various types of mice to create an experimental autoimmune MG (EAMG) model [114]. The results showed that the Th1-predominant C57BL/6 (B6) mice, but not the Th2-predominant Balb/c mice, were EAMG-susceptible. Mouse MHC, H-2 complex, shows haplotype-b in B6 and haplotype-d in Balb/c. Christadoss et al. demonstrated that the difference in MG-susceptibility between the different types of mice is due to differences in T-cell activity to the same antigenic stimulus, which is controlled by the I-A subregion of the MHC linked to the H-2 gene [115]. McIntyre and Seidman created B6.C-H-2bm12 (bm12) mice with three mutations in I-Aβ in B6 mice [116], which affected the epitope repertoire of murine $CD4^+$ T cells [117,118] and greatly reduced the incidence of MG [119].

Myasthenia gravis is also more likely to occur when autoimmune diseases run in the family. The frequency of MG increases efficiently in twins [120]. These events indicate that MHC antigens expressed on antigen-presenting cells are transmitted within the family, making the family susceptible to MG as well as other autoimmune diseases.

Myasthenia gravis is a T cell dependent, antibody-producing autoimmune disease. Therefore, as treatment, steroids, immunosuppressive drugs, and more recently, various biological agents have been used, making it even easier to treat and achieve efficacy.

(8) LYMPHORRHAGE

Myasthenia gravis is a disease in which T cell-dependent antibody production causes damage to neuromuscular junction signaling, and autoantibodies against various proteins involved in AChR assembly, mainly AChR antibodies, are thought to be involved in the pathogenesis of this disease. In fact, anti-MuSK, anti-LRP4, and anti-agrin antibodies are thought to be involved in this condition. In reality, however, seronegative MG exists and accounts for 10% of generalized MG [121]. Oda fixed ocular muscle AChR to wells and measured AChR antibody titers in the serum of patients with ocular muscle type MG, but reported that some cases were still negative [88]. Comparison of soluble IL2 receptor (sIL2R), a marker of T-cell activation, with AChR antibody titer shows a significant negative correlation, with lower antibody titer resulting in higher sIL2R [122]. This result suggests that T cells may be activated in MG cases with low AChR antibody titers. The ocular muscle type may have low antibody titers and T-cells may be activated. In cases of patients diagnosed with seronegative MG in which no antibody can be found no matter what is done with the known antibodies, is there an antibody that has not yet been found? Or is it just that the antibody is below the sensitivity of the assay?

In the past, when many MG patients died and autopsies were performed, lymphorrhage was a major theme [123], but since then, there has been controversy regarding the existence and pathological significance of lymphorrhage. In 1963, Fenichel et al. performed muscle biopsies on 37 MG patients and divided the tissue into three groups: 15 normal, 11 small muscle group showing denervated muscle, and 12 lymphocytic infiltration group. Most of the biopsied muscles were quadriceps muscle, and the time from onset to biopsy was 1 to 8 years. Lymphocytic infiltration was found at a high rate in muscle biopsies as well as autopsies, and was more common in cases with a short period of time after onset and thymic abnormalities [124,125]. Pascuzzi and Campa biopsied triceps muscle and found lymphorrhage in the muscle endplate, pointing to a possible involvement of cellular immunity in the pathogenesis of MG [126]. Furthermore, Maselli et al. biopsied the anconeus muscle of 8 MG patients and found cellular infiltration of the neuromuscular junction in 7 patients [127]. On the other hand, Nakano et al. found inflammatory cell infiltration around the endplate in 12 of 30 patients, but the degree was mild, less than 10% of the endplate, and they claimed that lymphorrhage is a nonspecific phenomenon [128]. Most of the muscle biopsy sites were external intercostal muscles.

In animal models of MG, an infiltrate of lymphocytes is seen around the neuromuscular junction during the acute phase, which occurs when the rats are injected with antigen, and then during the chronic phase, when AChR antibody titers increase, this infiltration disappears and morphological destruction of the neuromuscular junction is observed [129]. The same phenomenon is seen in the immunization of extracted and purified AChR [129] and in the passive transfer of AChR antibodies in serum [130-132].

Since the mid-20th century, research in this area has advanced and treatment methods have changed dramatically. Until then, there were no treatment methods available to deal with the rapidly progressing pathology of MG, and muscle tissue was observed at autopsy at the end of the spontaneous course of the disease. The literature of the time analyzed various muscles and documented their pathological findings in detail. With the advent of anti-cholinesteraseagents, thymectomy, and the use of steroids, many lives were saved. With the near elimination of deaths from the disease MG, muscle tissue specimens are often obtained from localized muscle tissue such as the pectoralis major and intercostal muscles at the time of thymectomy. As studies of experimental models have shown, the pathophysiology of the disease is variable. Depending on the time point in the clinical course of the disease and the site of the lesion, the patient's clinical picture may differ and the pathophysiology may be judged differently. The classic literature has repeatedly demonstrated the importance of lymphorrhage. We believe that in some cases of seronegative MG, lymphorrhage may occur at the neuromuscular junction, leading to the onset of the disease.

Conclusion.

Recent progress in epidemiological studies of childhood myasthenia and treatment methods has made it possible to compare the pathophysiology of MG around the world based on reports from various regions. The results have revealed subtle racial and regional differences in the frequency of autoimmune MG and CMS based on genetic differences, as well as diversity in the pathophysiology and treatment of myasthenia associated with the medical history fostered in each country and region.

Research on the pathophysiology of myasthenia has made great strides in the past half century, and a variety of treatment options are now available, including the administration of immunosuppressive agents and molecular biological agents. This review did not address treatment, but outlined the pathophysiology of myasthenia in general. A thorough understanding of the pathophysiology is necessary for appropriate treatment. Children differ significantly from adults in that they are growing and developing. The frequency of childhood MG is clearly less than that of adult-onset MG, and the basic diagnosis and treatment of MG is often chosen to mimic that of adult MG, assuming that the pathophysiology of the disease also reflects that in adults. However, it is clear, as mentioned above, that there is a unique pathophysiology of MG in children, and treatment methods should be selected accordingly. Further accumulation of research is expected.

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