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HLA in myasthenia gravis: From superficial correlation to underlying mechanism

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ABSTRACT

Myasthenia gravis (MG) is a rare autoimmune disease characterized by muscle weakness and abnormal fatigability. Like many other autoimmune diseases, genetic contribution to MG has been studied where the HLA system appears to play the most vital role. Although many correlations have been revealed in these studies, the underlying mechanism for them is still in the veil. Based on current evidence, we propose two synergetic mechanisms underlying the MG predisposition via HLA. In brief, the first advocates specific MHC II-peptide patterns that influence the efficacy of antigen presentation, and the second emphasizes the role of classical MHC alleles in shaping the TCR repertoire for MG predisposition. Besides, possible explanations for unresolved or controversial MG-related epidemiological phenomenon or clinical problems are addressed as well. Then, we discuss three factors influencing the effect of HLA on MG: gender discrepancy, inflammatory microenvironment, and epigenetic regulation. Lastly, from a provisional angle, we introduce several precautious treatments for people highly predisposed to MG. Although this is a review focusing on MG, the underlying mechanisms might be applicable in other autoimmune diseases as well.

1. Introduction

As the most prevalent neuromuscular junction disorder, MG is a B-cell driven, T-cell dependent, complement and antibody-mediated disease caused by autoantibodies (Abs) against components of the post-synaptic muscle endplate. According to the types of serum antibodies, MG can be classified into acetylcholine receptor (AChR) Ab positive, muscle-specific kinase (MUSK) Ab positive, lipoprotein-related protein 4 Ab positive, and serum antibody-negative MG [1]. Classified by the affected muscles, MG is divided into ocular MG (oMG) and generalised MG (gMG), the former of which may convert to the generalised form in some patients. Patients who are serum anti-AChR-Ab positive but without thymoma, are grouped into early-onset (EOMG) or late-onset (LOMG) forms [2]. Twin studies in the latest 50 years showed that the concordance of MG was around 35% in monozygotic twins, supporting a role of genetic factor in MG etiology [3]. Hence, the considerably large genetic contribution in MG predisposition is still worth to be studied.

The human leukocyte antigen (HLA) locus, also known as the major histocompatibility complex (MHC) locus spans around 4 Mbp on the short arm of chromosome 6 and can be divided into class II, III, I regions (Fig. 1b). MHC proteins, which are encoded by the classical HLA genes (A, B, C, DP, DQ, and DR), present the autoantigen peptide fragments on the cell membrane for recognition by T cells. Apart from the classical genes, other non-classic HLA genes, for example TNF-α, also participate the antigen presentation and regulate many other immunology processes [4]. Notably, the polymorphism mostly exhibits in the classical HLA genes and particularly within the region encoding the peptide-binding groove. Since antigen peptides bind to classical MHC molecules via the specific anchor residues with the peptide-binding pockets [5], some specific MHC-peptide patterns may be vulnerable to the autoimmune diseases. Besides, classic MHC molecules also play an important role in T cells selection in the thymus, where they present self-peptides to select T cells and eliminate those self-tolerant ones. In comparison to non-HLA genes, HLA plays a more significant role in the pathogenesis of MG [3,6]. Hence, in this review we start from HLA to explore the mechanisms underlying MG genetic predisposition. We also attempt to provide potential answers for some unresolved

Abbreviations: MG, myasthenia gravis; AchR, acetylcholine receptor; MUSK, muscle-specific kinase; Abs, autoantibodies; oMG, ocular MG; gMG, generalised MG; EOMG, early-onset MG; LOMG, late-onset MG; TAP2, transporter associated with antigen processing 2; TECs, thymic epithelial cells; EAMG, experimental autoimmune myasthenia gravis; MIR, main immunogenic region; EOMs, extraocular muscles; Ii, invariant chain; Treg, regulatory T; AIRE, autoimmune regulator; EB, Epstein–Barr; DCs, dendritic cells; CIITA, Class II transactivator; scFv, single-chain fragment variable; Cat, Cathepsin

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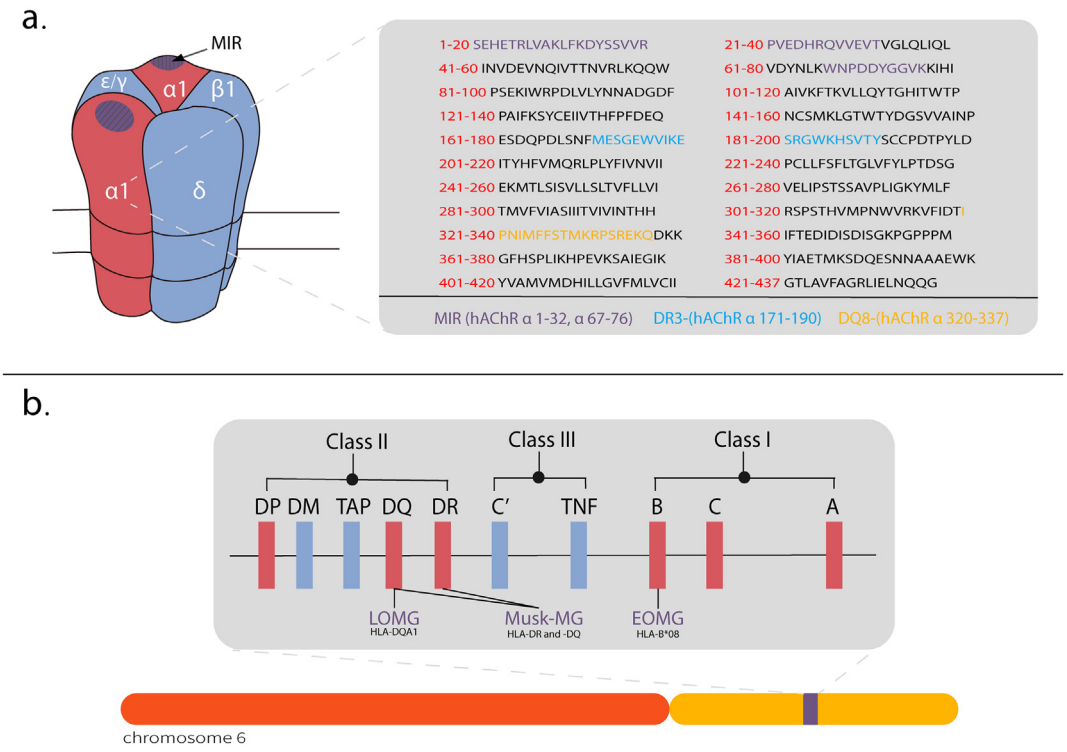

Fig. 1. Structure of muscular AChR and the HLA system. a. The muscle AChR is composed of five homologous subunits oriented like barrel staves around the ion channel, the fetal and adult forms of which are respectively α1γα1δβ1 and α1εα1δβ1. The synthesized hAChR α subunit, which is used for immunizing EAMG animals [48–50], is composed of 437 amino acid (α 1–210 extracellular, and α 211–437 intracellular). The human MIR at the extracellular apex of α1 subunit is sequence 1–32 and 67–76. α 171–190 and α 320–337 has been respectively linked with HLA-DR3 and HLA-DQ8. b. The human MHC on the short arm of chromosome 6. HLA-DQA1 is linked with LOMG, and HLA-B*08 with EOMG. Besides, HLA-DR and -DQ is related to Musk-MG. (main immunogenic region, MIR; early-onset myasthenia gravis, EOMG; late-onset myasthenia gravis, LOMG; muscle-specific kinase, Musk; complement genes, C′; tumor necrosis factors, TNF; transporter associated with antigen processing, TAP).

epidemiological or clinical problems in MG.

2. Characterization of HLA in MG

Along with the prevalence of genome-wide association studies in the recent decade, more than 300 susceptibility loci for autoimmune disease have been identified, among which the HLA region contributes to the majority of autoimmune diseases (e.g. type 1 diabetes and systemic lupus erythematosus) [7]. However, due to its low prevalence and the intrinsic heterogeneity to stratify subjects, to date, merely three large-scale genome-wide association studies regarding MG have been performed. HLA was the hottest region with EOMG [8,9] and LOMG [10]. Since EOMG and LOMG together constitute around 65% of MG population [11], and adding that other MG subgroups have also been associated with distinct HLA loci, from a single-nucleotide polymorphism perspective, HLA is indeed vital to the genetic predisposition to MG.

Based on the current evidence, several traits of HLA can be characterized in the background of MG (Table 1): 1) subtype specificity; 2) gender discrepancy; 3) ethnic and geographical disparity; 4) contribution of non-classic MHC molecules (Fig. 2).

HLA-B*08 is predisposing patients for EOMG, while HLA-DR and DQ alleles are predisposing for LOMG and MUSK-MG. As a common Caucasoid haplotype, ancestral haplotype 8.1 (HLA A1-B8-DR3-DQ2) is a multigene haplotype that has been reproducibly associated with multiple autoimmune diseases [12]. Previous studies have also verified the connection between haplotype 8.1 and MG (particularly patients with thymus hyperplasia) [13,14]. But hampered by the robust linkage disequilibrium across the HLA region, in a study of MG patients with thymus hyperplasia in 2004, the causative segment termed MYAS1 was located to a region covering TNF, HLA−B, HLA−C, and HLA-E encoding genes [15]. Since thymus hyperplasia is one of the characteristic histological features in EOMG [6], then a study in 2015 narrowed down to the haplotype with HLA-B8 and -DR3 for predisposing EOMG [16]. Moreover, other correlation studies concerning EOMG supported the participation of HLA-B*08 in EOMG predisposition as well [8,17,18]. Until recently, when controlling for the influence of the haplotype 8.1, HLA-B*08:01 was considered to be the unique genetic factor responsible for EOMG development [19]. In comparison to EOMG, other MG subtypes more favoured HLA II genes. LOMG has been linked to HLA-DQA1 in North American, Norwegian, and Italian populations [9,20], while MUSK-MG has been connected to several HLA-DR and -DQ alleles in different countries [21–25]. The conclusion can not be drawn for the rest subtypes of MG.

As many other autoimmune diseases, gender discrepancy also plays a profound role in HLA profiles for MG patients [26]. An mystery sourced from the MG epidemiology has haunted researchers for many years: the bimodal distribution with two incidence peaks–a strong female predominance in EOMG and a slight male predominance in LOMG [27]. To partially explain it, we advocate a hormone theory in which the former peak can be explained by a robust estrogen in young women and the latter peak by an age-related decline in male testosterone. Kaur et al. pinpointed three top ranked genes from the MG-associated genes affected by sex hormones: HLA-G, transporter associated with antigen processing 2 (TAP2) and HLA-DRB1 [28]. Interestingly, antigen peptide transporter 2, encoded by TAP2, directly participates the antigen presentation led by classic MHC molecules, which are under the control of HLA. Besides, when screening HLA I/II genes in EOMG, a stronger correlation between female patients and the HLA I gene-B*08 has been revealed [8,17]. Several attempts to explain the early female predominance have been made such as the polymorphisms occur in estrogen response elements of HLA genes [3], and estrogen could inhibit expression of HLA II genes in thymic epithelial cells (TECs) [29]. The

Table 1
The revealed correlations between HLA and myasthenia gravis.

Related HLA genes	Myasthenia gravis subtypes	Risk	Race	Year	References
HLA-DRB1(*)09	MG	Predisposing	Northern Chinese	2011	21,924,912
HLA-DRB1(*)08	MG	Protective			
HLA-DQA1*0101/2 and DQB1*0502 DQA1*0101/2-DQB1*0502	MG	Predisposing	Southern Iranian	2009	19,561,379
HLA-C*0701	MG	Predisposing	Swedish	2009	19,846,760
HLA class I A*31, B*08, B*39, B*40, C*15, C*17, and class II DRB1*09	MG	Predisposing	Venezuelan	2004	14,700,596
DQB1*06 and DQA1*02		Protective			
DRB1*03/DQB1*02 haplotype	Men with MG	Predisposing	Tunisian	2013	22,521,184
DRB1*04/DQB1*0302 haplotype	Women with MG	Predisposing			
DRB1*03 and DRB1*04 DRB1*03/DQB1*02 haplotype	ALL	Predisposing			
DRB1*13:01	EOMG and LOMG	Protective	Norwegian	2012	22,590,574
HLA-DQA1; rs9271871	LOMG	Predisposing			
HLA-B*08	EOMG	Predisposing			
HLA-DQA1; rs9271871	LOMG	Predisposing	North American/	2015	25,643,325
HLA-DQA1; rs601006	EOMG	Predisposing	Italian		
HLA-B*08	EOMG	Predisposing	North European	2012	23,055,271
HLA-B8-DR3 haplotype	EOMG	Predisposing	Swedish	2015	25,251,578
DQB1 *0503, *0604, *0502, and *0402	EOMG	Predisposing	Southeast Texas	2011	21,108,743
HLA-B*08:01	EOMG (who carry the ancestral haplotype 8.1)	Predisposing	Swedish	2018	29,037,440
HLA-B*08	MG (especially EOMG)	Predisposing	Saudi	2009	19,490,212
HLA-B*08	Postpubertal onset MG	Predisposing	Norwegian	2017	29,036,181
HLA-DRB1*04:04	Prepubertal onset MG	Predisposing			
HLA-A∗11:01:01, HLA-A∗24:02:01, and HLA-DPA1∗02:02:02	Juvenile-onset MG	Predisposing	Chinese	2019	30,595,166
HLA-A∗01:01:01, HLA-A∗02:03:01, HLA-C∗03:04:01, and HLA-DQB1∗06:02:01	Adult-onset MG	Predisposing			
DRB1*1302/DQA1*0102/DQB1*0604 and DRB1*0901/ DQA1*0301/DQB1*0303	Childhood-onset MG	Predisposing	Japanese	2004	15,003,812
DQB1*02	EOMG (especially women)	Predisposing	Turkish	2006	16,720,217
DQB1*0301	MG with thymoma	Predisposing			
HLA-B*50	Ocular Mg conversion to generalised MG	Predisposing	Turkish	2017	27,802,446
HLA-DQA1*03:02/DQB1*03:03:02 haplotype	Childhood-onset ocular MG	Predisposing	Southern Chinese	2012	22,503,410
HLA-B*4601/DRB1*0901 haplotype	Juvenile ocular MG	Predisposing	Southern Chinese	2015	25,953,150
DRB1*11/DQA1*0501/DQB1*0301 haplotype	Total (ocular plus generalised) MG and generalised MG	Protective	Iranian	2015	26,671,138
DRB1*04/DQA1*0301/DQB1*0302 haplotype	AChR-positive MG	Predisposing			
DRB1*16, DQA1*0102, and DQB1*05	MuSK-positive MG	Predisposing			
DRB1*14/DQA1*0104/DQB1*05 haplotype	MuSK-positive MG	Predisposing			
HLA-A02	MG with thymoma	Protective	French	2009	19,278,738
DQ A1*0401 and B1*0301	MG with thymoma	Predisposing	Northern Chinese	2012	21,917,268
DQA1*0103 and DQB1*0601	MG with thymoma	Protective			
HLA-DR11	MG (particular with thymoma)	Predisposing	Mexican Mestizo	2003	14,641,517
HLA-DRB1*14 and DQB1*05	MuSK-MG	Predisposing	Japanese	2016	27,000,234
HLA-DRB1∗14 and DRB1∗16	MuSK-MG	Predisposing	Turkish	2013	23,993,985
HLA DQB1*05	MuSK-MG	Predisposing	Serbian	2015	25,070,808
HLA DRB1*13	MuSK-MG	Protective			
HLA DRB1*03, DQA1*0501, and DQB1*0201	AChR-EOMG	Predisposing	Italian	2009	19,139,372
HLA DQB1*0502 and HLA-DQ5	MuSK-MG	Predisposing			
HLA-DR14-DQ5	MuSK-MG	Predisposing	Dutch	2006	16,769,963

late male predominance might be explained by a positive association between low testosterone levels and the risk of MG in males at an average age of 50 years [30]. However, little evidence supported the association between testosterone level and HLA genes. This finding might coincidently explain why the late male predominance is minor than the early female predominance.

Although MG patients with diversified ethnic background carried different HLA haplotypes, some common HLA loci still exist. On one hand, several loci (HLA-B*08 and HLA-DQA1) presented virtually in certain MG subtypes universally. On the other hand, similar HLA patterns were revealed in MG patients from geographically adjacent ethnic groups. For instance, common HLA profile was revealed in MUSK-MG patients from Turkey versus Serbia [23], and also Italia versus Netherland [24]. A northern Han Chinese MG population identified the same HLA-DRB1 locus as that revealed in Singaporean Chinese and Japanese [31]. The similarity in HLA profile conforms to the trajectories of global human migration, in which common ancestral haplotypes are carried by the people with the same ethnic background.

In addition to the genes encoding classical MHC molecules, other genes within the HLA region also contribute to MG predisposition. Genetic polymorphism studies have confirmed two other genes related to MG predisposition: one is TNF-α, which is situated in the HLA class III region; the other is TAP2, which locates within the HLA class II region. The patients with genetic polymorphisms showed an elevated serum level of TNF-a thus increased the risk of MG [3]: the TNF-α-308 allele 2 was associated to female EOMG patients with thymic hyperplasia [32], and the TNF-α-863 polymorphism has been associated to ocular MG with thyroid-associated ophthalmopathy [33]. As to TAP2, TAP2*0101 was positively associated with MG in EOMG patients [34], which might increase the efficiency of TAP on the endoplasmic reticulum membrane to present antigenic peptides to classic MHC molecules.

3. Mechanisms of HLA in MG pathogenesis

Thus far, studies concerning HLA in autoimmune diseases have

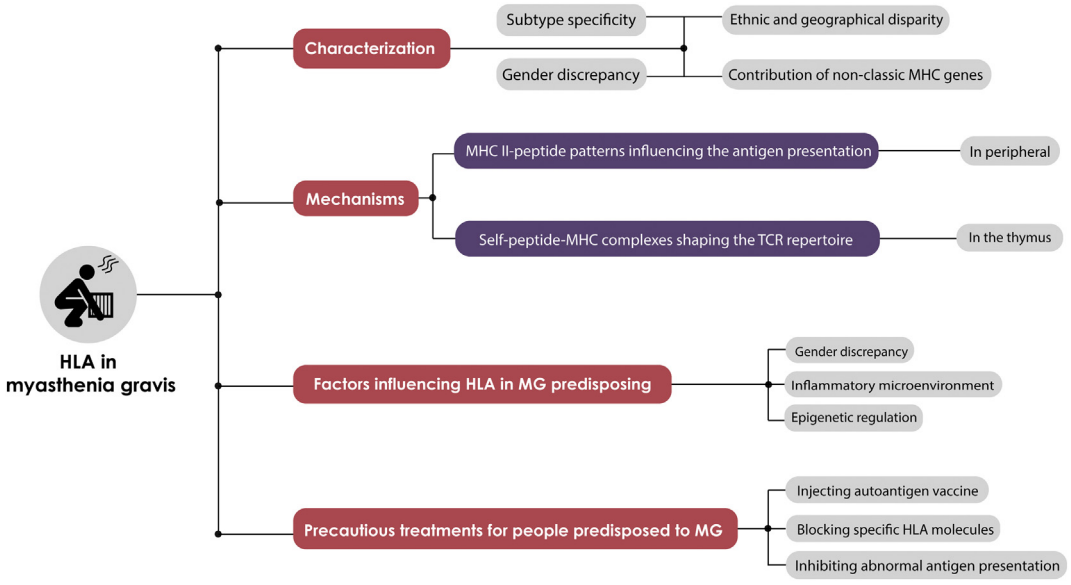

Fig. 2. Mechanisms of how the HLA system influences MG predisposition. (myasthenia gravis, MG; T-cell receptor, TCR).

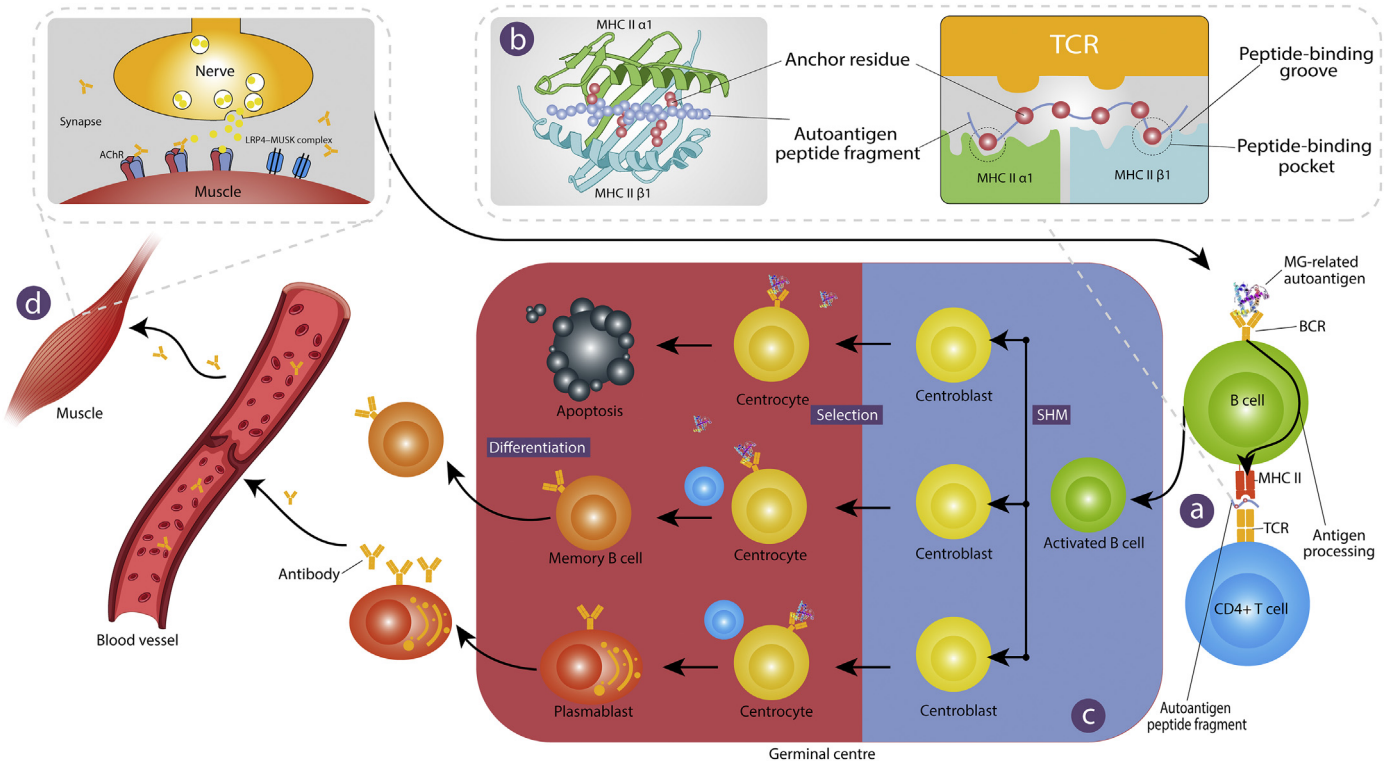

Fig. 3. The antigen presentation theory underlying the MG predisposition via HLA. a. In peripheral lymphoid organs, after MG related autoantigen processed by the B cell, the MHC II molecule on the B cell represents a linear antigen peptide to the TCR on a CD4+ T cell. b. On the peptide binding groove, the MG-related autoantigen peptide binds to the MHC II molecule via their specific anchor residues with the peptide-binding pockets of MHC II. c. After activated by CD4+ T cell, the B cell enters into germinal centre to undergone SHM for selecting the memory B cells or plasma cells with antibodies of much higher affinity to the autoantigen [104]. d. The produced autoimmune antibodies (such as antibodies to AChR, MUSK, LRP4) bind to the MG-related receptors on the muscle cells, which in addition to interrupt normal electrical signals but also activate complements to destruct these MG-related receptors. The debris of these receptors can enter the circulation to give rise to autoantigen recognition again. (myasthenia gravis, MG; B-cell receptor, BCR; T-cell receptor, TCR; somatic hypermutation, SHM; acetylcholine receptor, AChR; muscle-specific kinase, MUSK; lipoprotein-related protein 4, LRP4).

strengthened the role of classic MHC I and II molecules. Classic MHC molecules involved in autoimmune diseases often differ from normal ones by only a few amino acids that are predominantly located in the peptide-binding groove, and at times adjacent to key anchoring pockets, indicating the significance of intimate binding between peptides and T cells [35,36]. Hence, considering the classic MHC molecules' function in phased development of T cells, abnormality origins either from early thymic development or from peripheral immune responses [37]. After integrating hypotheses raised by other studies [38–40], we propose two synergetic mechanisms underlying MG pathogenesis via HLA. Given that MG is an antibody-mediated disease, follicular helper T cells and naïve B cells in peripheral lymphoid organs are essential for

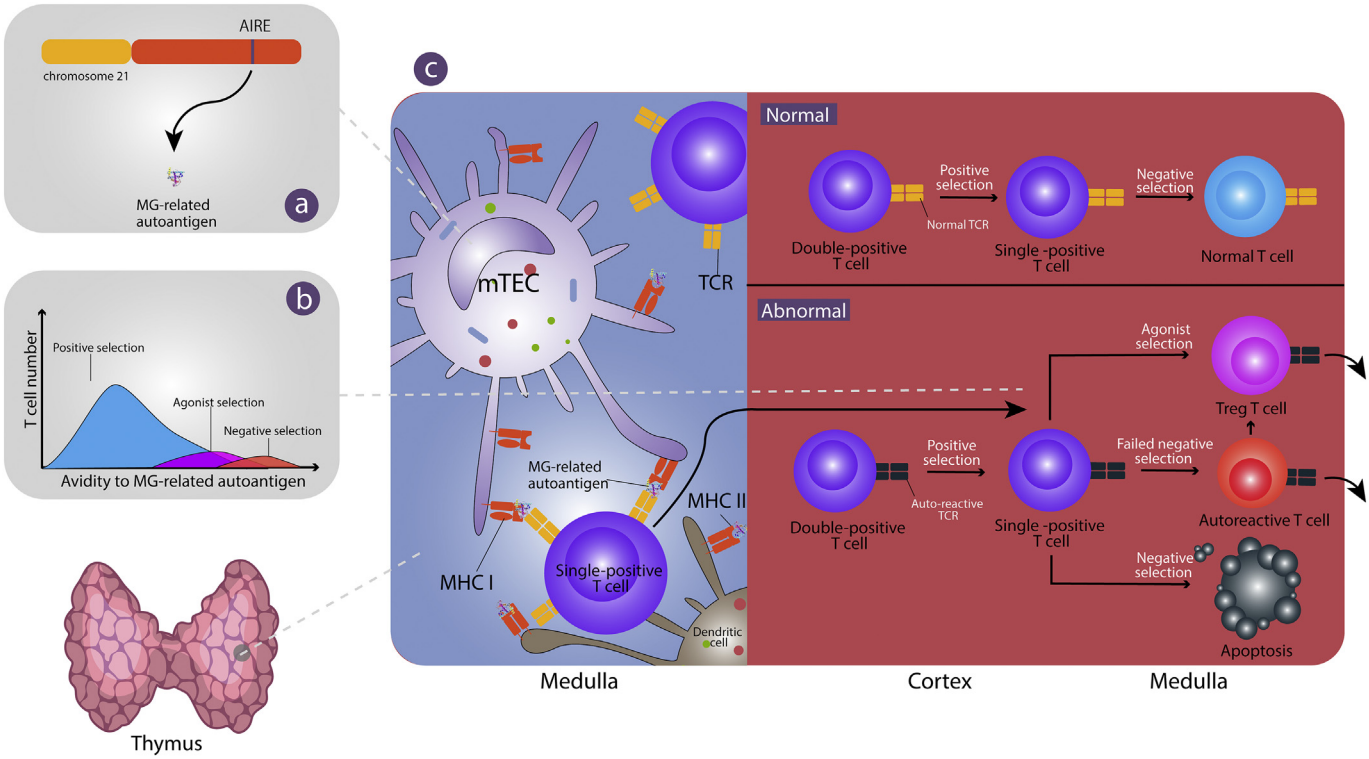

Fig. 4. The TCR repertoire shaping theory underlying the MG predisposition via HLA. a. The mTECs express AIRE and present MG-related self-antigens (such as AChR α) on MHC I to negatively select T cells. Besides, thymic dendritic cells can phagocytose mTECs to present these self-antigens on MHC II. b. a Poisson distribution model of T cells selection in normal condition [64]. c. T cells go through cortex and medulla to separately undergo positive and negative selection. The avidity between TCR of T cell and MHC of APC determines the destiny of a T cell: low avidity causes positive selection to empower them to succeed negative selection, high avidity causes negative selection by apoptosis, and moderate avidity causes agonist selection to differentiate potential autoreactive cells into Treg cells. Hence the TCR repertoire is shaped by these selections. If above selections fail, then autoreactive T cells may escape to the circulation to cause MG. (medullary thymic epithelial cell, mTEC; autoimmune regulator, AIRE; myasthenia gravis, MG; T-cell receptor, TCR; regulatory T, Treg).

the generation of high-affinity antibodies. So, the first pathogenic mechanism exists in the linked recognition of antigen by B cells and T cells, in which specific MHC II-peptide patterns may present autoantigenic peptides more efficiently to elicit MG (Fig. 3). The second pathogenic mechanism concerns the role of classic MHC molecules in shaping the TCR repertoire during T cells' thymic development, by which abnormal positive/negative selection may leave the T cell prone to cause MG (Fig. 4).

3.1. MHC II-peptide patterns influencing antigen presentation

Variation in different MHC II-peptide patterns can affect autoimmune disease' predispositions. By means of MHC fine-mapping, these patterns have been proved in rheumatoid arthritis [41] and type 1 diabetes [42,43], and the MHC II parts of which locate in the peptide-binding groove. This variation in MHC II-peptide patterns has also been confirmed in experimental autoimmune myasthenia gravis (EAMG) models by which MHC II transgenic mice were immunized with different AChR subunits or peptides. Compared to DQ6 transgenic mice, the DQ8 counterparts responded more intensely to Torpedo acetylcholine receptor and got higher clinical scores for EAMG [44]. In T cells from MG patients, the responses to hAChR peptide α146–162 also diverged in two groups with different HLA-DQ haplotypes [45].

AChR α subunit may be the most pathogenic autoantigen, and the specific anchor residues may at the main immunogenic region (MIR). After immunization with extracellular domains of the hAChR subunits (α, β, γ, δ and ε), Lewis rats responded mostly to α subunit [46]. In addition, the critical role of AChR α subunit was mostly revealed by the epitope studies of the MIR, which is a conformation-dependent region at the extracellular tip of α1 subunits of AChR (Fig. 1a). Previous MG clinical studies and EAMG experiments showed that at least half of the antibodies against AChRs targeted the MIR [47]. Concluded from these facts, MG-risk peptides are very likely on the α subunit of AChR and may be the MIR or epitopes near it. In humans and rats, Luo et al. found that both MIR epitopes recognized by their monoclonal antibodies (mAbs) were composed of two discontinuous sequences, which are adjacent in native conformation [48]. The MIR epitopes with high-affinity binding capacity determined by them are α 67–76 and α 1–14 in rats, and an additional sequence α 15–32 in humans.

Two specific MHC II-peptide patterns, DQ8-(hAChR α320–337) and DR3-(hAChR α171–190), play roles in the MG pathogenesis, and both cytoplasmic and extracellular AChR peptides can induce MG. When immunized with three cytoplasmic peptide sequences of hAChR (α320–337, α304–322, and α419–437), only DQ8 and DR3 mice developed EAMG (DQ8 most intensely) [49]. While in vitro study with T cells from DQ8 transgenic mice responded most fiercely to the α320–337, suggesting the connection between MHC-DQ8 molecules and hAChR α320–337. When immunized with segments from hAChR α-chain extracellular region (1 − 210) [50], α171–190 elicited the strongest response in HLA DR3 transgenic mice. Moreover, three (α36–49, α145–163, and α195–212) of five segments that elicited strong T cell response in HLA-DR3 mice showed high binding affinity to the MHC-DR3 molecule. It brings us another question: how do cytoplasmic AChR peptides trigger MG? Or if there any other AChR subunits trigger MG?

The transition from oMG to gMG may partially explain the pathogenic roles of different AChR subunits and cytoplasmic peptides in MG. Clinically speaking, ptosis and diplopia are the initial signs in over 50% of MG patients, among which 50–80% would convert to gMG [51]. A serial of studies conducted by Christadoss et al. explored the possible mechanism of the conversion from oMG to gMG. In HLA-DQ8, DR3, and

MHC II deficient transgenic and normal mice, AChR α subunit induced oMG in all strains but most significantly in the HLA-DQ8 mice, which subsequently proceed to gMG [52]. Then hAChR γ subunit was used to immune HLA-DQ8 mice, oMG and gMG presented in the HLA-DQ8 mice with AChRs destruction by the anti-AChR antibody and complement activation in the neuromuscular junction [53]. Later, hAChR ϵ-subunit was used to immune MHC-DQ8 and DR3 transgenic mice. HLA-DR3 mice showed significantly severe oMG and gMG phenotypes and higher proliferative responses in the lymph nodes than that of HLA-DQ8 mice [54].

From these animal studies, we can conclude that apart from the α subunit, other subunits of AChR are pathogenic for MG as well, and the pathogenicity is relevant to genotypes of HLA II. The initiating symptoms represented by oMG might be partially explained by the fetal AChR γ subunit which is selectively expressed in adult extraocular muscles (EOMs) [53] or two isoforms of hAChR α which are expressed in different organs [52]. In comparison to other skeletal muscles, EOMs have simpler neuromuscular junction structure and lower complement regulators thus are more vulnerable to be attacked by anti-AChR antibodies [55,56]. Subsequent complement-mediated destruction of postsynaptic AChR in EOMs may predispose other epitopes to the immune system and then trigger a generalised attack to the bulb and limb muscles, by which convert oMG to gMG. In line with this hypothesis, Lindstrom et al. proposed that an initial autoimmune response towards the MIR may spread to the whole AChR [57]. After immunized with chimera consisting of MIR and a protein resembling the extracellular domain of AChRs, the rats can produce serum antibodies against both extracellular and cytoplasmic domains of muscle AChRs, emphasizing the role of cytoplasmic AChR peptides in MG pathogenesis.

Another interesting question is that apart from the AChR originally expressed, could exogenous molecules mimicking AChR peptides induce MG? The incidence of childhood-onset MG in China is several folds higher than that in other countries [58]. This may partially be attributed to the administration of live-attenuated Japanese encephalitis vaccine that is not widely used in other countries [59]. They found a peptide (TWTYHGS) of this vaccine antigen was similar to AChR α168–174, and subsequently immunized a synthesized protein encompassing this peptide to mice, which later exhibited MG-like symptoms.

We propose it is the affinity between MHC II molecules and autoantigen peptides that palaces certain MHC II-peptide patterns at risk for MG. This affinity is relevant to the conformational plasticity of MHC II-peptide complex, which affects the dynamic of antigen presentation process [60]. It should be noted that in addition to the MHC II-peptide binding affinity, multiple co-factors such as the invariant chain (Ii), HLA-DM, and HLA-DO also contribute to the valid presentation of the antigen [61]. They are intracellular proteins that aid in antigenic peptide chaperoning and loading to the MHC II molecule when processing antigens. However, no MG study regard to these proteins has done yet, so we focus on the binding affinity of the MHC II-peptide complex. In EAMG study of HLA DR3-transgenic mice, the hAChR α subunit peptides that provoked strong T cell response basically corresponded to sequences that showed high binding affinity to the HLA-DR3 molecule [50].

3.2. Self-peptide-MHC complexes shaping the TCR repertoire

The selection in the thymus might shape TCR repertoire prone to MG. TCRs are selected in the positive and negative selection, and are already fixed when they matured from the thymus [62]. In thymic selection, the affinity/avidity between TCRs and self-peptide-MHC complexes uphold by medullary TECs determine the fate of unmatured T cells. Weak but enough avidity is required to protect thymocytes from 'death-by-neglect' and to promote the positive selection of naïve T cells, whereas strong avidity causes negative selection by apoptosis [63]. When the avidity is neither too strong nor too weak, agonist selection will re-direct potential autoreactive T cells to differentiate into regulatory T (Treg) cells [64]. Hence any abnormalities, whether originating from self-peptides or MHC molecules, occurring in these procedures may lead to aberrant TCR selection. For self-peptides, autoimmune regulator (AIRE) is a transcription factor that dictates the expression of specific muscular autoantigens in medullary TECs (mTECs), thus regulating negative selection of self-reactive T cells. The rs3761389 in AIRE has been associated to susceptibility of MG [65,66]. Besides, polymorphisms in the gene encoding α subunit in AChR, CHRNA1, also implicate the risk of MG development [66,67]. MHC molecules HLA-DRB1(∗)13 is an HLA genotype that is protective for several autoimmune diseases including MG. The protective mechanism might base on enhancing negative selection and the development of DR-driven Treg cells [68]. Still, more evidence is needed for supporting this hypothesis.

The special relationship between HLA I (HLA-B*08) and EOMG in humans was reviewed in the previous section. Another animal study with Newfoundland dog also proved that MHC class I genes had a closer relationship with EOMG than that of MHC class II genes [69]. For most autoimmune diseases driven by autoantibodies, e.g. systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes, and celiac disease, HLA II genes mostly play a significant role. However, for other autoimmune diseases not driven by autoantibodies, e.g. ankylosing spondylitis, psoriasis, Behcet's, HLA I genes are relatively predominant in the pathogenesis [70]. As an antibody-depended disease, this is not the case for EOMG. The close connection between HLA II genes and antibody-mediated disease is based on the co-activation between CD4+ T cells and activated B cells, which is essential for the robust production of antibodies (in comparison to activation by T independent antigens). But it is CD8+ T cells that interact with MHC I molecules, the function of which is not thus clear in autoimmunity. We think this HLA I-EOMG connection indicates a unique pathogenesis for EOMG mediated by CD8+ T cells in contrast to other MG subtypes. Clinical evidence showed that thymectomy is usually effective for EOMG but not for LOMG, and thymic hyperplasia coexisted more frequently with EOMG than LOMG [2]. Besides, thymic pathology has been found related to HLA-A3, HLA-A24, and HLA-B8 (but not HLA II alleles) in MG patients [71]. Taken together, it indicates that thymus hyperplasia might have unique meaning in EOMG pathogenesis, which might relate to classic HLA I genes.

Considering thymus facilitating T cell selection, a further hypothesis is that CD8+ Treg cells might be the main drive of EOMG. Nowadays, emerging evidence supports a role of CD8+ Treg cells in autoimmunity [72]. Without universally acknowledged definition to CD8+ Treg cell, several subgroups of it have been detected in mouse and human thymuses [73,74]. CD8+ Treg cells produce immunosuppressive cytokines, expressing inhibitory surface molecules, releasing cytotoxic enzymes, and degrading extracellular ATP [75]. They have also been found to suppress MG-associated T cell responses in EAMG mice [76]. In addition, we advocate an age-related TCR repertoire change that might explain the different effect of thymectomy in EOMG and LOMG. The dividing boundary between EOMG and LOMG is not definite, around 30–50 years old, which coincides with the thymic involution point when significant TCR repertoire reduction happens (age 40 [77]). By longitudinal investigation in humans, the diversity of TCR repertoire decreased much significantly in CD8+ than CD4+ T cells [78]. We think after thorough thymic involution starting from a young age, the pre-shaped MG-predisposing TCR repertoire is stagnate. Although it did not induce EOMG at an early age, it still can provoke LOMG when much older, which partially explains the lack of response of LOMG to thymectomy.

Virus and thymoma might induce MG via disturbing the shaping of TCR repertoire. Epstein–Barr (EB) virus has been associated with MG: high anti-EB virus IgG levels were correlated with EOMG; EB virus commonly presented in thymoma-infiltrating B cells of myasthenic patients [79]; and high prevalence in LOMG of oligoclonal expansions

in both CD8+ and CD4+ T cells hinted the involvement of viruses [80]. The virus might provoke persistent inflammation and initiate autoantigen sensitization in the thymus [81], which lead to the subsequent TCR repertoire change. As for thymomas, they are recorded in 10–15% of all patients with MG, and most notable in EOMG [82]. They may also disturb normal T-cell development: neoplastics expresses less MHC class II; most thymomas do not express AIRE; and the production of Treg cells is decreased in thymomas [83]. Moreover, neoplastic epithelial cells also variably express striational antigen epitopes, including epitopes of titin and various AChR subunits (but not whole receptors) together with reduced levels of MHC-class II. All of these may profoundly interfere with the selection of T cells and shape an autoantigenic TCR repertoire.

4. Factors influencing HLA in MG predisposing

HLA genes do not independently predispose patients to MG. Other factors also influence or “fine tune” this complicated process. In addition to the gender discrepancy, which has been articulated in the second part, herein we introduce two others that is notable in the context of MG: inflammatory microenvironment and epigenetic regulation.

Inflammatory microenvironment is indispensable for HLA pathogenesis for they are requisite for lymphocyte activation and can influence MHC expression. Autoreactive T Cells from patients with MG have been found characterized by elevated IL-17, IFN-γ, and GM-CSF (granulocyte-macrophage colony-stimulating factor) and diminished IL-10 [84]. Both Treg and conventional T cells in the thymus of MG were defective in downregulating IL-17 and TNF-α [85]. Hence, elevated pro-inflammation and downregulated anti-inflammation cytokines can cause a microenvironment that may activate CD4+ and CD8+ T cells in circulating [86] or in the thymus. In addition, the so-called “bystander lymphocytes” that are self-reactive can also become activated in this circumstance [87]. Genetic studies on MG also detected risk factors that influence MG predisposition. BAFF (a potent B-cell survival factor) and VAV1 (a key signal transducer for T- and B-cell activation) have been found influencing predisposition to MG via NF-κB pathway [88]. TNFAIP3-interacting protein 1 (TNIP1), a NF-κB signalling inhibitor, has been associated to EOMG [8]. Inflammatory cytokines may also influence MHC expression in MG. Through secretion of IL-1β, caspase-1 could lead to an upregulation of MHC class II on dendritic cells (DCs) [89], while caspase-1 inhibitor could ameliorates EAMG via innate DC IL-1-IL-17 pathway and consequently decreased the expression of MHC class II on DCs [90].

Epigenetic regulation of HLA in MG pathogenesis has also been proved in recent years. A MG monozygotic twins study found near two third of the differentially methylated CpGs were hyper-methylated in MG patients, most of which located within the 4 Mbp HLA locus [91]. Class II transactivator (CIITA) is one of the major determinants of tissue-specific MHC II expression, and the pIV CIITA promoter was heavily methylated in thymomas of MG patients, which might influence the IFN-γ-induced expression of CIITA [92]. Yang et al. examined the abnormal expression of long non-coding RNAs in peripheral blood mononuclear cells among MG patients [93] and found a long non-coding RNA, named IFNA-AS1, which regulates CD4+ T cell activation via downregulating HLA-DOB and HLA-DRB1 expression in MG patients [91]. Furthermore, they reversed the influence of IFNG-AS1 on CD4+ T cell activation by restoring HLA-DRB1 and found IFNG-AS1 expression levels were negatively correlated with HLA-DRB1 and HLA-DOB expression levels in MG patients.

5. Precautious treatments for people predisposing to MG

To date, three novel therapeutic techniques may provide inspiration for the development of precautious treatments for people predisposed to MG: injecting autoantigen vaccine, blocking specific HLA molecules, and inhibiting abnormal antigen presentation. Previously, Luo et al. found a vaccine consisting of cytoplasmic domains of human AChR subunits reduced the development of chronic EAMG in rats [94]. Then they improved this vaccine with adjuvant and found it did not induce EAMG this time [95]. The second method has been verified in both mAb and single-chain fragment variable (scFv) specific to HLA alleles. mAbs against peptides corresponding to the tip of the MHC antigen-binding groove of alleles DQB1*02, *03, *05, and *06 inhibited the in vitro proliferation of AchR-specific T cells from MG patients [96]. To increase the efficacy of mAb, Ayyar et al. humanized a murine mAb which was capable of blocking MG-associated DQB1 allele and reformatted it into scFv. The scFv exhibited superior binding affinity than the original mAb and blocked the proliferation of T cells of MG-patients typed DQB1*0601 [97]. For the third method, Cathepsin (Cat) S is an enzyme that helps load the antigen into the MHC complex in antigen presentation. Yang et al. found that Cat S null mice showed weak responses to immunodominant AChR peptides, and Cat S inhibitor suppressed IFN-γ production in lymphocytes from EAMG mice, suggesting Cat S inhibitors could be tested for their therapeutic potentiality in MG [98].

6. Conclusions and perspectives

From the correlations between HLA and MG found in many studies, we have tried to explain the mechanisms underlying MG pathogenesis. Herein, we proposed two independent and synergetic mechanisms underlying MG predisposition via HLA. The first advocates that some MHC II-peptide patterns can develop MG by influencing antigen presentation, and the second emphasizes the role of MHC alleles in shaping the TCR repertoire for MG predisposition. Concluded from our analysis, it appears that T cells are more important than B cells in the genetic predisposition to MG. Since MG is a typical B-cell mediated disease, the function of B cells in the predisposition to MG should not be ignored, in that the B cells from MG patients might be naturally defect [99–101]. Hence, we believed that both B cells and T cells contribute to the MG predisposition, but T cells are predominant during this process. Since the pathogenesis may comprise large heterogeneity, subgroup analysis towards MG patients is highly recommended. Lastly, although we emphasised specific peptide-MHC patterns may exist in predisposing MG, considering TCR also having its own polymorphism, we prefer to extend this to a broader pattern composed of peptide-MHC-TCR. For example, a common motif of TRBV29 has been found in the T cells from DQ5-positive MUSK-positive MG patients [102].

In this filed, many questions still remain to be answered. First is the gender discrepancy that might be partially explained by a hormone theory consisted of estrogen and testosterone. Female predominance in EOMG might be explained by the estrogen response elements within HLA genes. But the underlying mechanism for how testosterone affects the risk of MG is still unclear. In addition, CD8+ Treg cell is a promising field for investigating the pathogenesis of EOMG. We cannot disregard the epistasis effect on HLA in MG predisposition [103], and other unknown genes might influence HLA as well. More MHC II-peptide patterns might be identified on the AChR α subunit, and to define detailed amino acid residues of MHC II in the peptide-binding groove might be helpful to figure out the affinity dynamics underlying it. Given autoimmune diseases have many pathogenetic mechanisms in common or analogue, the potential precautious treatment devised for MG might be extended to other diseases as well.

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