Research advancement in immunopathogenesis of myasthenia gravis

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Abstract: Myasthenia gravis (MG) is an autoimmune neuromuscular junction disease mediated by antibodies against the acetylcholine receptor (AChR). The etiology and immunopathogenesis of MG remain unclear. Recent research has shown the involvement of autoantibodies, lymphocytes, cytokines and chemokines, in the pathogenesis of MG. Systematic factors are also demonstrated, such as inheritance and endocrine. This review indicates the research development in immunopathogenesis of MG.

Keywords: myasthenia gravis; immunological pathogenesis

1 Introduction

Myasthenia gravis (MG) is an uncommon autoimmune neuromuscular junction disease characterized by painless, fluctuating, and fatigable weakness of some specific muscle groups. Ocular weakness with asymmetric ptosis and binocular diplopia is the most typical initial presentation. Although the course is variable, most patients with initial ocular weakness develop bulbar or limb weakness within the first 3 years of MG. Although the mechanism of MG remains unclear, the proposition that MG results from antibody-mediated and T cell-dependent immunological attack on the endplate region of the postsynaptic membrane is widely accepted.

Studies on the animal model of experimental autoimmune myasthenia gravis (EAMG) lead to a further understanding of the immunopathogenesis of MG. Effects of autoantibodies, functional imbalance of lymphocytes, abnormal expressions

of cytokines and chemokines, and systematic factors can all contribute to the development of MG.

2 The effects of autoantibodies

At present, there are substantial evidences to support that MG is an autoimmune syndrome caused by the failure of neuromuscular transmission, which results from the binding of autoantibodies to proteins involved in signaling at the neuromuscular junction (NMJ). These proteins include the nicotinic acetylcholine receptor (AChR), and less frequently, a muscle-specific tyrosine kinase (MuSK) involved in AChR clustering. Much has been known about the mechanisms that maintain self-tolerance and modulate anti-AChR antibody synthesis, AChR clustering, and AChR function as well as those leading to neuromuscular transmission failure upon antibody binding.

Besides the AChR-specific antibody, antibodies against skeletal muscle antigens also exist in some MG patients. Among these antigens, ryanodine receptor (RyR) and titin are the 2 major antigens for non-AChR antibodies. Both of them are Ca^{2+} -releasing channel of the sarcoplasmic reticulum. Antibodies for RyR and titin are found mainly in thymoma MG patients and some late onset MG patients, and correlate

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with severe MG disease. The RyR antibodies recognize a region near the N-terminus of RyR which is important for channel regulation, and inhibit Ca^{2+} release *in vitro*. However, Skeie GO *et al.*^[1] have stated that the proposition that antibodies against the intracellular antigens RyR and titin are pathogenic *in vivo* still lacks sufficient evidence.

Lately, in order to investigate the relations between autoantibodies and excitation-contraction (E-C) coupling in MG patients, Maruta T *et al.*^[2] have developed a novel method to detect the autoantibodies against dihydropyridine receptor (DHPR). The titer of DHPR antibodies exerts no significant correlation with autoantibodies to acetylcholine or ryanodine receptors, which shows that the DHPR antibody is another marker for thymoma in MG, and it might play a role in clinical symptoms related to E-C coupling.

3 The functional imbalance of lymphocytes

The weakness of MG patients is mediated by T helper cell (Th)-dependent autoantibodies against neuromuscular epitopes. So far, analysis of Th phenotypes or antigen specificities has yielded very few clues to pathogenesis. Tackenberg B *et al.*^[3] adopted an alternative antigen-independent approach, and analyzed T cell receptor (TCR) Vbeta usage/expansions in blood from 118 MG patients. Their results have demonstrated persistent clonally-expanded CD4^+ B helper T cell populations in the blood of MG patients. The unexpected CD4^+ expansion may provide a valuable clue for MG immunopathogenesis. Moreover, Ben-David H *et al.*^[4] suggest that $\text{CD8}^+ \text{CD28}^-$ regulatory cells play a partial role in the process that the dual APL suppresses EAMG-associated T cell responses. Research in Th17 cells, a newly identified IL-17-producing Th subset^[5,6], has provided new insights into the development of autoimmune diseases and immune responses, and has thereby led to revision of the classic Th1/Th2 paradigm^[7,8]. In addition, the $\text{CD4}^+ \text{CD25}^+$ regulatory T cells (Tregs) in peripheral blood of MG pediatric patients are increased in number but their functions are not strengthened. Previous reports^[9] have indicated that the pathogenesis of MG in children may be correlated with the impairment of immunotolerance of the peripheral blood Tregs. Kong QF *et al.* have studied the immunoregulatory effects of

bone marrow stromal cells (BMSC) on EAMG and determined that the development of EAMG is accompanied with an imbalance among Th1, Th2, Th17, and Treg cell subsets^[10].

The pathogenic roles of B cells and autoantibodies in central and peripheral nervous system disorders, as reviewed here, provide a rationale for investigating whether depletion of B cells by new agents can ameliorate the clinical symptoms and potentially restore the immune function. Preliminary clinical studies and case reports suggest that B cell depletion may be a viable alternative approach to the treatment of autoimmune neurological disorders^[11].

4 Abnormal expressions of cytokines and chemokines

Many reports have indicated that Th1, Th2, Th17 and Treg cells are all involved in the pathogenesis of EAMG, via a complex interaction with their cytokines. Interleukine-6 (IL-6), a cytokine secreted by Th2 and Th17 cells, is likely to play an important role in the pathophysiology of MG when it is overproduced. Moreover, its possible role as a modulating factor in either proliferation of pleomorphic adenoma cells or their differentiation into myoepithelioma has also been discussed. Recent research^[12] has presented a rare case of parotid myoepithelioma in MG patient and proposed a possible IL-6-mediated relationship between MG and parotid myoepithelioma. Similarly, Tüzün E *et al.*^[13] have found the beneficial effect of etanercept treatment on MG patients who had low levels of IL-6 and $\text{IFN-}\gamma$ in plasma. On the other hand, Xiao BG *et al.*^[14] have found no improvement of clinical symptom or the alteration of immunological parameter in MG patients after subcutaneous injection of IL-10-treated dendritic cells (DC), suggesting the limitation of IL-10-DC in the treatment of MG. Yapici Z *et al.*^[15] suggest that in juvenile MG patients, a high level of IL-10 is associated with the production of anti-acetylcholine receptor antibody and the treatment response. Increasing data have shown that $\text{IFN-}\gamma$, IL-10-, and $\text{TGF-}\beta$ -modulated DC provide a promising strategy in treatment of experimental allergic encephalomyelitis and EAMG through different manners^[16]. IL-10 is secreted by several hemopoietic cells. It could suppress the Th1-mediated immune response, and stimulate B cell differentiation

and the humoral immune response. Recently, Alseth EH *et al.* have indicated a direct linkage between IL-10 and MG pathogenesis, although the complex role of this multi-faceted cytokine *in vivo* has not yet been fully elucidated^[17].

Also, chemokines participate in the regulation of immune and inflammatory responses by interacting with their receptors, which are primarily expressed on immune and inflammatory cells such as B and T lymphocytes and antigen-presenting cells. Therefore, chemokines and their receptors are considered to mediate inflammation and tissue damage in autoimmune disorders. Chemokine receptor (CCR) genotypes have been identified recently, and the importance of their genetic polymorphism in some autoimmune and infectious disorders is also discussed. Besides, there is few evidence concerning the association between an increased risk of MG and CCR2-64I or CCR5Delta32 polymorphism. However, the increased anti-AChR antibody titer in the patients with CCR2 G/G genotype suggests that the *CCR2* gene plays a role in the pathophysiology of MG^[18]. Feferman T *et al.* have demonstrated that the chemokine IFN- γ inducible protein 10 (IP-10) and its receptor CXCR3 are overexpressed in MG and EAMG. Furthermore, they have also studied the modulation of rat EAMG by interfering the CXCR3/IP-10 signaling. Suppression of EAMG suggests that inhibition of CXCR3/IP-10 signaling may be a potential therapeutic strategy for MG^[19]. The development and function of Th17 cells are influenced in part by cytokines TGF- β , IL-23 and IL-6, but the mechanisms underlying recruitment and activation of Th17 cells during initiation of autoimmunity remain unclear. Bai Y *et al.* have shown that Th17 cells can promote humoral autoimmunity via a novel mechanism that involves CCL2^[20]. Meraouna A *et al.* have hypothesized that molecules that are dysregulated in MG thymus and could be normalized by glucocorticoids may play a key role in thymic pathogenesis. These results suggest that the high level of CXCL13 production by epithelial cells could be responsible for germinal center formation in MG thymus^[21].

5 Systematic factors

Systematic factors, such as inheritance and endocrine, are also demonstrated to be related to the pathogenesis of

MG. Since MG is much more prevalent in women than in men, Nancy P *et al.*^[22] investigated the potential changes in estrogen receptor (ER) expression in thymic hyperplasia. They have found an increase in ER alpha expression on thymocytes, and increases in both ERs expressions on T cells from peripheral blood mononuclear cells, indicating that the signals provided by thymic and peripheral microenvironments are distinct. Moreover, the dysregulation of ER expression in MG lymphocytes could affect the homeostasis and influence the progression of the autoimmune response.

Yilmaz V *et al.*^[23] have found polymorphisms of *IFN- γ* , *IL-10*, and *IL-12* genes in MG, suggesting that the gene polymorphism of some cytokines may contribute to susceptibility or to antibody production in MG although these findings need to be confirmed by further studies. Wang W *et al.*^[24] have reported that the C57BL/6 mice genetically deficient in IL-12/IL-23 and IFN- γ are susceptible to EAMG, suggesting a pathogenic role of non-Th1 cells. Aranka L *et al.* studied the clinical and molecular genetic aspects of genetically determined neuromuscular disorders of some Roma families in Hungary. However, results revealed no difference in the molecular genetic findings between the Caucasian and the Roma spinal muscular atrophic (SMA) patients^[25].

6 Conclusion

In the past years, studies on MG have proved that MG is an autoimmune disease that affects the transmission of signals from nerves to muscles, and the basic pathogenesis of MG is the production of anti-AChR antibodies, resulting from the response of the autoreactive T lymphocytes to AChR. However, the molecular mechanisms of MG and the production of autoreactive T lymphocytes remain elusive. The difficulty in elaborating the immunological pathogenesis of MG by possible approaches indicates the complexity of the problem. Although currently no studies have provided any ideal theory for clarifying the occurrence and development of MG, research does illustrate some of the underlying factors of MG and has provided some novel therapeutic strategies for MG treatment.

References:

- [1] Skeie GO, Aarli JA, Gilhus NE. Titin and ryanodine receptor antibodies in myasthenia gravis. *Acta Neurol Scand* 2006, S183: 19-23.
- [2] Maruta T, Yoshikawa H, Fukasawa S, Umeshita S, Inaoka Y, Eda Hiro S, *et al.* Autoantibody to dihydropyridine receptor in myasthenia gravis. *J Neuroimmunol* 2009, 208(1-2): 125-129.
- [3] Tackenberg B, Kruth J, Bartholomaeus JE, Schlegel K, Oertel WH, Willcox N, *et al.* Clonal expansions of CD4⁺ B helper T cells in autoimmune myasthenia gravis. *Eur J Immunol* 2007, 37 (3): 849-863.
- [4] Ben-David H, Sharabi A, Dayan M, Sela M, Mozes E. The role of CD8⁺CD28⁺ regulatory cells in suppressing myasthenia gravis-associated responses by a dual altered peptide ligand. *Proc Natl Acad Sci U S A* 2007, 104(44): 17459-17464.
- [5] Bi YJ, Liu GW, Yang RF. Th17 cell induction and immune regulatory effects. *J Cell Physiol* 2007, 211: 273-278.
- [6] Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. *Allergol Int* 2008, 57: 121-134.
- [7] Kramer JM, Gaffen SL. Interleukin-17: a new paradigm in inflammation, autoimmunity, and therapy. *J Periodontol* 2007, 78: 1083-1093.
- [8] Liu R, La Cava A, Bai XF, Jee Y, Price M, Campagnolo DI, *et al.* Cooperation of invariant NKT cells and CD4⁺CD25⁺ T regulatory cells in the prevention of autoimmune myasthenia. *J Immunol* 2005, 175: 7898-7904.
- [9] He XT, Liu WB, Feng HY, Zhang Y, Huang X, Meng R, *et al.* The role of CD4⁺ CD25⁺ T cells in the mechanism of myasthenia gravis in children and adults. *Chin Med J* 2008, 88(45): 3189-3191.
- [10] Kong QF, Sun B, Wang GY, Zhai DX, Mu LL, Wang DD, *et al.* BM stromal cells ameliorate experimental autoimmune myasthenia gravis by altering the balance of Th cells through the secretion of IDO. *Eur J Immunol* 2009, 39: 800-809.
- [11] Dalakas MC. B cells in the pathophysiology of autoimmune neurological disorders: a credible therapeutic target. *Pharmacol Ther* 2006, 112(1): 57-70.
- [12] Sassano P, Paparo F, Ramieri V, Colangeli W, Verdino G. Interleukine-6 (IL-6) may be a link between myasthenia gravis and myoepithelioma of the parotid gland. *Med Hypotheses* 2007, 68(2): 314-317.
- [13] Tüzün E, Meriggioli MN, Rowin J, Yang H, Christadoss P. Myasthenia gravis patients with low plasma IL-6 and IFN- γ benefit from etanercept treatment. *J Autoimmun* 2005, 24 (3): 261-268.
- [14] Xiao BG, Duan RS, Zhu WH, Lu CZ. The limitation of IL-10-exposed dendritic cells in the treatment of experimental autoimmune myasthenia gravis and myasthenia gravis. *Cell Immunol* 2006, 241(2): 95-101.
- [15] Yapici Z, Tüzün E, Altunayoğlu V, Erdoğan A, Eraksoy M. High interleukin-10 production is associated with anti-acetylcholine receptor antibody production and treatment response in juvenile myasthenia gravis. *Int J Neurosci* 2007, 117 (11): 1505-1512.
- [16] Duan RS, Link H, Xiao BG. Long-term effects of IFN- γ , IL-10, and TGF- β -modulated dendritic cells on immune response in Lewis rats. *J Clin Immunol* 2005, 25(1): 50-56.
- [17] Alseth EH, Nakkestad HL, Aarseth J, Gilhus NE, Skeie GO. Interleukin-10 promoter polymorphisms in myasthenia gravis. *J Neuroimmunol* 2009, 210(1-2) :63-66.
- [18] Kim HS, Kim DS, Lee EY, Sunwoo IN, Choi YC. CCR2-64I and CCR5Delta32 polymorphisms in Korean patients with myasthenia gravis. *J Clin Neural* 2007, 3(3): 133-138.
- [19] Feferman T, Aricha R, Mizrahi K, Geron E, Alon R, Souroujon MC, *et al.* Suppression of experimental autoimmune myasthenia gravis by inhibiting the signaling between IFN- γ inducible protein 10 (IP-10) and its receptor CXCR3. *J Neuroimmunol* 2009, 209(1-2): 87-95.
- [20] Bai Y, Liu R, Huang D, La Cava A, Tang YY, Iwakura Y, *et al.* CCL2 recruitment of IL-6-producing CD11b⁺ monocytes to the draining lymph nodes during the initiation of Th17-dependent B cell-mediated autoimmunity. *Eur J Immunol* 2008, 38(7): 1877-1888.
- [21] Meraouna A, Cizeron-Clairac G, Panse RL, Bismuth J, Truffault F, Tallaksen C, *et al.* The chemokine CXCL13 is a key molecule in autoimmune myasthenia gravis. *Blood* 2006, 108(2): 432-440.
- [22] Nancy P, Berrih-Aknin S. Differential estrogen receptor expression in autoimmune myasthenia gravis. *Endocrinology* 2005, 146(5): 2345-2353.
- [23] Yilmaz V, Tütüncü Y, Baris Hasbal N, Parman Y, Serdaroglu P, Deymeer F, *et al.* Polymorphisms of interferon- γ , interleukin-10, and interleukin-12 genes in myasthenia gravis. *Hum Immunol* 2007, 68 (6): 544-549.
- [24] Wang W, Milani M, Ostlie N, Okita D, Agarwal RK, Caspi R, *et al.* C57BL/6 mice genetically deficient in IL-12/IL-23 and IFN- γ are susceptible to experimental autoimmune myasthenia gravis, suggesting a pathogenic role of non-Th1 cells. *J Immunol* 2007, 178 (11): 7072-7080.
- [25] Aranka L, Peter M, Jeno K, Katalin R, Gyula T, Eموke E, *et al.* Genetically determined neuromuscular disorders of some Roma families living in Hungary. *Ideggyogy Sz* 2009, 62(1-2): 41-47.

重症肌无力的免疫发病机制研究进展

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摘要: 重症肌无力是一种由抗乙酰胆碱受体的抗体介导的神经肌肉接头处的自身免疫病。其发病机制尚未阐明。自身抗体、淋巴细胞免疫调节紊乱、细胞因子表达失衡及系统因素在重症肌无力的发病机制中均起重要作用。本综述旨在探讨重症肌无力的免疫学发病机制。

关键词: 重症肌无力; 免疫发病机制