Advances in Research on the Relationship Between Intestinal Flora and Myasthenia Gravis

Running Title: Correlation of Intestinal Flora and Myasthenia Gravis

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ABSTRACT: Human intestinal flora refers to a large and diverse microbial population present in the digestive tract of the human body, which plays a significant role in the establishment of human immune homeostasis and the normal function of the immune system. Myasthenia Gravis is an autoimmune disease of the neuromuscular junction, mainly involved in the anti-acetylcholine receptor antibody, cellular immune dependence, and complement[1]. At present, studies have found that the intestinal flora of Myasthenia Gravis is different from that of healthy people. Probiotic therapy has been shown effective in the experimental autoimmune Myasthenia Gravis animal models. This article reviews the relationship between intestinal flora and Myasthenia Gravis, to provide new ideas for further study of the pathogenesis and clinical treatment of Myasthenia Gravis.

KEYWORDS: intestinal flora; myasthenia gravis; research progress; autoimmune disease; EAMG

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INTRODUCTION

Myasthenia Gravis (MG) is an autoimmune disease of the neuromuscular junction mainly mediated by anti-acetylcholine receptor (AChR) antibody, cellular immune dependence, and complement, which often manifests as morbid fatigue of part or whole-body skeletal muscle[1]. The specific etiology and pathogenesis of this disease are still unclear, and there is no effective treatment to cure it. At present, studies have shown that the disorder of intestinal flora can cause a variety of autoimmune diseases[2, 3]. Many scholars have found that there are significant differences between intestinal flora and healthy people in MG patients, and probiotics have been shown effective in the treatment of experimental autoimmune myasthenia gravis (EAMG) animal models. This article reviews the relationship between intestinal flora and myasthenia gravis.

INTESTINAL FLORA OVERVIEW

Intestinal flora concept

The human intestinal flora refers to a large and diverse microbial population present in the digestive tract of the human body. It contains at least 10^{14} Bacteria in the human gastrointestinal tract, and the total weight is about 1 kilogram[4]. The number of genomes encoding the protein is More than 100 times more human genomes[5-7]. Among the approximately 200 common bacteria in the human gut, the most important genus are Bacteroides, Clostridium, Faecalibacterium, Eubacterium, Ruminococcus, Peptidococcus, Peptidostreptococcus, and Bifidobacterium[8-10]. These major intestinal bacteria are associated with type 1 diabetes, obesity, inflammatory bowel disease, Alzheimer's disease, Parkinson's disease, neuromyelitis optica spectrum disorders, chronic urticaria, Crohn's disease, multiple sclerosis, myasthenia gravis, Irritable bowel syndrome, gestational diabetes and many other human diseases[11-33], see table 1

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for details.The number, composition, and state of the intestinal flora are always changing dynamically. Even in the same individual or different individuals, or individuals in the same family, the diversity of the flora is very different[34]. It is generally believed that the intestines of newborns are sterile, after birth, various microorganisms enter the gastrointestinal tract through the mouth, anus, and colon, and the microbiota in the newborn is relatively stable to about one year old[35]. As the age changes, the intestinal flora in the human body will also change. Studies have shown that with the increase of age, the beneficial function of the age-related intestinal flora decreases, and the probability of inflammation and disease in the body increases, especially for older people over the age of 90, the chances of their occurrence are greater[36]. Under normal circumstances, the intestinal flora and the host interact with each other to form a relatively stable dynamic balance, which plays a vital role in the digestion, absorption, metabolism, and immune regulation of the human body. When the proportion is imbalanced, displaced, and the body's immunity is low, the balance of intestinal micro-ecology is broken, which triggers immune disorders and successively causes various diseases of the body[37-39].

Intestinal flora and human immunity

With the development of the Human Microbiome Project (HMP)[40] people's research on the intestinal flora has been deepened, and it has been found that the intestinal flora is closely related to human health. The intestinal flora is even considered to be "another organ" of our body. The establishment and the perfection of the immune system are almost simultaneous. Our growth and development have always accompanied their interaction with our immune system. The composition and metabolites of intestinal microbes not only promote the development of the host's immune system but also regulate the body's immune system. The interaction between the intestinal flora and the immune system is two-way. The result is to maintain homeostasis; that is, the body's immune system can both immune to the

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gastrointestinal symbiotic microbes and respond appropriately to protect the body from pathogens[34]. Studies have shown that the intestinal flora has different mechanisms and uses multiple pathways to promote the differentiation and function of intestinal regulatory T cells (Tregs) and effector T cells and promote IgA conversion and IgA secretion of B cells. The segmented filamentous bacteria can promote the development of Th17 cells and play a vital role in the induction of slgA. The sphingolipid of fragile Bacillus can inhibit the proliferation of iNKT cells and contribute to the health of the colon, lactic acid in the gastrointestinal tract. The presence of Bacillus promotes barrier integrity and maintains a Th1 / Th2 / Th3 cytokine balance. These extensive studies have revealed that the intestinal flora has a broad and long-lasting effect on the development and function of innate immune cells and adaptive immune cells in the intestine. Heterologous signals derived from the flora can closely regulate the development of intestinal-associated immune tissues and activation of immune cells[41-46]. It can be seen that the intestinal flora plays a significant role in the establishment of human immune homeostasis and the normal function of the immune system.

RELATIONSHIP BETWEEN INTESTINAL FLORA AND MG

Correlation between intestinal flora and MG

The cause of MG has both environmental and genetic factors and the specific factors that cause susceptibility to remain challenging to determine. It is known that changes in the intestinal flora can affect the body's various physiological functions by regulating the body's immune system. Whether the changes in the intestinal flora are related to the pathogenesis of myasthenia gravis has caused many scholars to think and pay attention. German Moris et al.[47] used microbial phylogenetic analysis of MG patients and control stool samples through the 16S rRNA gene map, Bifidobacterium ITS region map, and qPCR to find out that the relative proportion of MG patients with Bacteroides and Bifidobacteria was low. The proportion of Bacteroides and

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Desulfovibrio is increased. Dongxu Qiu et al.[48] compared the fecal microbiota of MG patients with age and gender-matched healthy controls, the pedigree of intestinal microflora, the changes in short-chain fatty acids (SCFAs), and found that they were associated with healthy people.

In comparison, the intestinal microflora of the MG group changed in the relative abundance of the bacterial taxonomic group, and the bacterial richness decreased sharply, especially in the Clostridium genus. The absolute amount of the healthy control group was three times higher than that of the MG group; the content of SCFAs in the MG group was significantly lower than that in the healthy control group. In addition, they also found that intestinal flora imbalance is closely related to the level of inflammatory biomarkers in the serum of MG patients. These findings indicate that MG patients have disorders of the diversity, composition, and function of fecal microbial communities, suggesting that changes in our intestinal flora are closely related to MG production. To investigate whether disturbed gut microbiome might contribute to the onset of MG, Peng Zheng et al[49] did plenty of research in mice. Germfree (GF) mice are colonized initially with MG microbiota (MMb) or healthy microbiota (HMb) and then immunized in a classic mouse model of MG. The MMb mice demonstrate substantially impaired locomotion ability compared with the HMb mice. Studies by the above scholars have shown that the diversity, composition, and function of the fecal microbial community in MG patients are abnormal, and the disorder of intestinal flora may trigger the occurrence of MG. See table 2 for relationship between intestinal flora changes and MG.

Possible mechanisms of intestinal flora and MG

The gastrointestinal tract is a complex ecosystem that contains a large number of microorganisms that are symbiotic with the host and affect human nutrition, metabolism, and

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immune function[7]. Its dysregulation may be involved in the pathogenesis of a variety of autoimmune diseases (such as multiple sclerosis, rheumatoid arthritis, etc.) [2, 3]. MG is an antibody-mediated T cell-dependent autoimmune disease whose pathogenesis is closely related to high levels of AChR antibodies[50]. Studies have shown that the production of AChR antibodies is associated with imbalances in Th1 cells, B cells, and $Foxp3^+$ T regulatory cells (Treg) [51, 52]. Among them, $Foxp3^+CD4^+$Treg cells play a vital role in maintaining self-tolerance and immune homeostasis and preventing the development of MG. $Foxp3^+CD4^+$Treg cells affect the number of autoreactive T cells and inhibit self-reactive B cells producing AChR antibodies, thereby reducing the severity and progression of the disease[53]. In MG patients, the abnormal number and function of $Foxp3^+CD4^+$ Treg cells have become a major focus of many studies on the pathogenesis of MG[54-56]. The number of $Foxp3^+CD4^+$Treg cells in the lamina propria of the colon was significantly higher than that of any other organ[57], which was significantly affected by the composition of the intestinal flora. Studies have found that colonization of sterile mice of Clostridium difficile strains increases the number of $Foxp3^+CD4^+$Treg cells in the lamina propria of the colon, and a mixture of 17 Clostridium isolates isolated from healthy individuals by researchers. It can actively induce $Foxp3^+CD4^+$Treg cells in human colon[48]. Intestinal microflora can affect the number of $Foxp3^+CD4^+$Treg cells and the T cell receptor (TCR). The TCR on $Foxp3^+CD4^+$Treg cells can recognize subpopulations of commensal bacteria and induce naive $CD4^+$T cells to differentiate into Antigen-specific $Foxp3^+CD4^+$Treg cells increase the number of $Foxp3^+CD4^+$Treg cells[58, 59]. Existing studies have shown that the proportion of Bifidobacteria and Clostridium in the intestinal microflora of MG patients is significantly lower than that of healthy people[47, 48]. Moreover, some scholars have found that the use of bifidobacteria and lactobacilli in the treatment of experimental autoimmune myasthenia gravis (EAMG) rat model can lead to an increase in serum levels of transforming growth factor-beta (TGFβ), and Percentage of

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regulatory T cells (Treg) in peripheral blood leukocytes[60]. Therefore, we hypothesized that the decrease in the number of Foxp3 $^{+}$ CD4 $^{+}$ Treg cells caused by the disorder of the intestinal flora and the abnormal function might lead to the occurrence of MG or the aggravation of symptoms, and the specific mechanism remains to be further studied. (See Figure 1 and Figure 2 for details.)

Treatment of intestinal flora and MG.

It is the wish of most researchers to open up new ways to suppress immunopathological changes and block the progression of MG. Existing studies have shown that probiotics have a strain-specific beneficial effect in regulating multiple immune diseases. Chang-Suk Chae et al[61] have demonstrated that IRT5 probiotics, a mixture of 5 probiotics, could suppress various experimental disorders in mice model. They found that oral administration of IRT5 probiotics significantly reduced the clinical symptoms of EAMG, such as weight loss, body tremors, and grip strength. Alessandra Consonni et al.[60] studied the clinical efficacy of two Lactobacillus and two Bifidobacterium probiotic strains in the Lewis-induced EAMG model, and the disease symptoms of EAMG were effectively alleviated. Elena Rinaldi et al[62] considered that selected probiotic strains could be evaluated as adjuvant therapy in clinical trials to restore autoimmune tolerance interrupted in patients with myasthenia gravis. This series of studies suggests that probiotic therapy is expected to be a new treatment and method for the treatment of myasthenia gravis in the future.

CONCLUSION

In summary, the existing research indicates that the community diversity, composition, and function of the intestinal flora in MG patients have changed. The intestinal flora is closely related to the occurrence of MG. The decrease in the number of Foxp3^{+}CD4^{+}Treg cells caused

by the disorder of intestinal flora and dysfunction may lead to the occurrence of MG or the aggravation of symptoms. At present, scholars have found that probiotic therapy can effectively alleviate the symptoms of EAMG. Therefore, we can start from the gut microbiota and focus on discovering the biomarkers of MG that may be present in the gut flora, which will help to diagnose MG better and better understand the relationship between gut flora and MG. In the future, using probiotic therapy to regulate intestinal flora may also be an effective treatment for MG.

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FIGURES

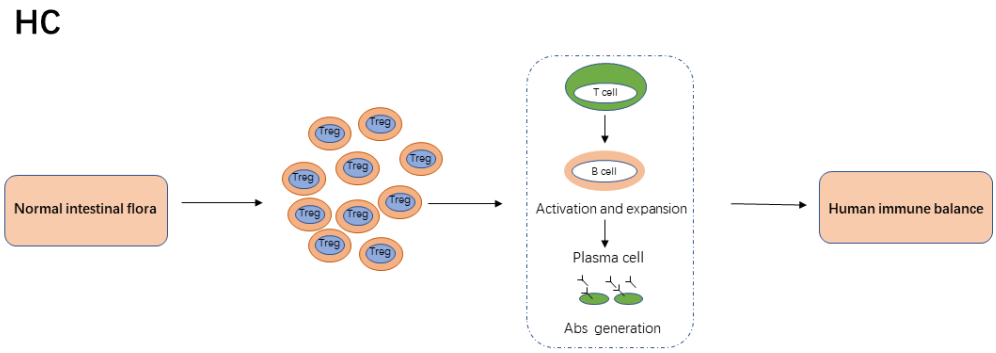

Figure 1

The intestinal flora of healthy people is normal, and the number of Treg cells is normal, which is sufficient to play the role of autoimmune suppression and maintain the immune balance of the body.

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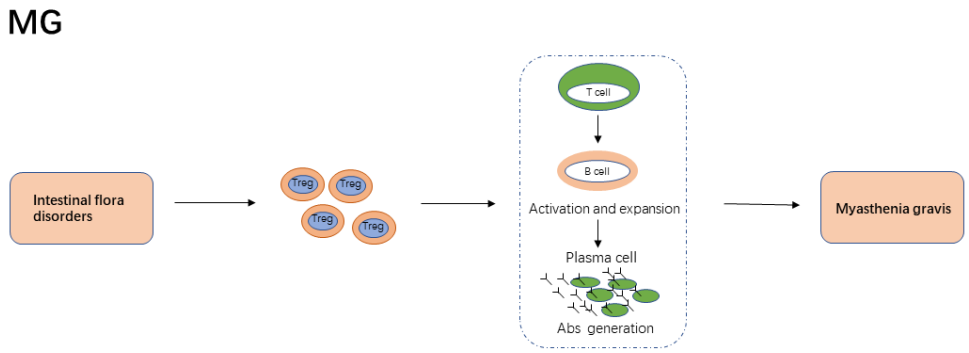

Figure 2

Disturbances of the intestinal flora lead to abnormal numbers of Treg cells, resulting in disturbances in autoimmune balance and increased autoimmune antibodies in the body, which may lead to the development of myasthenia gravis or exacerbation of symptoms.

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TABLES:

Table 1: The main genus in human intestinal flora and their related diseases

Gut microbiota	Main related diseases	Reference
Bacteroides	Obesity, Type 1 diabetes, Myasthenia Gravis	27, 28,49
Clostridium	Alzheimer's Disease,Inflammatory Bowel Diseases,Type 1 diabetes, Myasthenia Gravis	19,23, 28,48
Faecalibacterium	Chronic Urticaria,Crohn's disease, neuromyelitis optica spectrum disorders, Parkinson's disease, multiple sclerosis	13,17, 18, 30, 31
Ruminococcus	Irritable bowel syndrome, Parkinson's disease,Gestational diabetes mellitus(GDM)	16, 21,33
Peptidococcus	Inflammatory bowel disease,Crohn's disease	11,22
Peptidostreptococcus	Oral cancer,colorectal cancer,Gastric Cancer, Kawasaki Syndrome	14,15,29, 32
Bifidobacterium	Chronic pancreatitis, Gastric cancer, multiple sclerosis,fatty liver,Nasopharyngeal Carcinoma, Type 1 diabetes	12,20,24,25, 26, 28

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Table 2: Relationship between intestinal flora changes and MG

Researchers	Gut microbiota	MG	Feature
German Moris[47]	Verrucomicrobiaceae,Bifidobacteriaceae, Coriobacteriaceae, Leuconostocacceae and Flavobacteriaceae	↓	Compared to the healthy group, the gut microbiota of the MG group was changed in terms of the relative abundances of bacterial taxa, the current research results of intestinal flora are not completely consistent[47-49].
	Acidaminococcaceae, Desulfovibrionaceae and Pasteurellaceae	↑	
Dongxu Qiu[48]	Clostridium,Eubacterium and F. prausnitzii	↓	
	Streptococcus and Parasutterella	↑	
Peng Zheng[49]	Fusobacteria, Bacteroidetes	↑	
	Actinobacteria	↓	