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Review

Achalasia: The Current Clinical Dilemma and Possible Pathogenesis

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Achalasia is a primary esophageal motility disorder manifested by dysphagia and chest pain that impair patients' quality of life, and it also leads to chronic esophageal inflammation by food retention and increases the risk of esophageal cancer. Although achalasia has long been reported, the epidemiology, diagnosis and treatment of achalasia are not fully understood. The current clinical dilemma of achalasia is mainly due to its unclear pathogenesis. In this paper, epidemiology, diagnosis treatment, as well as possible pathogenesis of achalasia will be reviewed and summarized. The proposed hypothesis on the pathogenesis of achalasia is that genetically susceptible populations potentially have a higher risk of infection with viruses, triggering autoimmune and inflammation responses to inhibitory neurons in lower esophageal sphincter.
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Key Words
Diagnosis; Epidemiology; Esophageal achalasia; Etiology; Therapeutics

Introduction

Achalasia is a primary esophageal motility disorder, characterized by incomplete relaxation of the lower esophageal sphincter (LES) and aberrant peristalsis in the esophageal body.[1] It not only causes dysphagia and chest pain that impairs patients' quality of life,[2] but also leads to chronic esophageal inflammation by food retention and eventually increases the risk of esophageal cancer.[3] Although achalasia was first reported in 1674, it is still not fully understood.[4] These warrant further exploration of the pathogenesis of achalasia. In this review, evidence-based pathogenesis will be presented.

Epidemiology

Achalasia used to be considered as a rare motility disease, with an annual incidence and prevalence of 1.63/100 000 and 10.82/100 000, respectively.[5] However, the data might be significantly underestimated due to difficulties in identifying patients with achalasia. It was estimated that approximately 27-42% of patients with achalasia used to be misdiagnosed because of their overlapping symptoms such as heartburn and chest pain.[6,7] A recent American epidemiological survey conducted in areas with a high prevalence of high-resolution manometry (HRM) found that the prevalence and annual incidence of achalasia were 162.1/100 000 and 26.0/100 000 respectively, which were at least 2- to 3-fold greater than previously

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estimated.[8] Therefore, given the poor penetration of the most advanced diagnostic criteria and technology, it is safe to say that the actual number of achalasia patients worldwide remains unknown.

Diagnosis

The diagnosis of achalasia is based on an appropriate clinical presentation such as dysphagia, reflux, and chest pain, and typical findings on multiple complementary tests including endoscopy, barium esophagram, HRM, and functional lumen imaging probe (FLIP).[2] In these examinations, endoscopy can be used to rule out structural lesions, esophageal inflammation, and tumors,[9] and it presents manifestations such as esophageal dilatation, effusion, and food impaction in achalasia.[10] The typical "beak sign" in barium esophagram is also strong evidence for the auxiliary diagnosis of achalasia.[11] With HRM, achalasia can be divided into 3 types according to the motor patterns of the esophageal body: type I without peristalsis and pressurization, type II seen with ≥ 20% pan-esophageal pressurization, and type III with ≥ 20% premature contractions. Abnormal integrated relaxation pressure is a common feature of these 3 subtypes.[12] FLIP, a new device for evaluating the expansion of esophagogastric junction,[13] has gradually become a potential complementary diagnostic tool for achalasia.[14,15] However, patients with achalasia in the earlier stage do not necessarily have such typical findings. Diagnosing achalasia by using these tools alone has a limited yield.[10,16] Further understanding of much earlier stage in the pathogenesis of achalasia is needed to achieve an accurate diagnosis of this disease.

Treatment and Prognosis

The treatment options of achalasia include drug therapy (calcium channel blockers, nitrates, and phosphodiesterase inhibitors), endoscopic therapy (peroral endoscopic myotomy, botulinum toxin injection, and pneumatic dilation), surgery (laparoscopic Heller myotomy) and others that can relieve the obstruction of LES. Among them, pharmacotherapy is less commonly used in clinical practice because of its transient efficacy and many side effects.[17,18] Although botulinum toxin injection can provide some relief in most patients, regular reinjections are still required, yet with diminishing benefits over time.[19] Esophageal myotomy through endoscopy and laparoscopy, a commonly used, well-established, safe and effective treatment for achalasia, provides symptom relief with a success rate of over 90%,[20,21] but the impairment of the esophagogastric junction would result in gastroesophageal reflux disease in about 30% of the postoperative patients.[22,23] In addition, approximately one-third of achalasia patients need repeated treatments.[2] Therefore, it is of great value to further explore the radical treatment, especially the non-invasive radical treatment, of achalasia.

Pathogenesis

The current clinical dilemma of achalasia is mainly due to its unclear pathogenesis. Therefore, further clarification of the pathogenesis of achalasia is essential. Numerous heterogeneous studies on the pathogenesis of achalasia have proved that achalasia is caused by the neurodegeneration in the LES. Neurodegeneration is defined as the selective loss of inhibitory neurons in the myenteric plexus of the distal esophagus.[2] Various contributing factors could lead to the neurodegeneration, including susceptible individuals with genetic background and the environmental factors which evoke a series of inflammation and immune response. Taking account of all the established evidence involved in achalasia, we proposed a hypothesis on the pathogenesis which was plotted in the Figure, where susceptible individuals with genetic background, are affected by viruses or other environmental factors, which subsequently triggers an autoimmune response that involves many mediators such as cytokines, chemokines, autoantibodies, complements, and extracellular proteolytic enzymes. Correspondingly, mast cells, eosinophils, and T lymphocytes cross-talk with each other to mediate inflammation, causing the degeneration or loss of myenteric nerve plexus inhibitory neurons.

Herein we reviewed the characteristics of neurodegeneration of LES in achalasia and the precipitating factors involved in the process of neurodegeneration in order to deepen the understanding of achalasia and provide enlightenment for potential therapeutic targets of achalasia.

Neurodegeneration in the Lower Esophageal Sphincter

Neurodegeneration is characterized by the selective loss of inhibitory neurons in the myenteric plexus of the distal esophagus and the dramatic reduction in important mediators involved in the release and transmission of these neurons. These mediators include nitric oxide (NO), vasoactive intestinal peptide (VIP), and interstitial cells of Cajal (ICCs).

NO, the main inhibitory neurotransmitter that leads to the relaxation of the Auerbach plexus, is predominantly produced by neuronal nitric oxide synthase (nNOS).[24] The number of nNOS-positive cells in LES was significantly lower in patients with achalasia than that in controls.[25-27] Sivarao et al[28] demonstrated that the

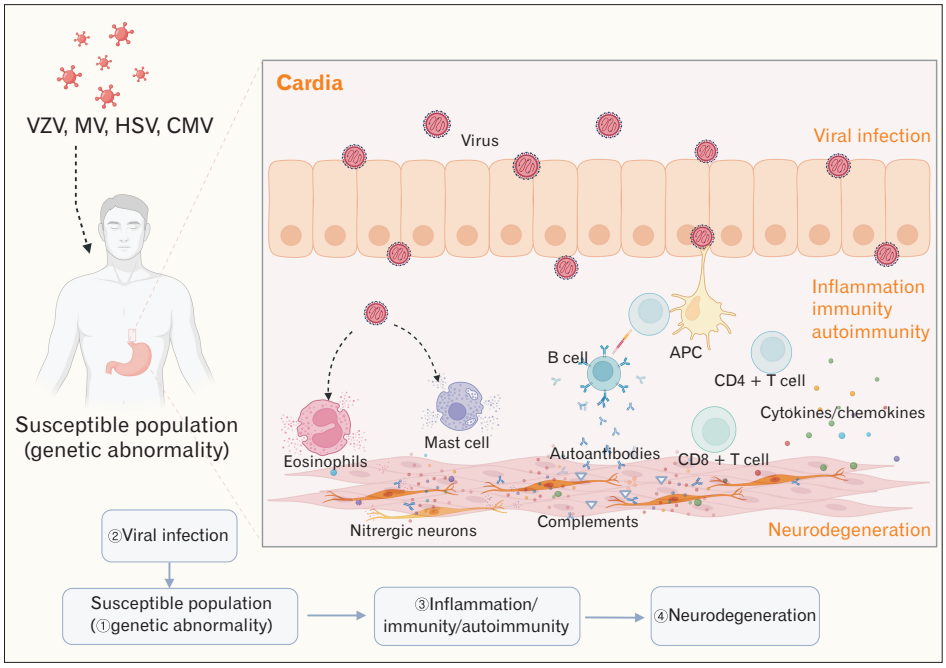

Figure. The possible pathogenesis hypothesis of achalasia: susceptible individuals with genetic background, are affected by viruses or other environmental factors, which subsequently triggers an autoimmune response that involved many mediators such as cytokines, chemokines, autoantibodies, complements, and extracellular proteolytic enzymes. Correspondingly, mast cells, eosinophils, and T lymphocytes cross-talk with each other to mediate inflammation, causing the degeneration or loss of myenteric nerve plexus inhibitory neurons. VZV, varicella-zoster virus; MV, measles virus; HSV, herpes simplex virus; CMV, cytomegalovirus; T cell, thymus-dependent lymphocyte; B cell, bursa dependent lymphocyte; APC, antigen-presenting cell.

reduction of nNOS could cause achalasia-like LES dysfunction and esophageal body peristalsis disorder through establishing an nNOS gene knockout mouse model.

VIP is a pivotal neuropeptide released by the inhibitory neurons that causes the relaxation of the distal esophageal wall and LES. Aggestrup et al[29] examined VIP-containing nerves in the lower esophagus in patients with achalasia and controls, and conspicuously fewer VIP-immunoreactive nerve fibers were found in the smooth muscle of patients. Similarly, the lack of VIP immunoreactivity in achalasia patients was observed through an immunohistochemical study of the myenteric plexus.[30] Furthermore, Guelrud et al[31] administered intravenous doses of exogenous VIP and placebo to patients with achalasia and healthy controls, and compared LES pressure (LESP) by esophageal manometry. They found that exogenous VIP improved LES relaxation and decreased LESP in achalasia patients without affecting LESP in healthy volunteers, suggesting the relaxation effect of VIP on the lower esophageal sphincter in achalasia.[31]

ICCs are the cells originated from mesenchymal that occur within and around the muscularis in the gastrointestinal tract. Apart from serving as pacemakers, they are also involved in the transfer of neurotransmitters including substance P, vesicular acetylcholine transporter, and nNOS.[27] Previous histopathology studies have demonstrated a reduced number of ICCs in LES of achalasia patients, and the reduction of ICCs would concur with the number of nNOS-positive cells.[32,33] It should be noted that the number of ICCs differed among different achalasia subtypes and was related to patients' clinical prognosis.[27]

The Precipitating Factors Contributing to Neurodegeneration of Lower Esophageal Sphincter

Although the degeneration of inhibitory neurons in patients with achalasia appears evident, the causes are still unknown. Many factors might contribute to the neurodegeneration of achalasia: (1) genetic abnormality, (2) viral infection, and (3) inflammation and immunity.

Genetic abnormality

The occurrence of achalasia in siblings[34,35] and even in identical twins[36] has been reported by previous studies, suggesting a background of genetic abnormality. In addition, achalasia has also been confirmed to be associated with known genetic diseases such as Down's syndrome,[37] Parkinson's disease,[38] and Allgrove's syndrome.[39] Therefore, genetic factors are involved in the occurrence and development of achalasia. The related genes include immune-related and neurodegeneration-related genes. These genetic abnormalities could cause the susceptibility to viral infection and neurodegeneration after viral infection.

Immune-related genes. The human leukocyte antigen (HLA) class II gene, an immune-related gene cluster, is located on the short arm of chromosome 6 (6P23.1) and consists of HLA-DP, HLA-DR, and HLA-DQ.[40] Genetic association analysis studies

Table 1. The Association Between Human Leukocyte Antigen Class II Genes and Susceptibility to Achalasia

Researcher	Population	Methods	Gene	Results
Wong et al,[41] 1989	Achalasia (n = 40) Control (n = 979)	HLA phenotyping	HLA-DQw1	HLA-DQw1 heightened the risk of achalasia by 4.2 times in Caucasians and 3.6 times in blacks.
De la Concha et al,[44] 1998	Achalasia (n = 40) Control (n = 275)	HLA phenotyping	HLA-DQA1*0101 HLA-DQaP	Achalasia was most strongly associated with HLA-DQA1*0101 and 2 HLA-DQaP heterodimers.
Ruiz-de-León et al,[42] 2002	Achalasia (n = 92) Control (n = 275)	HLA typing; autoantibodies determination	HLA-DQA1*0103 HLA-DQB1*0603	The patients with achalasia showed a significantly higher frequency of HLA-DQA1*0103 and DQB1*0603, in which found greater prevalence of the antiplexus antibodies.
Gockel et al,[45] 2014	Achalasia (n = 1068) Control (n = 4242)	Genotyping performed on the immunochip; genetic association analysis	HLA-DQβ1 insertion	An eight-residue insertion at position 227-234 of HLA-DQβ1 showed the strongest association with achalasia.
Becker et al,[46] 2016	Multicenter study (Poland, Sweden, Central Europe, Spain, Italy)	Genotyping and imputation	HLA-DQβ1 insertion (rs28688207)	The HLA-DQβ1 insertion was a strong achalasia risk factor and displayed a geospatial north-south gradient among Europeans (lowest in the northern and highest in the southern).
Furuzawa-Carballeda et al,[43] 2018	Achalasia (n = 91) Control (n = 234)	High-resolution HLA typing based on Sanger and NGS	HLA-DRB1*14:54 HLA-DQB1*05:03	The HLA class II alleles HLA-DRB1*14:54:01 and DQB1*05:03:01 and the extended haplotype were risk factors for achalasia in mixed-ancestry Mexican individuals.
Vackova et al,[47] 2019	Achalasia (n = 347) I:II:III (89:210:48)	genotype-phenotype analysis	HLA-DQβ1 insertion (rs28688207)	The frequency of the HLA-DQB1 insertion differed among achalasia subtypes, being most prevalent in type I (14.6%), followed by type II (9.5%) and III (6.3%).
Li et al,[48] 2021	Achalasia (n = 330) Control (n = 2073)	Whole-exome sequencing; array-based genome-wide association analysis	HLA-DPB1 missense variants (rs1126511)	Common missense variants rs1126511 (HLA-DPB1) were reproducibly associated with an increased risk of achalasia.

HLA, human leukocyte antigen.

have found a trend in increasing frequency of the HLA-DR and DQ alleles such as HLA-DQw1,[41] HLA-DQA1*0103, HLA-DQB1*0603,[42] HLA-DRB1*14:54, and HLA-DQB1*05:03[43] in patients with achalasia (Table 1).[44-46] Vackova et al[47] performed the first genotype-phenotype analysis to investigate the frequency of an insertion variant (rs28688207) in HLA-DQB1, and found that the distribution of the insertion was significantly different across the HRM subtypes, being the most prevalent in type I, followed by type II and III. A whole-exome sequencing study conducted by Li et al[48] also identified a missense variant (rs1126511) in the HLA region in patients with achalasia.

The protein tyrosine phosphatase N22 (PTPN22) gene, another immune-related gene, which encodes a lymphoid-specific phosphatase that downregulates T cell activation,[49] is associated with systemic lupus erythematosus, rheumatoid arthritis and other autoimmune diseases.[50] Santiago et al[51] performed a case-control study with 231 nonrelated Spanish patients of white ethnicity who were diagnosed with achalasia and 554 healthy controls, all genotyped for PTPN22 C1858T using TaqMan chemistry. The frequency of the 1858T allele was found to be higher in female achalasia patients than in healthy controls.[51]

Besides, other case-control studies, which compared the genotype of achalasia patients and controls, also demonstrated gene polymorphisms of cytokines such as interleukin-10 (IL-10) promoter,[52] IL-23 receptor,[53] and IL-33.[54]

Neurodegeneration-related genes. NOS, ICCs, and VIP play important roles in the transmission of neurotransmitters that mediate contraction and relaxation of the esophageal sphincter. Therefore, the abnormalities of related genes can also increase the risk of achalasia.

VIP is one of the main neurotransmitters implicated in the relaxation of the LES. Paladini et al[55] investigated 5 single nucleotide polymorphisms mapping in the human VIP receptor 1 gene in 104 achalasia patients and 300 controls. Achalasia patients showed a significant difference in allele, genotype, and phenotype distribution of single nucleotide polymorphism rs437876 mapping in intron 4, and this association was almost entirely owing to those patients with achalasia onset late in life.

Another inhibitory neurotransmitter, NO, which mediates the relaxation of smooth muscle, can be produced by 3 different NOS isoforms: nNOS, inducible NOS (iNOS), and endothelial NOS (eNOS).[56] A case-control study on 183 patients with achalasia and 366 healthy subjects showed that eNOS4a4a, iNOS22GA, and nNOS29TT genotypes were more common in achalasia patients than those in controls. This result suggested that the polymorphisms of eNOS, iNOS, and nNOS genes were risk factors for achalasia.[57]

ICCs are involved in multiple neurotransmitter transmission in the LES region and their loss could lead to the development of different gastrointestinal motor disorders.[26] The c-kit gene polymorphisms (rs2237025 and rs6554199) might affect the transcription activity of ICCs in the lower esophageal sphincter. Furthermore, a Turkish cohort study supported the hypothesis that the T allele of the c-kit rs6554199 polymorphism may be associated with achalasia.[58]

Viral infections

Esophagus is the only part covered by squamous epithelium in the human digestive tract, which makes it susceptible to infection by multiple neurotropic viruses such as varicella-zoster virus,[59] herpes simplex virus type 1 (HSV-1),[60] cytomegalovirus.[61] These viruses can be latent in ganglion cells in the squamous epithelium and trigger inflammation after activation.

The presence of viral infection in achalasia has been confirmed by multiple studies. A few case-control studies found that the serum antibody titers of measles virus,[62] varicella-zoster virus,[63] and HSV-164 of achalasia patients were significantly higher than those of healthy controls. Moreover, viral DNA can be detected in saliva and LES muscle of achalasia patients (Table 2).[63-70] The relationship between viral infection and achalasia has also been demonstrated in vitro. After ex vivo stimulation with HSV-1 antigens, the proliferation of lymphocytes from LES of achalasia patients and the release of T helper 1 type cytokines such as IFN-γ and IL-2 were found to be significantly higher.[64,66,67]

However, the controversing association between viruses and achalasia has also been reported.[30,71,72] A recent study by Moradi et al[72] failed to detect any significant relationship between achalasia and papillomavirus, adenovirus, and neurotropic viruses in the cases. It is possibly due to the analysis of peripheral blood samples rather than LES muscle samples in the study. In addition, the technique used in the study might be insufficient. Therefore, further large-sample and high-level studies are still needed to explore the role of viruses in achalasia.

Inflammation and immunity

When viral infection occurs in susceptible populations, inflammation and immunity might be activated and cause sustained damage to myenteric neurons of the distal esophagus.

Infiltration of immune cells. Mast cells, eosinophils and lymphocytes could be recruited and degranulated by the body

Table 2. Viral Infection in Achalasia

Researcher	Population	Methods	Viruses	Results
Jones et al,[62] 1983	Achalasia (n = 18) Control (n = 12)	Complement fixation test; haemagglutination inhibition test	MV	Serum antibody titers against MV increased 3 fold in patients with achalasia.
Robertson et al,[63] 1993	Achalasia (n = 58) Control (n = 40)	Complement fixation test; in situ DNA hybridisation	VZV HSV-1 CMV	The incidence of VZV antibodies was significantly greater in achalasia. VZV was detected in 3/9 achalasia. No positive results were obtained for HSV-1or CMV.
Niwamoto et al,[65] 1995	Achalasia (n = 12) Control (n = 116)	PCR amplification; automated DNA sequence analysis	HSV	92-bp fragments were identified in nearly all specimens, which were identical to a single HSV sequence.
Birgisson et al,[71] 1997	Achalasia (n = 13) Control (n = 15)	PCR amplification; automated DNA sequence analysis	HSV-1/2, CMV, EBV, VZV, HHV-6, MV, HPV	No amplified products were seen in the achalasia specimens or controls corresponding to any of the virus sequences tested.
Castagliuolo et al,[66] 2004	Achalasia (n = 15) Control (n = 8)	Esophageal mononuclear cell proliferation assay; ELISA	HSV-1	The prevalence of circulating anti–HSV-1 and HSV-2 antibodies proved similar in the 2 groups. After incubation with HSV-1, mononuclear cells from achalasia patients showed a 3.4-fold increase and a 1.4-fold increase in interferon-gamma release.
Facco et al,[67] 2008	Achalasia (n = 59) Control (n = 38)	ELISA; esophageal mononuclear cell proliferation assay	HSV-1	HSV-1 DNA was detected in both patients (63%) and control (68%). Being exposed to HSV-1 increased monocyte proliferation and released IFN-γ and IL-2 in achalasia.
Villanacci et al,[30] 2010	Achalasia (n = 12) Control (n = 7)	IHC; in situ DNA hybridisation	HSV HPV	All patients were completely negative for the presence of both HSV and HPV.
Lau et al,[64] 2010	Achalasia (n = 151) Control (n = 118)	PCR; RT-qPCR; ELISA	HSV-1	There was no difference in the positive rate of serum HSV-1 between achalasia and control, but higher in saliva (7.9%). HSV-1 stimulation increased IFN-γ expression by 61.33 times in achalasia.
Moradi et al,[72] 2018	Achalasia (n = 52) Control (n = 50)	PCR; RT-PCR	Neurotropic and non-neurotropic viruses	No association between the virus and achalasia was detected.
Kanda et al,[68] 2021	Achalasia (n = 11) Control (n = 6)	RT-qPCR	HSV-1	The expression of HSV1-miR-H1 target ATG16L1 was significantly reduced in the LES of achalasia.
Naik et al,[69] 2021	Achalasia (n = 15) Control (n = 5)	Nested-RCR; RT-PCR; immunocytochemistry	VZV	VZV DNA was detected in 80% of the saliva and VZV transcripts were detected in 87% of the LES in achalasia. VZV late proteins (gE, gH, and ORF40p) were detected in enteric neuronal cell bodies and nerve fibers.
Gaber et al,[70] 2022	Achalasia (n = 6769) Control (n = 27 076)	Correlation analysis	VZV HPV	The presence of any of the autoimmune conditions and viral infections (VZV and HPV) were associated with increased odds of achalasia.

MV, measles virus; VZV, varicella-zoster virus; HSV-1/2, herpes simplex virus type 1/2; CMV, cytomegalovirus; PCR, polymerase chain reaction; EBV, Epstein-Barr virus; HHV-6, human herpes virus 6; HPV, papillomavirus; ELISA, enzyme linked immunosorbent assay; RT-qPCR, reverse transcription quantitative real-time PCR; IHC, immunohistochemistry; ATG16L1, autophagy-associated 16-like protein 1; LES, lower esophageal sphincter.

Table 3. Autoantibodies in Achalasia

Researcher	Population	Methods	Autoantibodies	Results
Goin et al,[91] 1999	Chronic chagasic patients with achalasia (n = 19); without achalasia (n = 14); non-chagasic patients with idiopathic achalasia (n = 25); normal control (n = 20)	ELISA	Autoantibodies against M2 mAChR	There was a strong association between the existence of circulating anti-M2 mAChR antibodies and the presence of achalasia in chagasic patients.
Latiano et al,[90] 2006	Achalasia (n = 41) Control (n = 200)	Indirect immunofluorescence; immunoblotting	Antineuronal antibodies	10 of 41 (24.4%) patients presented antineuronal antibodies.
Kraichely et al,[92] 2010	Achalasia (n = 70) Control (n = 161)	Radioimmunoprecipitation assays; ELISA; indirect immunofluorescence	GAD65 antibody	The overall prevalence of neural autoantibodies in achalasia was significantly higher, especially the GAD65 antibody (21.4%).
Mukaino et al,[89] 2018	Achalasia (n = 28) CIPO l (n = 14)	LIPS	Anti-gAChR antibodies	There is a significant prevalence of anti-gAChR antibodies in patients with Achalasia (21.4%).
Priego-Ranero et al,[93] 2022	Achalasia (n = 36) Control (n = 22), EGJOO (n = 6); TD (n = 16)	Immune blot/line assay	Anti-GAD65 autoantibodies; Anti-PNMA2 autoantibodies	Most of the achalasia sera had anti-GAD65 (83%) and anti-PNMA2 (90%) autoantibodies.

ELISA, enzyme linked immunosorbent assay; M2 mAChR, M2-muscarinic acetylcholine receptors; GAD65, glutamic acid decarboxylase-65; CIPO, chronic intestinal pseudo-obstruction; LIPS, luciferase immunoprecipitation system assay; gAChR, ganglionic acetylcholine receptor; EGJOO, esophagogastric junction outflow obstruction; TD, transplant donors; PNMA2, paraneoplastic antigen Ma2.

after viral infection and produce direct or indirect killing effects on normal cells.

A series of histopathological studies have been conducted to investigate the inflammatory cell infiltration in the LES region of achalasia patients. By comparing the LES specimens between 116 patients with achalasia and 20 controls, Liu et al[33] found that the number of mast cells in patients with achalasia increased significantly. The number of mast cells negatively correlated with the number of interstitial cells of ICCs and nNOS. A significantly higher proportion of mast cell degranulation in LES muscle in patients with achalasia was also found when compared to the controls.[73] Similarly, infiltration and degranulation of eosinophils have also been reported in patients with achalasia.[74-77] As for lymphocytes, there was considerable heterogeneity among studies. Raymond et al[78] concluded that T lymphocytic inflammatory infiltrates of varying intensity were present along the nerve fascicles and around ganglion cells in 90% of the cases of achalasia. Clark et al[79] further found that a T-cell-rich inflammatory response was predominantly composed of CD3-positive T cells, most of which were also CD8-positive. However, Döhla et al[80] showed that the majority of myenteric inflammatory cells in patients with achalasia were CD4-positive T cells with an overall CD4/CD8 ratio of 1.82.

Up-regulation of cytokines, chemokines and complements. Cytokines, chemokines and complements could be induced after viral infection. Cytokines and chemokines such as IL, IFN, and TNF mediate the recruitment and migration of immune cells, which have been proved to play an important role in the pathophysiology of auto-inflammatory disorders, pro-inflammatory disorders, and neurological disorders.[81] Complements, a system of interacting serum proteins, can kill viruses and eliminate apoptotic cells by forming membrane-attacking complexes, which is essential for host defense and immune surveillance.[82]

Studies have demonstrated the elevation of cytokines and chemokines in achalasia, suggesting that inflammation is an indispensable part of the pathogenesis of achalasia. Palmieri et al[83] performed the first genome-wide expression profiling of mRNA in LES tissue samples from patients with achalasia and controls. Toll-like receptor 4 and IL-18 were found to be significantly higher in LES specimens of patients with achalasia. Serological analysis studies also found that pro-inflammatory cytokines (IFN-γ, IL-17, and IL-22), anti-inflammatory cytokines (IL-4 and TGF-β), and chemokines (monokine induced by IFN-γ and IFN-γ induced protein-10) were significantly upregulated in patients with achalasia, the most prominent of which were proinflammatory related factors.[84-86]

Besides, complements might also involve in the pathogenesis of achalasia. Im et al[87] collected blood samples from 5 patients with achalasia and 5 healthy controls. By using serum proteomic analysis, they found that several complements including C4B5, C5, and C3 were upregulated in patients with achalasia. Histopathological studies also found that in patients with achalasia, complements were heavily deposited on ganglion cells of the intestinal myoplexus. As a participant in the inflammatory response, complements may be inappropriately activated through certain viruses or immune complexes formed by the combination of viruses and antibodies, to form membrane-attacking complexes that damage neurons or other tissues.

Autoantibodies and extracellular proteolytic enzymes. Viral infection can also lead to the production of in situ antigens and might induce the production of autoantibodies and extracellular proteolytic enzymes, subsequently producing indiscriminate killing effects. A matched case-control study including 6769 patients with achalasia and 27 076 controls found that the incidence of autoimmune diseases in patients with achalasia was significantly higher than that in controls, supporting the hypothesis that achalasia has an autoimmune component.[70]

With the use of immunohistopathological analysis, a study found that matrix metalloproteinase-9, an extracellular proteolytic enzyme, increased significantly in the intestinal myoplexus of esophageal tissues of achalasia and co-expressed with achalasia autoantigens glutamic acid decarboxylase-65 and paraneoplastic antigen MA2 in situ.[88] A corresponding increase in other autoantibodies, such as anti-ganglionic acetylcholine receptor antibodies,[89] antineuronal antibodies[90] and so on (Table 3),[91-93] was also detected in patients with achalasia by using indirect immunofluorescence and immunoblotting assay. The above researches provided new insight into autoimmune mechanisms in the immune pathology of achalasia.

Conclusion

Achalasia is a primary esophageal motility disorder with unclear epidemiology, complicated diagnosis and no radical treatment. The difficulties in the diagnosis and treatment of achalasia are largely due to its unclear pathogenesis. One possible hypothesis is that genetically susceptible populations potentially have a higher risk of infection with viruses, triggering autoimmune and inflammation responses to inhibitory neurons in LES. However, it is hard to prove the hypothesis and explore the specific pathways due to the absence of well-established cell and animal models. Furthermore, considering the clinical heterogeneity of achalasia, studies focusing on the differences in pathogenesis among different subtypes are also necessary.

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