

Review Article

Autoimmune Inflammation in Achalasia

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Abstract

Idiopathic Achalasia is an archetype esophageal motor disorder. It is an inflammatory pathology produced by the loss of ganglion cells of the esophageal Auerbach's plexus. Although the etiology is unknown, it is conceivable to hypothesize an etio- and physiopathogenic mechanism for idiopathic Achalasia. Early event that triggers the disease appears to be the product of a constant offense produced by a neurotropic infectious agent, which induces a persistent inflammation at the myenteric plexus. Nonetheless, not all of the patients infected with virus progress to the disease. Thus, only those individuals with genetic predisposition to maintain a severe chronic inflammation and defects in tolerance mechanisms will evolve to Achalasia. Moreover, in most of the cases illness is accompanied by the presence of antinuclear antibodies and myenteric antibodies that are suggested to participate in the destruction of the Auerbach's plexus. In this review we will focus on the evaluation of the inflammation that suggests that Achalasia is an autoimmune disease associated with a viral infection.

Keywords: Achalasia; Autoimmunity; Inflammation

Introduction

Primary Achalasia is one of the most common and study causes of motility disorder of the esophagus and is characterized by myenteric plexitis leading to neuronal loss. It is characterized by the absence of coordinated esophageal peristalsis and incomplete relaxation of the Lower Esophageal Sphincter (LES) during deglutition. Symptoms include dysphagia typically for solids and liquids, but the patients may also have regurgitation of ingested food, retrosternal pain, burning, pulmonary aspiration, varying degrees of weight loss, cough and nutritional deficiencies [1]. The incidence is 1 in 100,000 habitants per year, while prevalence is 8 to 9 cases per 1,000,000 people worldwide [2]. The diagnosis of idiopathic Achalasia is confirmed with High-Resolution Manometry (HRM) which is the current gold standard while the esophagram with barium swallow and endoscopy are also used [2].

Etiology and physiopathology

Several aspects has suggested that Achalasia is an autoimmune disease associated with a viral infection; chronic inflammatory infiltrates, predominantly enriched in lymphocytes that damage the ganglion cells of the Auerbach's plexus; the presence in the patient's serum of organ-specific autoantibodies; the association in the same

patient with other well-established autoimmune diseases that often occurs in the association with one another, either within single individual or family; and genetic factors and polymorphisms that might play an important role in the development and progression of Achalasia. This manuscript will be focused on the autoimmune inflammation in Achalasia.

Inflammation

Proteomic studies and serum profile analysis has established that elevated concentrations of pro inflammatory cytokines (IL-22, IL-17, IL-12, IL-6 and TNF- α), C4-B5, C3, cyclin-dependent kinase 5, α 2-macroglobulin and anti-GAD65 antibodies are up-regulated in Achalasia patients than in healthy controls [3-6], as well as the terminal complement complex C5b-C9, C9 and IGM are deposited within or at the ganglion cells of the myenteric plexus, validating the theory of immune-mediated response and neural degeneration components of the disease pathogenesis [7].

Histopathological hallmark of Achalasia is degeneration of the myenteric plexus in the esophageal tissues. Neuritis and ganglionitis are evident in the early stages of the disease, leading to a progressive loss of ganglion cells and fibrosis. It has found inflammatory infiltrates of varying intensity around myenteric neurons, contrasting with the control groups with normal myenteric plexus [8-12]. The characterization of the intraganglionic infiltrate show a predominance of CD3+, CD4+, CD25+ and CD8+ T lymphocytes in all the diseased tissues, as well as CD20+ B cells and activated eosinophils (positive major basic protein and eosinophils-derived neurotoxin) but to a lesser degree, with detection of occasional plasma cells along the nerve fascicles and around the ganglion cells [8,9,11,13-15]. Moreover, T and B inflammatory infiltrates predominate in tissues with advanced stage disease (>10-year symptom history) [13]. Contact of mast cell infiltration with Interstitial Cells of Cajal (ICC) has recently associated with nitrergic nerves, S-100-positive cells, ICC and neuronal degeneration in Achalasia [12] [16-18]. The number of mast cells is higher in type I than type III Achalasia. Additionally, patients with a history of autoimmune disease or viral infection has greater mast cell infiltration in the LES muscle [12]. It has also demonstrated

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that mast cells express the 3-O-sulphated heparan sulphate receptor that mediates the entry of HSV-1 but not HSV-2 [19], and thus mast cells played an important role in host defenses against HSV infection through TNF- α and IL-6 expression [20].

Some tissue and circulating cells of the adaptive immune response have also been immunophenotyped in patients with Achalasia, including CD4 effect or T cells. In the muscle of the lower esophageal sphincter, it has been detected an increased expression of interleukin-22 (IL-22) particularly along the myenteric plexus [8,5] and in peripheral cells of patients with Achalasia. IL-22, synthesized by Th22 and Th17 cells. It is an initiator of the innate immune response against many infectious disease (bacterial, viral, and fungal infections) at epithelium. The Th22 cells do not express IL-17, IL-4, and IFN- α , IL-23R nor CD161. This CD4 T cells differentiate from naïve T cells (transcription factor: Aryl Hydrocarbon Receptor (AHR)) in response to TNF- α and IL-6 signals, and subsequently synthesizes and secretes IL-22, IL-13 and IL-26 [5,21].

The IL-17A is produced by Th17 cells, CD8 T cells, $\gamma\delta$ T cells, neutrophils, macrophages and a variety of epithelial and parenchymal cells [22]. IL-17A is a key mediator of auto immune diseases. Under both physiological and pathological conditions Th17 cells induce B cell proliferation and differentiation into immunoglobulin-secreting cells and produce a wide range of proinflammatory cytokines such as IL-17A, IL-17F, IL-21 and IL-22, IL-1 β , IL-6, IL-8, TNF- α , IL-23, G-CSF, GM-CSF, and chemokines (CXCL1, CXCL2, CCL2, CXCL5 CCL20) [23]. This endorses the diapedesis of neutrophils and monocytes. The increased infiltration of Th17 cells is associated with enlarged IL-21 concentrations and Activation-Induced cytidine Deaminase (AID) expression [24]. AID is the key enzyme that controls Ig class switching and somatic hypermutation [25], suggesting that IL-17 indirectly promotes lymphoid neogenesis and supports the development of humoral immune responses contributing to antibody-mediated response. The percentage of tissue and circulating Th17 cells is conspicuously higher in patients with Achalasia compared to controls [5,26].

Thus, it is not preposterous to propose that interferon-gamma (IFN- γ) also might play a role in the pathogenesis of the disease, promoting cell-mediated immunity and activating mononuclear cells. Produced by activated CD4 effect or T cells and Natural Killer cells (NKs), IFN- γ regulates various immune and inflammatory responses. Specifically, this cytokine promotes immune modulation, and it has anticancer and antimicrobial activities. It inhibits type I collagen synthesis and induces the production of chemokines and their receptors [23]. Abnormally high percentages of circulating and tissue IFN- γ /CD4+ T cells has been determined in patients with Achalasia when compared with controls [8,27].

Following HSV-1 antigen exposure augmented IL-1 β -, IL-2- and TNF- α -cell expression has also been found in tissue from Achalasia patients as compared with controls [27].

TGF- β 1 has immunosuppressive effects on T, B and NK cells; controls cellular growth, proliferation and differentiation of fibroblasts; down-modulates fibronectin, proteoglycans, type I and III collagen synthesis as well as apoptosis. In the myenteric plexus from patients with Achalasia, it has been detected significantly higher levels of TGF- β 1 compared with tissues from controls [8].

IL-4 is a pleiotropic cytokine involved in the regulation of immune response. It inhibits the synthesis of pro-inflammatory cytokines,

promotes Th2 cell differentiation, and inhibits Th1 cells. It is a B cell stimulator factor, and is a down-regulator of apoptosis.

IL-4 is a fundamental cytokine during the fibrosis process. Th2 cells suppress autoimmune disease mediated by Th1 cells [28].

Previous studies by our group have been determined that the peripheral cells and tissue of patients with Achalasia has significantly higher IL-4-expressing cell percentage compared with controls [8].

IL-13, a Th2 related cytokine, is a key mediator in fibro proliferative disorders. It acts through the regulation of type I collagen gene. IL-13 has also associated with the increase of esophageal body contractility in Achalasia [29]. On the other hand, IL-4/IL-13-expressing CD4+ T cells play important roles in polarization of macrophages/dendritic cells to the M2a phenotype, important for recovery from acute kidney injury through activation of JAK3/STAT6 signaling [30,31]. M2a macrophages are considered beneficial in reparative processes with respect to ongoing injury. Nonetheless, sustained activity may result in the continuous production of various wound-healing growth factors, ultimately becoming a pathological process leading to fibrosis [30-34]. IL-13-producing cell percentage in patients with Achalasia is similar to IL-4 [8].

Regarding regulatory cells, it is worth mentioning that, in healthy individuals, Tregs constitute 5% to 15% of peripheral and tissue CD4+ T cells. Tregs modulate the natural course of protective immune responses maintaining immune tolerance, in order to limit tissue damage, allergy, inflammation, cancer and autoimmunity. Additionally, Treg-mediated suppressive activity includes the synthesis of granzyme B, adenosine, CAMP and perforins; production of IL-10, and IL-35; depletion of IL-2; expression of suppressor molecules such as CTLA-4, GITR, LAG-3, IL-19 and TGF- β 1; and Antigen Presenting Cells (APCs) decrease functions, that otherwise promote energy or apoptosis of effect or T cells. Thus, the Tregs play a key role in metabolic and genetic regulation, in tissue repair (i.e., hair follicles regeneration) and homeostasis.

Severe inflammation determined in patients with Achalasia apparently induces an increase in the percentage of circulating and tissue Tregs cells when compared to controls [8].

In addition to Tregs, a circulating naïve/transitional T1 regulatory B cells have been shown to support development of immune tolerance [35]. Blair and colleagues have defined these latter cells as a regulatory B cell pool (1% to 3% of whole splenic B cells) with many subtypes that display a common CD19+/CD24hi/CD38hi phenotype in autoimmune diseases [36,37]. These IL-10-producing B cells suppress the differentiation of Th1 cells that requires CD80/CD86 interactions with target CD4+ T cells. Moreover, Tregs promote to the polarization and maintenance of Tregs and may control organ-specific inflammation [38]. A higher cell number of IL-10-producing B cells were determined in esophageal tissue of patients with Achalasia compared with control group [8].

Lastly, it is known that dendritic plasmacytoid regulatory cells (termed pDCregs) are a subpopulation of immune cells that express the Indoleamine 2,3-dioxygenase (IDO) enzyme that is responsible for mediating tryptophan metabolism, producing kynurenine, the first breakdown product, and a natural ligand for the AHR which suppresses T effect or cell activity and promotes polarization of naïve CD4+/CD25-/Foxp3- T cells to CD4+/CD25+/Foxp3+ regulatory T cells instead of Th17 cells and apoptosis of effector T cells. IDO-

mediated increased of L-kynurenine stops Th1 proliferation at mid-G1 phase; promotes Tregs activation and differentiation and the pro-apoptotic activity to endorse immune tolerance. IDO favors Th2 immune response and it has a selective role in its differentiation. In addition, IDO contributes to the immune responses to pathogens through the activation of TLRs (LPS, CPG/ODN and other bacterial antigens) and it subsequently modulates the inflammation [39-43]. Large numbers of IDO-expressing CD123 pDC cells has been determined in patients with Achalasia compared with control tissues.

Extracellular matrix turnover is a modulated process that involved Matrix Metalloproteinases (MMPs) activity and their inhibitors (TIMPs). Particularly, MMP-9 (92 kDa gelatinase) and MMP-2 (72 kDa gelatinase) play major role in tissue repair and remodeling and both have been described in LES from patients with Achalasia [8]. Moreover, it has been determined that the circulating levels of activated MMP-9/proMMP9 and MMP-9/MMP-2 proteoforms are increased in patients with Achalasia compared with controls. In this vein, MMP-9- and MMP-2-expressing cells were conspicuously higher in Achalasia than in control biopsies from transplant donors. Besides, in tissues with more MMP-9-expressing cells a severe damage in the Auerbach's plexus was observed. Thus, MMP-9 may contribute to the extracellular proteolysis of PNMA2, Ri, GAD65, and VIP (novel MMP-9 substrates) that might play role in the pathogenesis of organ-specific autoimmune disease including Achalasia (remnant epitopes generating autoimmunity paradigm) [8].

Conclusion

Evidence suggests that Achalasia may be caused by a viral infection with neurotropic viruses that have predilection for squamous epithelium, most importantly herpes simplex virus type 1 causing a chronic latent infection. This virus may act as a disease-initiating factor for chronic inflammation of the myenteric plexus ganglion cells. The inflammation in Achalasia is the keystone in the development and establishment of the disease. It contributes to the loss of immunological tolerance, probably through the processing of autoantigens' (REGA paradigm) inducing an autoimmune response in individuals with genetic predisposition.

References

- Kahrilas PJ, Bredenoord AJ, Fox M, Gyawali CP, Roman S, Smout AJ, et al. The Chicago Classification of esophageal motility disorders, v3.0. *Neurogastroenterol Motil.* 2015;27(2):160-74.
- Pandolfino JE, Gawron AJ. Achalasia: a systematic review. *JAMA.* 2015;313(18):1841-52.
- Cauble EW, Rife C, Singh ER, Arevalo LF, Freeman J, Lopes-Virella MF, et al. Evidence that achalasia involves a systemic inflammatory response. *Gastroenterol.* 2012;142(5):S97.
- Im SK, Yeo M, Lee KJ. Proteomic identification of proteins suggestive of immune-mediated response or neuronal degeneration in serum of Achalasia patients. *Gut Liver.* 2013;7(4):411-6.
- Wang Z, Zhang J, Mi J, Ma H, Zhao D. Expression and significance of interleukin-17 and interleukin-22 in the serum and the lower esophageal sphincter of patients with Achalasia. *Saudi J Gastroenterol.* 2018;24(4):242-8.
- Clayton S, Cauble E, Kumar A, Patil N, Ledford D, Kolliputi N, et al. Plasma levels of TNF- α , IL-6, IFN- γ , IL-12, IL-17, IL-22, and IL-23 in achalasia, eosinophilic esophagitis (EoE), and Gastroesophageal Reflux Disease (GERD). *BMC Gastroenterol.* 2019;19(1):28.
- Storch WB, Eckardt VF, Junginger T. Complement components and terminal complement complex in oesophageal smooth muscle of patients with achalasia. *Cell Mol Biol (Noisy-le-grand).* 2002;48(3):247-52.
- Furuzawa-Carballeda J, Aguilar-León D, Gamboa-Domínguez A, Valdovinos MA, Nuñez-Álvarez C, Martín-del-Campo LA, et al. Achalasia--An Autoimmune Inflammatory Disease: A Cross-Sectional Study. *J Immunol Res.* 2015;2015:729217.
- Raymond L, Lach B, Shamji FM. Inflammatory aetiology of primary oesophageal achalasia: an immunohistochemical and ultra structural study of Auerbach's plexus. *Histopathol.* 1999;35(5):445-53.
- Clark SB, Rice TW, Tubbs RR, Richter JE, Goldblum JR. The nature of the myenteric infiltrate in achalasia: an immunohistochemical analysis. *Am J Surg Pathol.* 2000;24(8):1153-8.
- Sodikoff JB, Lo AA, Shetuni BB, Kahrilas PJ, Yang GY, Pandolfino JE. Histopathologic patterns among achalasia subtypes. *Neurogastroenterol Motil.* 2016;28(1):139-45.
- Liu ZQ, Chen WF, Wang Y, Xu XY, Zeng YG, Lee Dillon D, et al. Mast cell infiltration associated with loss of interstitial cells of Cajal and neuronal degeneration in Achalasia. *Neurogastroenterol Motil.* 2019;31(5):e13565.
- Gockel I, Bohl JR, Doostkam S, Eckardt VF, Junginger T. Spectrum of histopathologic findings in patients with achalasia reflects different etiologies. *J Gastroenterol Hepatol.* 2006;21(4):727-33.
- Savarino E, Gemignani L, Zentilin P, De Bortoli N, Malesci A, Mastracci L, et al. Achalasia with dense eosinophilic infiltrate responds to steroid therapy. *Clin Gastroenterol Hepatol.* 2011;9(12):1104-6.
- Jin H, Wang B, Zhang L-I, Zhao W. Activated eosinophils are present in esophageal muscle in patients with achalasia of the esophagus. *Med Sci Monit.* 2018;24:2377-83.
- Gockel I, Bohl JE, Eckardt VF, Junginger T. Reduction of Interstitial Cells of Cajal (ICC) associated with neuronal nitric oxide synthase (n-NOS) in patients with achalasia. *Am J Gastroenterol.* 2008;103:856-64.
- Zarate N, Wang XY, Tougas G, Anvari M, Birch D, Mearin F, et al. Intramuscular interstitial cells of Cajal associated with mast cells survive nitrergic nerves in achalasia. *Neurogastroenterol Motil.* 2006;18(7):556-68.
- Boeckxstaens GE. Novel mechanism for impaired nitrergic relaxation in achalasia. *Gut.* 2006;55(3):304-5.
- Campadelli-Fiume G, Cocchi F, Menotti L, Lopez M. The novel receptors that mediate the entry of herpes simplex viruses and animal alpha herpes viruses into cells. *Rev Med Virol.* 2000;10(5):305-19.
- Aoki R, Kawamura T, Goshima F, Ogawa Y, Nakae S, Nakao A, et al. Mast cells play a key role in host defense against herpes simplex virus infection through TNF- α and IL-6 production. *J Invest Dermatol.* 2013;133(9):2170-9.
- Xin N, Namaka MP, Dou C. Exploring the role of interleukin-22 in neurological and autoimmune disorders. *Int Immunopharmacol.* 2015;28(2):1076-83.
- Abadja F, Sarraj B, Ansari MJ. Significance of Th17 immunity in transplantation. *Curr Opin Organ Transplant.* 2012;17(1):8-14.
- Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. *J Autoimmun.* 2008;31(3):252-6.
- Deteix C, Attuil-Audenis V, Duthey A, Patey N, McGregor B, Dubois V, et al. Intra-graft Th17 infiltrate promotes lymphoid neogenesis and hastens clinical chronic rejection. *J Immunol.* 2010;184(9):5344-51.
- Durandy A. Activation-induced cytidine deaminase: a dual role in class-switch recombination and somatic hypermutation. *Eur J Immunol.* 2003;33(8):2069-73.
- Furuzawa-Carballeda J, Boon L, Torres-Villalobos G, Romero-Hernández F, Ugarte-Berzal E, Martens E, et al. Gelatinase B/Matrix Metalloproteinase-9 as Innate Immune Effector Molecule in Achalasia. *Clin Transl Gastroenterol.* 2018;9(11):208.
- Facco M, Brun P, Baesso I, Costantini M, Rizzetto M, Berto A, et al. T cells in the myenteric plexus of achalasia patients show a skewed TCR repertoire and react to HSV-1 antigens. *Am J Gastroenterol.* 2008;103(7):1598-609.
- Wynn T. Type 2 cytokines: mechanisms and therapeutic strategies. *Nat Rev Immunol.* 2015;15(5):271-82.
- Fukaura K, Ihara E, Ogino H, Iboshi Y, Muta K, Xiaopeng B, et al. Mucosally Expressed Cytokines are Associated with the Esophageal Motility Function. *Digestion.* 2018;98(2):95-103.

30. Lu J, Cao Q, Zheng D, Sun Y, Wang C, Yu X, et al. Discrete functions of M2a and M2c macrophage subsets determine their relative efficacy in treating chronic kidney disease. *Kidney Int.* 2013;84(4):745-55.
31. Zhang MZ, Wang X, Wang Y, Niu A, Wang S, Zou C, Harris RC. IL-1/IL-13-mediated polarization of renal macrophages/dendritic cells to an M2a phenotype is essential for recovery from acute kidney injury. *Kidney Int.* 2017;91(2):375-86.
32. Ricardo SD, van Goor H, Eddy AA. Macrophage diversity in renal injury and repair. *J Clin Invest.* 2008;118(11):3522-30.
33. Ikezumi Y, Suzuki T, Karasawa T, Hasegawa H, Yamada T, Imai N, et al. Identification of alternatively activated macrophages in new-onset paediatric and adult immunoglobulin A nephropathy: potential role in mesangial matrix expansion. *Histopathol.* 2011;58(2):198-210.
34. Perdigoto AL, Chatenoud L, Bluestone JA, Herold KC. Inducing and administering Tregs to treat human disease. *Front Immunol.* 2015;6:654.
35. Ding T, Yan F, Cao S, Ren X. Regulatory B cell: new member of immunosuppressive cell club. *Hum Immunol.* 2015;76(9):615-21.
36. Blair PA, Noreña LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity.* 2010;32(1):129-40.
37. Tebbe B, Wilde B, Ye Z, Wang J, Wang X, Jian F, et al. Renal transplant recipients treated with calcineurin-inhibitors lack circulating immature transitional CD19+CD24hiCD38hi regulatory B-Lymphocytes. *PLoS One.* 2016;11(4): e0153170.
38. Lemoine S, Morva A, Youinou P, Jamin C. Human T cells induce their own regulation through activation of B cells. *J Autoimmun.* 2011;36(3-4):228-38.
39. Thebault P, Condamine T, Heslan M, Hill M, Bernard I, Saoudi A, et al. Role of IFN- α in allograft tolerance mediated by CD4+CD25+regulatory T cells by induction of IDO in endothelial cells. *Am J Transplant.* 2007;7(11):2472-82.
40. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol.* 2010;185(6):3190-8.
41. Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control immune responses. *Trends Immunol.* 2013;34(3):137-43.
42. Vorobjova T, Uibo O, Heilman K, Uibo R. Increased density of tolerogenic dendritic cells in the small bowel mucosa of celiac patients. *World J Gastroenterol.* 2015;21(2):439-52.
43. Ikezumi Y, Suzuki T, Yamada T, Hasegawa H, Kaneko U, Hara M, et al. Alternatively activated macrophages in the pathogenesis of chronic kidney allograft injury. *Pediatr Nephrol.* 2015;30(6):1007-17.