

# Immunological Challenges to the Development of Chagasic Mega Syndromes



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## Abstract

Chagas' disease is one of the most serious parasitic diseases in Latin America, with a social and economic impact that far outweighs the combined effects of other parasitic diseases such as malaria, leishmaniasis and schistosomiasis. Chagas' disease presents two well-defined phases, the acute phase and the chronic phase. The acute phase lasts for approximately two or three months. After this phase, the patient enters an asymptomatic state, which characterizes the beginning of the chronic phase. In the chronic phase of the disease, the destruction of components of the enteric nervous system leads to the development of megaesophagus and megacolon. Mega syndromes are characterized as dilatation of the organ associated with an inflammatory infiltrate that is the main responsible for the destruction of the enteric neurons. This review aims at organizing the data previously known about the challenges faced by the immune system in the presence of Chagas disease and the establishment of the chagasic megaesophagus and megacolon in an attempt to discover which is the key mechanism that defines the installation and the protection against the digestive form of this pathology.

**Keywords:** Chagas' disease; Chagasic megacolon; Immune system

## Introduction

The first suspicion of the existence of the digestive form of Chagas disease came in 1916, when Carlos Chagas himself observed that during the acute infection, some adults exhibited a marked dysphagia for foods whose ingestion needed to be accompanied by water. Patients reported that food transit was interrupted in the esophagus, causing immense pain. Even the ingestion of fluids could be difficult, being sometimes impossible, and in this way, the need for it to be administered in small doses. This phenomenon, without pathogenic explanation, was then termed "Mal do engasgo" [1]. The digestive forms of Chagas' disease are present prominently in the regions below the equatorial line, with esophagopathy occurring in approximately 7 to 10% of cases and colopathy in 3 to 7%. The chagasic megacolon affects, above all, the sigmoid and the rectum. It may manifest as an isolated disease, but is often found associated with megaesophagus or Chagas' heart disease. It is more common to adult (30 to 60 years) and more incident in the male sex. As the first symptom of mega colon is constipation, both the clinical and the anatomical diagnosis are usually late, after the establishment

of the dilatation [2]. Optical light microscopy shows chronic, focal and diffuse inflammatory infiltrates in the muscularis of the mucosa, in the sub mucosa and in the muscular layers, lesions of the enteric nervous system, especially of the my enteric plexus, periganglionite and focal or diffuse gang lionitis with intense regressive phenomena of the neurons arriving at the complete destruction of the nervous ganglia of the my enteric plexus, with consecutive fibrosis, ulcerations and chronic inflammation of the mucosa, focal or diffuse in more advanced cases, reaching the sub mucosa, inter muscular interstitial fibrosis, focal or diffuse, due to myositis, periganglionitis and ganglionitis [3]. Ultra structural changes of my enteric plexus consist of lesions, usually focal, of all components of the ganglia: neurons, Schwann cells and nerve fibers. Therefore, it is common, in the same ganglion, the existence of neurons, sometimes deeply damaged alongside others morphologically intact. In severe cases, the lesion may be diffuse and the ganglion ends up being replaced by dense fibrous connective tissue [4]. It is permissible to admit a progression of the lesions of the plexus, which worsen proportionally to the duration and degree of the mega. The accumulation of feces in

the colon causes light dilation and compression of the mucosa. Compression, in turn, leads to ischemia, and secondarily to degeneration, necrosis and ulceration of the mucosa. In the mucosa so ulcerated begins a secondary inflammatory process and independent of the inflammation induced by Chagas' disease itself. This inflammatory process reaches my enteric plexus already previously damaged by *T. cruzi*, further aggravating the destruction of the enteric nervous system (SNE). In turn, the sub mucosal plexus suffers the consequences of the lesions of my enteric plexus, due to the synaptic relations between them. Inflammation secondary to stasis added to destruction of the plexus and interstitial components evolves into interstitial fibrosis of the sub mucosa and inter muscular conjunctiva. In turn, increasing the resistance of the medium requires more effort in the muscle fibers for contraction. Over time, hypertrophy and regressive changes in muscle fibers occur. The latter is consequence of disorders of metabolic changes of muscle cells and the interstitium induced by inflammation itself (vascular changes, edema, cellular infiltrate and fibrosis) that interposes between them. Because the sub mucosal plexus is closely related to muscle cells, it is easy to understand how myositis and its sequelae can further damage the lymph nodes [5]. The scarcity of parasites in relation to the intensity and extent of the lesions in the chronic phase of the disease led several authors to evaluate the involvement in autoimmune factors of the pathogenesis of the chagasic lesion. Some authors point in the existence of a cross-reaction between autologous components and *T. cruzi* antigens. Studies using an experimental infection model by *T. cruzi* suggest that during the acute phase of infection there would be a polyclonal activation responsible for the release of self-reactive clones that would persist for long periods in the host, leading to the appearance of lesions [6]. Although parasitism is scarce in relation to the intensity and extent of the lesions, several studies leave no doubt as to the presence of the parasite in the tissues of chagasic patients [7], found that the cardiac inflammatory process was particularly evident in parasitized muscle cells. Barbosa et al. [8] demonstrated, through autopsies of patients with diffuse chagasic myocarditis, the presence of *T. cruzi* amastigote forms into heart samples as well as extra-cardiac tissues. In the last decade, using polyclonal anti- *T. cruzi* to detect parasite antigens in heart tissues of patients with chagasic cardiopathy, Higuchi et al. [9] have shown a close correlation between the presence of parasite antigens and the intensity of the inflammatory infiltrates. Another methodology used is the polymerase chain reaction (PCR) technique, through which *T. cruzi* DNA is detected in inflammatory lesions in patients with Chagas' and Chagas' cardiomyopathy with megaesophagus [10,11].

The inflammatory process of the chronic phase of Chagas disease always shows signs of cellular activity. In the chagasic megaesophagus, inflammatory infiltrates are composed of 72-93% of CD3-IR T lymphocytes, 6-29% of CD68-IR macrophages and 1-4% of CD20-IR B lymphocytes. About 1-35% of the inflammatory infiltrate cells in the muscle layers express TIA-

1 (T-cell intracellular antigen) a protein found in cytotoxic lymphocytes and Natural Killer cells. Natural Killer CD57-IR cells were rarely observed. These findings suggest that cytotoxic T lymphocytes may be involved in the pathogenesis of chagasic megaesophagus as well as in the development of heart disease present in some individuals with chronic infection [12]. Corbett et al. [13] analyzing tissues of chagasic patients with megacolon demonstrated the presence of Natural Killer cells, thus suggesting their participation in the continuation of the chronic phase inflammatory process of the megacolon of chagasic patients. Lemos et al. [14], studying patients with the digestive tract of Chagas disease, performed peripheral blood analysis of these individuals to verify the circulating lymphocyte phenotype. A significant decrease in the number of CD3/CD4-IR T lymphocytes and CD19-IR B lymphocytes was observed. The number of CD4-IR T lymphocytes / number of CD8-IR T lymphocytes was decreased from individuals with advanced megaesophagus, thus demonstrating a more significant decrease in the number of CD4-IR T lymphocytes, which is not observed in cardiac non-mega-chagasic patients [15]. As a marker for activation of T lymphocytes in mega-bearing patients, an anti-HLA-DR antibody was used and in this way elevated levels of activated T lymphocytes were demonstrated, which had already been observed in patients with Chagasic or even asymptomatic heart disease. Still in mega-bearing patients, there was also a decrease in the percentage of CD4/CD28-IR cells, which initially suggested that the lack of the CD28 co-stimulation molecule could in some way lead to failures of immune resistance mechanisms and contribute to progression of the disease. This hypothesis is in part corroborated by the studies of Miyahira et al. [16] that evaluated the role of the CD28-CD80/CD86 co-stimulation pathway to the resistance of mice to *T. cruzi* infection. This study concluded that this pathway is essential for the organism to develop resistance to *T. cruzi*, since the abolition of this pathway led to a decrease in the production of interferon and the activation of CD8-IR lymphocytes, resulting in an exacerbation of parasitemia.

Eosinophils are important cells in resistance to *T. cruzi* infection and their role in chronic phase pathology has been considered by several authors [17,18]. Eosinophils synthesizes and release several bioactive mediators and are important to both intestinal physiology and defense against various pathologies [19,20]. These cells have intra-cytoplasmic granules that may vary due to the degree of maturation and activation of the cell. These granules have four main substances: primary basic protein (MBP), cationic eosinophilic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN). These proteins give the eosinophil a high capacity for cell destruction. For many years eosinophils have been believed to possess only pro-inflammatory action, but it is now known that these cells are also capable of secret immuno-mediators that may participate in the modulation of the inflammatory process. In the intestine, eosinophils are found mainly associated with observed lesions and acute and chronic inflammatory processes,

and their role in defense against parasites is well known [21-24], using mice infected with *T. cruzi*, evaluated the kinetics of eosinophil release by bone marrow, suggesting a role as these cells in the parasite resistance process. Molina and Kierszenbaum performed a series of studies in which associations between eosinophils and the pathological changes induced by *T. cruzi* were demonstrated. In myocardial studies of chagasic patients, deposits of a neurotoxin derived from eosinophils in the myocardium of chagasic patients were observed, as well as the presence of activated eosinophils. A correlation between eosinophil concentration and severity of inflammatory lesions in the myocardium and skeletal muscles was also demonstrated. Later these same authors, using cultures of cardio myocytes infected with *T. cruzi*, showed that eosinophils and neutrophils play an important role in the destruction of *T. cruzi* in myocardial cells [19,25,26].

Another cell of the immune system that plays an important role in the evolution of Chagas' disease is the mast cell. Mast cells are multi-functional cells, being important both in intestinal physiology and in defense against pathological processes. These cells release pro inflammatory molecules, such as histamine and tumor necrosis factor alpha (TNF- $\alpha$ ). Mast cells are also capable of increasing the permeability of the intestinal epithelium in situations of chronic stress, inflammatory processes and parasitic infections through mechanisms not yet known [27]. Mast cells function as the main link between the immune system and the enteric nervous system, detecting, encoding and transmitting information about these systems. Signals sent by mast cells in response to an invading agent act both on the immune system and on sensory neurons [22,28,29]. The evaluation of the role of mast cells in the pathology induced by *T. cruzi* infection has already been the subject of several studies. Almeida et al. [30], working with mice infected with *T. cruzi*, showed in the stomach of these animals a reduction of acetylcholine levels and an increase in histamine levels, probably due to the large number of mast cells in the gastric wall. Postan et al. [31] through in-vitro studies have suggested that the presence of mast cells is directly related to the development of fibrosis in cardiomyocytes infected by *T. cruzi*. Recently, Freitas et al. [28] demonstrated that the presence of serotonin is closely related to the concentration of mast cells in the colon of chagasic patients and that this would represent the main form of communication between the immune system and the enteric nervous system. This would represent a great possibility of pharmacological intervention in inflammatory bowel diseases where intestinal transit is compromised due to an intense inflammatory reaction.

## Conclusion

We believe in the existence of an interconnection between the immune and neuro endocrine systems. This link would promote a bi-directional information exchange about the immune system and the enteric nervous system. Mast cell activation, besides performing roles in gastrointestinal physiology, plays a crucial

role in the inflammatory process, being one of the main encoders of intestinal signs that will culminate in motor responses, visceral perceptions and activation of cells of the immune system in gastrointestinal pathologies.

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