Role of Autoantibodies in the Physiopathology of Chagas’ Disease

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Summary

Chagas’ disease is a serious health problem in Latin America. Between 25 to 30% of the infected patients develop the chronic form of the disease, with progressive myocardial damage and often, sudden death.

Adrenergic or cholinergic antibodies with G-protein coupled membrane receptor activity may be present in the sera of these patients. The present study discusses the etiology and the contribution of antibodies to the physiopathology of Chagas’ disease.

Introduction

Chagas’ disease, about 100 years after being first described, still constitutes a severe health problem in Latin America, causing around 50,000 deaths a year, with approximately 18 million infected people1.

Although the incidence of Chagas’ disease decreased due to the public health efforts to eradicate the vector and better control of the quality of transfusions of blood and blood components2, currently, the main concern is regarding the group of people already infected by T. cruzi, a part of which will develop the chronic chagasic cardiopathy (CCC), the most frequent clinical form and of most severity.

There are around 2 million chagasic patients in the chronic phase in Brazil. This phase constitutes a late clinical form of infection by the T. cruzi, causing progressive intestinal and/or myocardial damage. Approximately 30% of the patients with positive serology for Chagas’ disease develop some type of expression of the chronic heart disease. The chronic chagasic cardiopathy has a progressive and fibrosing myocardial inflammation as the main morphological substrate. Histological studies show inflammatory and degenerative alterations at different stages of evolution. The inflammatory reaction is of the lympho-mono-plasmocytary type. The myofibrils show varied degrees of degeneration, which can become atrophy and necrosis in some areas and present hypertrophy in others. Concomitantly, fibrosis foci are found, which are caused by the repair process and the more numerous and extensive they are, the older the infection3.

The pathogenesis of this anatomic substrate is currently still debatable and the causes that lead to the differential evolution to one of the clinical forms are yet to be elucidated.

Many of the mechanistic studies concerning the pathogeny of Chagas’ disease reported in the beginning of the 90s point out to the participation of two main mechanisms in the genesis of this marked inflammatory process: 1) autoimmune aggression, associated mainly to the antigenic mimicry of the T. cruzi and, 2) persistent, low-intensity parasitism in the cardiac fibers4-7.

However, it has been difficult to prove that the autoimmunity associated to the antigenic mimicry is the definite cause of Chagas’ disease. On the other hand, it is almost impossible to rule out the possibility that autoimmunity is not involved in the process of this disease. Controversies have been created in the literature, in the form of Editorials8-12, reporting inconclusive evidence for the autoimmune theory associated to the antigenic mimicry of the T. cruzi in the pathogenesis of Chagas’ disease. These authors point out that most of the studies defending the autoimmune theory merely documented antigenic mimicry phenomena between the T. cruzi and the host’s tissues, without establishing a clinical-biological correlation with the chronic chagasic cardiopathy. Similarly, the role of the parasite in the cardiac lesions is questioned13-16. Recent studies have demonstrated that the T. cruzi-DNA is not exclusive of the chronic cardiac form and can also be detected in the asymptomatic forms15. The low parasite load found in several other organs16 and the presence of the parasite cannot always be correlated with the degree of myocarditis15. In this model, the disease is the reflex of the parasite replication; however, if the latter occurs in several organs and it is the sole responsible for the pathogenesis, then why do the inflammatory lesions with a higher functional degree of destruction occur only in the heart?

These data may suggest that just the presence of the T. cruzi in the tissue may not be enough of a stimulus to cause a diffuse myocarditis with significant functional loss. Another mechanism, related to the involvement of the autonomous nervous system in Chagas’ disease was reported by Köberle, who, in the 50s, observed lesions in ganglia and autonomous cardiac nervous fibers16. However, it is appropriate to
consider that, in all investigations regarding the involvement of the autonomous nervous system in Chagas’ disease, the anatomical assessment was limited to the control of heart rate as a marker of the parasympathetic influence and eventual sympathetic autonomic disorders can occur without being detected by these methods\(^\text{17}\).

Similarly, the relative sympathetic hyperactivity, postulated by Köberle, was not demonstrated. On the contrary, several subsequent studies showed that neuronal destruction can occur in sympathetic ganglia, although it is generally less intense than the parasympathetic denervation\(^\text{18-19}\).

On the other hand, patients with heart failure secondary to Chagas’ disease can present decreased levels of norepinephrine, in contrast to those with heart failure of other etiologies, as shown in previous studies by our group\(^\text{20}\). These data corroborate the study published by Simões et al\(^\text{21}\) that showed a sympathetic denervation at ventricular level. Nevertheless, another study showed an increment in serum levels of norepinephrine\(^\text{22}\).

Other research groups brought new and distinct contributions to the knowledge of the pathology of Chagas’ disease, indicating the complexity of the involvement of the autonomous nervous system in this disease. Thus, Machado et al\(^\text{23-24}\) demonstrated, in biochemical and histochemical studies carried out in rats inoculated with Trypanosoma cruzi, that the cardiac sympathetic innervation disappears during the acute phase, as a consequence of the destruction of nervous fibers in parallel with the intense myocarditis. This destruction is not accompanied by neural death or significant inflammatory alterations in the upper cervical ganglion, suggesting that the lesion of the sympathetic nervous endings is a local phenomenon\(^\text{25}\). After the acute phase, a gradual and complete recovery of the sympathetic cardiac innervation occurs. The parasympathetic innervation presents analogous behavior: marked destruction of nervous fibers and decrease in heart acetylcholine (ACh) levels in the acute phase, followed by re-innervation and return to normal ACh levels in the chronic phase\(^\text{26-27}\). However, Figueirêdo Silva et al\(^\text{28}\) demonstrated decreased ACh levels in the experimental model of chronic chagasic rat, when ACh release was stimulated by KCL. Dysautonomy can also be observed in chagasic patients that present the undetermined form\(^\text{29}\).

According to some authors\(^\text{30-31}\), the marked decrease in the cardiac neuronal population at the several evolution phases of the disease\(^\text{18, 12-35}\), although it does not constitute a primary and independent lesion mechanism, suggests specificity of this process in the infection by T. cruzi and can contribute as a secondary and amplifying mechanism of the lesion caused by the inflammatory process\(^\text{31}\).

Although the mechanism of autonomic dysfunction, in the chronic phase of Chagas’ disease, has yet to be clarified, recent reports on the existence of circulating antibodies with the capacity of binding to cholinergic (Ac-M) as well as adrenergic (Ac-β) receptors\(^\text{36-40}\) could conciliate the neurogenic alterations and the immunological aggression as interactive and relevant physiopathological factors\(^\text{17}\). Thus, Ribeiro et al\(^\text{41}\) showed that the presence of Ac-M and alterations in the vagal modulation occur regardless of the ventricular dysfunction.

But why and when do these circulating antibodies appear in the natural history of Chagas’ disease? That is, are they generated primarily against T cruzi antigens or secondarily to the myocardial damage?

From the end of the 70s on, systems of cross-reaction between molecularly defined proteins of T cruzi and those of the mammal host have been described.

Teixeira et al\(^\text{42}\) described that rabbits immunized with the ribosomal protein of the T cruzi developed a myocarditis that was compatible with an autoimmune response. Evidence that emphasized the relevance of the ribosomal protein P of the T cruzi in the immune response was established by subsequent studies\(^\text{43-44}\). One of the pioneers in this field was Mariano Levin’s group 1989\(^\text{45}\), by showing that the sera of individuals with chagasic cardiopathy had higher titers, when compared to the sera of the individuals with Chagas’ disease of undetermined form, of antibodies against an epitope of the ribosomal protein P of the T cruzi (P0, P1 and P2), very similar to the corresponding human ribosomal protein\(^\text{46}\). The precise identification of the components of the T cruzi, similar to the specific ones of the cardiac tissue presenting molecular mimicry, became crucial for the study of the autoimmune pathogenesis of the chronic chagasic cardiopathy.

At the same time, the 80s and the beginning of the 90s saw a considerable progress regarding the outlining of the structure of β-adrenergic receptors. Synthetic peptides that corresponded to the primary sequences of β1 and β2-adrenergic receptors were used as antigens to track receptor-specific antibodies in European patients with idiopathic dilated cardiomyopathy\(^\text{46-48}\).

Different experimental models were used to characterize these autoantibodies that were present in approximately 30% of the patients with idiopathic dilated cardiomyopathy\(^\text{49}\). At this time, literature evidence showed that the IgG from mice infected by T cruzi was also capable of interacting with myocardial muscarinic cholinergic receptors\(^\text{50}\). Following the same steps related to the studies with β-adrenergic receptors, autoantibodies that presented reactivity against myocardial muscarinic cholinergic receptors were also described in total serum of chronic chagasic patients\(^\text{51-54}\). The presence of functionally active autoantibodies with reactivity against muscarinic cholinergic receptors in chagasic patients with different degrees of cardiac involvement (asymptomatic patients with normal ECG/ECHO, asymptomatic patients with normal ECG and echocardiogram alterations, symptomatic patients with ECG/ECHO alterations, but no significant functional alterations and, finally, severely symptomatic patients with cardiac conduction and mechanical alterations) was first reported by a group from Rio de Janeiro\(^\text{55-57}\). This group showed that the presence of the functional antibodies does not depend on the degree of cardiac involvement.

Evidence of the participation of antibodies in the physiopathology of chronic chagasic cardiopathy

Antibody-mediated induction of ventricular dysfunction

Evidence based on animal experiments has shown that the antibodies interact with the cell constituents, causing them to have potential influence on cardiac metabolism.
and contractility. It is known that the interruption of the β-adrenergic pathway can affect the heart contraction force and the myocardial relaxation. Therefore, Leite\textsuperscript{18} showed that the sera of chronic chagas’ic patients decrease, in a dose-dependent way, the amplitude of contraction of the atrial myocardium in rabbits. They also decrease the contractility and the temporal parameters of contraction. The sera of patients without myocardial dysfunction did not significantly alter any of the measured parameters. Similarly, Savio-Galinberti et al\textsuperscript{19} observed that the antibodies with adrenergic effect impaired the performance of the cardiac muscle in the preparation of isolated heart. Another study by the same group showed that the antibodies, now with a muscarinic effect, decreased the L-type calcium current by the non-competitive activation of the muscarinic receptor subtype 2\textsuperscript{20}. The impairment of this current by the antibodies with adrenergic or muscarinic effect could contribute, at least partially, to the myocardial dysfunction present in the chronic phase of chagas’ic patients. Another study that showed cardiac involvement as a consequence of the autoimmune process generated by antibodies with adrenergic or cholinergic effect was the one carried out by Gimenez et al\textsuperscript{61}, which used mice immunized with a plasmid encoding for the M2 and β\textsubscript{1}-adrenergic receptors.

In this study, the authors found not only antibodies against the second loop of the M2 and β\textsubscript{1}-adrenergic receptors, but also against the third intracellular loop of the M2 receptor. Binding assays showed an increment in the expression of the M2 receptors that was almost 2-fold higher when compared to controls and a decrease in β\textsubscript{1} receptors, as well as signs of autonomic deregulation in the immunized animals. Myofibrillar disarray and fibrosis were other important findings in these animals, as a consequence of the prolonged exposition to the antibodies.

A well-designed study carried out by Jahns et al\textsuperscript{62} showed that the IgGs with β-adrenergic effect have a direct participation in ventricular dysfunction. These authors propose that the damage caused by the antibodies results from an autoimmune mechanism, considering that the antibodies of rats that were immunized with peptides corresponding to the second extracellular loop of the β\textsubscript{1}-adrenergic receptor, when transferred to healthy rats, were capable of inducing the same cardiac alterations observed in the previously immunized rats.

Electrophysiological role of antibodies

One of the first studies to propose that a humoral component could be involved in the genesis of arrhythmia in chagas’ic cardiopathy was published by De Carvalho et al\textsuperscript{70}. At this time, they described that sera from rabbits infected with \textit{T. cruzi} generated electrocardiographic disorders in the isolated rabbit heart. Three years later, the same group confirmed this hypothesis by showing that the antibodies originated from chronic chagas’ic patients that presented complex arrhythmias decreased the heart rate and caused atrioventricular blocking in isolated rabbit heart\textsuperscript{63}. Following the same line of thought, Costa et al\textsuperscript{65} characterized 58 serum samples of patients with CCC and described that some of them, with β-adrenergic effect, blocked the conduction through communicating junctions in a culture of cardiomyocytes of newborn rats, suggesting another mechanism through which these antibodies can contribute to the occurrence of arrhythmias.

Another study that supports the arrhythmogenic role of antibodies was published by Fukuda et al\textsuperscript{63}, in which rabbits were immunized with the peptide of the second extracellular loop of the β-adrenergic receptor, showing early post-potentials and a decrease in density of the potassium currents in the M cells of these animals.

Between 1994 and 1995, Mauricio Rosenbaum’s group studies\textsuperscript{64-65} demonstrated the presence of antibodies in patients with CCC and the possible involvement of these antibodies in the patients’ arrhythmias. This group showed a correlation between the type of conduction disorder and the characteristic of the IgGs found in the chagas’ic patients’ sera. It is noteworthy that the IgGs of patients who presented ventricular arrhythmias increased the frequency of heart rate and the cAMP production (75%), whereas the IgGs of patients with sinus node dysfunction decreased the frequency of heart rate and increased the synthesis of phosphatydilinositol (76.9%), in rat cardiomyocyte culture\textsuperscript{64}.

Up to 2007, all studies associated the role of antibodies with conduction disorders, focusing mainly on the atrioventricular conduction dysfunction, without considering that the ventricular repolarization involvement could be mediated by these antibodies and could be related to the inducement of sudden death, which is one of the main causes of death in patients with CCC\textsuperscript{66}. Thus, Medei et al\textsuperscript{62} showed, for the first time, that patients with CCC who had antibodies with muscarinic activity presented higher QT interval dispersion, when compared to chronic chagas’ic patients that did not have this type of antibody in their sera. It is worth mentioning that these antibodies with muscarinic effect, when perfused in isolated rabbit heart under controlled heart rate, increased the QT interval.

Conclusions

The aforementioned studies showed the involvement of antibodies in the physiopathology of chronic chagas’ic cardiomyopathy, either direct or indirectly and new therapeutic options can be proposed based on this knowledge.

Currently, the management of the chronic chagas’ic patient with cardiomyopathy is being carried out similarly to patients with cardiopathies of other etiologies, with the use of betablockers having been validated in these patients\textsuperscript{68}.

What would be the appropriate therapeutic option for refractory patients?

Therapeutic options, previously used for the treatment of other diseases, could be considered in the management of chronic cardiomyopathy that is refractory to conventional treatments.

A possible therapeutic alternative to prevent or perhaps repair the deleterious effects of the antibodies in these patients would be the “immunoabsorption of immunoglobulins”. This technique consists in the removal of IgG through plasmapheresis, passing the blood through an immunoaffinity column to which the desired antibodies adhere. This
procedure, described by Berta et al., has been used in patients with myasthenia gravis. Later, this technique was used in patients with dilated cardiomyopathy, removing antibodies with beta-adrenergic activity, resulting in satisfactory survival outcome and attaining satisfactory hemodynamic parameters of the patients.

Recently, some studies have shown the benefits of this technique in animal models and the possibility of using it in patients with Chagas’ disease.

Another therapeutic option could be the use of stem cells for repair. This new strategy is being intensely investigated in animal models as well as in clinical assays.

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References


