Chronic Chagas’ disease –
From pathogenesis to treatment regimes
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Abstract

Chagas’ disease, caused by Trypanosoma cruzi infection, was discovered nearly 100 years ago (1909) by the Brazilian physician Carlos Chagas. Chronic Chagas’ disease is still ranked as the most serious parasitic disease in Latin America. Infected patients remain lifelong parasite carriers. With a latency of 10 to 30 years, nearly one third of parasite carriers develop life-threatening complications: the majority develop Chagas’ heart disease (90%). Gastrointestinal disorders (megaesophagus, megacolon) and neuronal afflictions mainly affecting the parasympathetic nerve system were found in the others. Chagas’ heart disease presenting with sudden death, heart failure, malign cardiac arrhythmia, and thromboembolism is currently the major cause of morbidity and mortality in Latin America, enormously burdening economic resources and dramatically affecting patients’ social and employment situations. Chagas’ disease is starting to become a worldwide problem due to migration, international tourism and parasite transfer by blood contact, intrauterine transfer and organ transplantation. In this review, we reflect on the epidemiology and etiopathology of Chagas’ heart disease. We summarize the mechanisms that have been suggested to drive Chagas’ heart disease, mainly those based on autoimmunity phenomena. In this context, we focus on autoantibodies directed to G-protein coupled receptors. Following the autoimmunity story in chronic Chagas’ heart disease – and in addition to antiparasitic therapy, the treatment of heart failure, arrhythmia and thromboembolism and under study strategies such as heart transplantation and cell therapy – we describe regimes that use peptides and aptamers for autoantibody removal or neutralization. At present, such regimes are mostly proposed for beta-1-receptor autoantibodies in patients with dilated cardiomyopathy but, in principle, they can be adapted for patients with chronic Chagas’ heart disease who are positive for comparable autoantibodies.

Key words: autoantibodies, Chagas’ heart disease, epidemiology, G-protein coupled receptors, pathogenesis, treatment, Trypanosoma cruzi

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1. Introduction

Chagas’ disease (American: trypanosomiasis) is an endemic parasitic disease that mainly occurs in Latin American countries and is caused by the flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). The disease was named in honor of the Brazilian physician Carlos Chagas (born in 1879 in Oliveira, died in 1934 in Rio de Janeiro) who, in 1909, discovered a new trypanosome species in the intestine of the triatomine bug, which he named *T. cruzi* in honor of his mentor Oswaldo Cruz. A biographical sketch of Carlos Chagas was recently published in memory of the discovery of *T. cruzi* one hundred years ago [1].

*Trypanosoma cruzi* (Fig. 1) is commonly transmitted to humans and other mammals by the blood-sucking triatomine bug (Reduviidae), also colloquially referred to as the kissing bug (Fig. 2), which can transmit *T. cruzi* throughout its lifetime (up to 2 years). As cartooned in Figure 3, besides infected humans, more than 100 mammals, including dogs, cats, rats, sloths, armadillos, and bats, are known to be parasite reservoirs. Due to triatomine bug cannibalism, *T. cruzi* can also be spread throughout triatomine populations. Birds and reptiles do not carry *T. cruzi*. Once infected with *T. cruzi*, subjects become lifelong parasite carriers and pass through several stages of the disease, as illustrated in Figure 4, which is based on some excellent reviews [2-6]. Despite lifelong parasite persistence, two thirds of patients remain asymptomatic. Of the remaining third, and sometimes only after decades, 90% can develop heart disease, which causes enormous socio-economic problems in Latin American countries. The other 10% are affected by gastrointestinal diseases and neuronal afflictions.

Consequently, initiatives and control programs were started, which include 1) initiatives for interrupting the domestic and peridomestic transmission cycles by chemical control of the vectors, animal reservoirs, and infected humans, 2) initiatives for improving housing conditions and health education, and 3) screening for infected blood donors, who form one of the main non-vector routes of transmission.

For subjects who are already infected, strategies for an earlier diagnosis, improved monitoring of the progression of the disease, and optimal treatment guidance are essential elements of disease control. In our view, improvements in the understanding of the pathogenesis of chronic Chagas’ disease in general and specifically of Chagas’ heart disease are the most important conditions for guaranteeing this.
Chronic Chagas’ heart disease

Figure 2: Triatomine bug (Reduvidae) (Reproduced from [25] with permission of F. Torrico and M. Castro; Universidad Mayor de San Simon, Cochabamba, Bolivia and E. van der Enden; ITGPRESS, Antwerpen, Belgium)

Figure 3: Sylvatic and domestic network of infection with Trypanosoma cruzi including the pathogen (Trypanosoma cruzi), vector (Reduvidae) and hosts (more than 100 wild and domestic animals). Beside in humans, pathology of Chagas’ cardiomyopathy was seen in dogs.

Figure 4: Time course from Trypanosoma cruzi infection to chronic Chagas’ disease
2. Epidemiology

The geographic distribution of the triatomine vector and consequently the area of infection risk extend from the southern USA down to southern Argentina. The main endemic area of Chagas’ disease covers more than 20 countries from Mexico down to northern Argentina (Fig. 5). As indicated in Table 1 (adapted from Dias et al. [7] and Salvatella [8]), there were 30 million cases of infection in the 1980s, with an annual rate of 700,000 newly-infected subjects and more than 45,000 fatalities. Following successful multinational initiatives for interrupting Chagas’ disease transmission, in 2006 it was estimated that there were 28 million people at risk, 15 million infected cases, and an annual incidence and mortality of 41,200 and 12,500, respectively. The number of endemic areas decreased from 21 countries in the 1980s to 18 countries at present.

Chagas’ disease, which historically was a disease of poor, rural areas, proliferated due to continuous rural-urban migration, which was widespread throughout urban centers such as Sao Paulo, with about 300,000 infected individuals, and Rio de Janeiro and Buenos Aires, with more than 200,000 infected individuals [9].

For the United States, only a very small number of autochthonous vector-borne cases of infection have been reported, located in the south [10].

Due to the international migration of Latin Americans, Chagas’ disease is increasingly becoming a worldwide problem for health systems. For the USA, it was estimated that there are more than 300,000 infected people, based on the immigrant population of 23 million Latin Americans and the known prevalence of *T. cruzi* in their countries of origin [11]. Figure 6 shows that “Europe is not spared” from Chagas’ disease [12]. In Spain, with nearly 1,700,000 immigrants, 87,000 individuals could be infected. For the remainder of Europe, with a total of 500,000 immigrants, nearly 3,000 people were estimated to be infected. For Australia and Canada with 85,000 and 157,000 immigrants, 3000 and 5000 infected subjects were calculated, respectively [13]. Since unknown *T. cruzi* carriers can serve as blood donors, about 100 million people are at risk of becoming infected via contaminated blood [14,15]. Other groups at risk of *T. cruzi* infection via contact

Table 1: Changes in some epidemiological parameters following the interruption of Chagas’ disease transmission, 1999-2006; adapted from [7] and [8]

<table>
<thead>
<tr>
<th></th>
<th>1990</th>
<th>2000</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annual death (thousand)</strong></td>
<td>&gt; 45</td>
<td>21</td>
<td>12,5</td>
</tr>
<tr>
<td><strong>Cases of infection (million)</strong></td>
<td>30</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td><strong>Annual incidence (thousand)</strong></td>
<td>700</td>
<td>200</td>
<td>41,2</td>
</tr>
<tr>
<td><strong>Population at risk (million)</strong></td>
<td>100</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td><strong>Distribution (countries)</strong></td>
<td>21</td>
<td>19</td>
<td>18</td>
</tr>
</tbody>
</table>

Figure 5: Endemic area of Chagas’ disease (Reproduced from Wikipedia public domain)
with the blood of infected subjects are occupational groups such as social and healthcare employees. Consequently, blood donor screening began in 2007 in the USA [16]. Since 2005 [17], Spanish regulatory law requires that all at-risk donors (people born in an endemic area, people whose mothers were native to an endemic area, people who have undergone blood transfusions in an endemic area) are screened for Chagas’ disease or otherwise be excluded from donation. Chagas’ disease is also being increasingly recognized as an emerging public health problem in other European countries [18,19]. However up to now, strategies for detecting *T. cruzi*-infected blood such as established in Spain did not exist for other European countries.

International tourism is increasingly becoming another route for the worldwide spread of Chagas’ disease. In endemic areas, it has been reported that diaplacental and/or perinatal transfer from the mother to her fetus can contribute to *T. cruzi* transmission [20]. Outside Latin America, one case of the congenital transmission of *T. cruzi* has been documented in Spain [21].

Europe is colonized by different subfamilies of the triatomine bug. However, there is no indication of *T. cruzi* transmission by triatomine bugs in Europe. As previously reported [22], fleas, flies, bedbugs, mosquitoes and lice have been suggested as possible candidates for *T. cruzi* transmission in Europe. Some subspecies of ticks (Ixodida) are potential *T. cruzi* carriers; some of these live in Europe. However, at present, there is no indication that insects transfer *T. cruzi* to humans in Europe.

Figure 6: Estimated number of Chagas’ disease (infected) patients in Europe. (Color code denotes expected frequency). (Reproduced from [12] with permission of Oxford University Press, License Number: 2692410596257)
3. Etiopathology

3.1 The vicious cycle of Trypanosoma cruzi infection

The life cycle of *T. cruzi* involves stages in the digestive tract of the triatomine bug (secondary host), which is the vector, and stages in the blood and tissues of mammals (host, reservoir). Parasites freely circulating in the host’s blood are unable to replicate, but after they colonize phagocytic and non-phagocytic cells of host tissues, large quantities of *T. cruzi* are produced via replication and are subsequently released into the blood. After infection, the parasites exhibit clear tropism for heart, skeletal, and smooth muscle cells, as well as neuronal cells, which can act as reservoirs for the parasites. *Trypanosoma cruzi* parasites are taken up by triatomine bugs when they suck human blood or blood from any other contaminated mammal; they then multiply in the gut of the bugs. After a bug has fed on blood, it excretes the parasite in its feces onto the skin of the host, from where it can enter and continue the vicious cycle of *T. cruzi* infection. A bug’s infectious feces pass via the bug’s bite or other small wounds into human blood, but the parasite can also pass from the feces through intact mucous membranes, especially those in the mouth and eyes. The feces remain infectious for a long time, most likely even when they are outside of the bug. Consequently, infection via the ingestion of food contaminated with infected feces has been reported.

However, genetic variations in the parasite and its hosts could be responsible for the regional differences found in the incidence of this disease [23,24].

3.2 Acute Chagas’ disease

The acute stage of Chagas’ disease can be symptomless or it can present with only mild clinical symptoms and therefore remain undiagnosed. A typical sign that is often ignored due to its non-specificity is chagoma, a local infection characterized by swelling around the bug’s bite. If the route of parasite entry is through the conjunctiva of the eye, patients present after 4 to 12 days with a more typical symptom called Romaña’s sign [25], which comprises conjunctivitis, unilateral palpebral edema and pre-auricular lymphadenopathy (Fig. 7). However, other symptoms are less common and only 5-10% of patients present with fever, malaise and lymphadenopathy.

In a small number of patients, especially children, hepatosplenomegaly, myocarditis, and meningoencephalitis are occasionally seen. The mortality rate due to acute Chagas’ disease is 2-6%, which is mainly caused by myocarditis and meningoencephalitis [3,4,5,26,27].

![Figure 7: Child with Romana's sign (also named chagoma); unilateral painless periorbital swelling associated with the acute stage of Chagas' disease. (Reproduced from [25] with permission of F. Torrico and M. Castro; Universidad Mayor de San Simon, Cochabamba, Bolivia and E. van der Enden; ITGPRESS, Antwerpen, Bergium)](image-url)
3.3 Mechanisms of parasite control

Both humoral and cellular immune responses participate in parasite control, but their highly complex interactions are far from being clarified [28]. The humoral immune response comprises CD8+ T cells and macrophages, as well as IFN-γ secretion, which seem to be essential players. Perforin/granzyme-dependent killing of infected cells and FAS-mediated apoptosis, as well as the macrophage production of IL-12, for induction of T. cruzi resistance, must be considered [29,30]. Other important players in parasite defense via regulation of the immune response and due to their cytotoxic properties are cytokines such as e.g. IFN-γ and TNF-α and NO [31-37]. In contrast, there is evidence to show that CD8+ T cells can lose their activity and that immune suppression elements come directly from T. cruzi, causing ineffective parasite control by the immune system, which could be a reason for the incomplete parasite eradication and consequent life-long parasite persistence that result in chronically infected patients [38].

3.4 Chronic Chagas’ disease

Typical signs of chronic Chagas’ disease are positivity for anti-T. cruzi antibodies and – by using modern analytical equipment such PCR techniques – the detection of parasites in patient tissue samples [39,40]. Despite this permanent parasitic load in all patients, only one third of patients progress from the asymptomatic phase to the symptomatic stage of chronic Chagas’ disease (Fig. 4).

3.4.1 Asymptomatic chronic Chagas’ disease (latency stage, indeterminate stage)

Asymptomatic patients are only diagnosed by chance or by screening for T. cruzi antibodies, for example in the case of enrolment into the blood donor system or in preparation for surgery. The heart and the gastrointestinal tract have no distinct pathological findings on ECG, sonography, or radiology. However, small focal inflammatory lesions have been detected in tissue samples of the heart, skeletal muscle and the gastrointestinal tract from asymptomatic patients [41-43]. The asymptomatic stage might be interrupted by episodes showing non-specific characteristics of acute infection. In particular, patients with immunosuppressive disorders, such as HIV-positive patients, show such episodes [3,4,44].

3.4.2 Symptomatic chronic Chagas’ disease (symptomatic stage)

Genetic variability has been discussed as being responsible [23,24,45] for whether patients are asymptomatic or develop heart disease, gastrointestinal disease or neuronal disorders, but this debate has not yet been concluded [46]. With respect to the relationship between the HLA polymorphism and the manifestation of chronic Chagas’ disease, associations were observed between distinct HLA alleles and an increased risk of developing chronic Chagas’ disease in some studies [47,48], but others denied finding any such relationships [49].

3.4.2.1 Chagas’ heart disease

Chagas’ heart disease becomes manifest in men and women with a comparable frequency, and it mainly begins between the ages of 30 and 50 years old. In line with the diagnostic options available in endemic areas, cardiac arrhythmia, found by Holter ECG examination, is often the first clinical indication of the development of Chagas’ cardiomyopathy [50,51]. With increasing severity, right bundle branch block, left anterior hemiblock, ventricular extrasystoles, sinus bradycardia, auricular fibrillation and complete atrioventricular block were found to be the most frequent symptoms [3,5,25]. Consequently, for
newly diagnosed chronic Chagas’ patients based on \textit{T. cruzi} antibody positivity, it was recommended that patients should undergo a medical history interview, a physical examination, and a resting 12-lead ECG with a 30-second lead rhythm strip [52]. In the case of normality, examinations should be repeated annually. Where Chagas’ heart disease is diagnosed, a comprehensive cardiac evaluation is recommended, which should include Holter ECG examination and echocardiography, which is clearly more sensitive than ECG [53,54]. Cardiac MRI can successfully complete the diagnosis and aid planning of the management of chronic Chagas’ disease [55,56]. From a clinical point of view, myocarditis, thromboembolic events, sudden cardiac death and congestive heart failure are typical of advanced Chagas’ heart disease. However, about 30% of Chagas’ heart patients die from sudden cardiac death without any characteristic signs of advanced Chagas’ heart disease.

Chagas’ cardiomyopathy is characterized by progressive heart enlargement resulting from chamber dilatation. Figures 8A (photograph of explanted chagasic hearts) and 8B (thorax radiography) show typically enlarged chagasic hearts. Whereas microscopic parasite detection was found to be successful in only 10-20% of cardiomyopathic hearts, DNA amplification tests showed the appearance of parasites in almost all patients with Chagas’ heart disease [57,58].

However, the severity of cardiomyopathy did not correlate with the occurrence of parasite DNA. Therefore, direct heart damage in relation to parasite load and inflammation does not seem to be the only mechanism responsible for heart damage. The histopathological pattern of the chagasic heart shows nests of focal inflammation with T cells and

![Figure 8: A) Explanted hearts of patients with chronic Chagas disease demonstrating increasing cardiomegaly. (reproduced with permission of E. van der Enden, Institut voor Tropische Geneeskunde Antwerpen, Belgium). B) Cardiomegaly of a chronic Chagas’ patient with implanted pacemaker, demonstrated by thorax radiography (Reproduced with permission of R. Araujo, Santa Barbara Hospital, Sucre, Bolivia)](image)
varying numbers of B cells and macrophages, diffuse interstitial fibrosis, and a disturbed morphology of the myocytes. The conduction system in the heart also shows alterations [59-61].

The often apically aneurysmatic Chagas’ heart is thought to be the cause of thrombus formation, which may lead to thromboembolic events in the brain and lungs, and which are thought to be responsible for the high rate of sudden death in Chagas’ heart disease. However, the main life-threatening complication of chronic Chagas’ disease is the continuous progress towards severe heart failure. Nearly 60% of patients die due to cardiomyopathy.

3.4.2.2 Chagas’ gastrointestinal disease (megaesophagus and megacolon)

Malnutrition caused by swallowing problems and regurgitation leading to weight loss, as well as obstipation with abdominal pain, mark the progress of chronic Chagas’ disease into megacolon (Fig. 9) and megaesophagus conditions. Radiological investigations employing barium as the contrast agent can be used for early diagnosis; however, a simple radiological investigation is often sufficient.

3.4.2.3 The nerve system in chronic Chagas’ disease

Damage to the parasympathetic nervous system could be the main driver of alterations in the vegetative nervous system in chronic Chagas’ disease [62]. Damage to the parasympathetic nervous system starts in the acute phase and proceeds into the chronic phase. The central nervous system, however, is mostly affected during the acute phase of the disease. Rare cases of changes in the psyche of chronically infected Chagas’ patients are also a sign of nerve damage during the chronic phase of the disease [3].

4. Pathogenesis of chronic Chagas’ heart disease

Several hypotheses – extensively summarized by Gironés et al. [28] and Engman and Leon [63] – have been formulated:

A: **Primary damage of the neuronal system** with denervation of the autonomous

![Figure 9: Photograph of the megacolon of a patient with chronic Chagas disease. (Reproduced with permission G. Valda, Santa Barbara Hospital Succre, Bolivia)](image_url)
parasympathetic system in the heart. This neuronal damage starts in the acute phase of the disease and accelerates in the chronic stage, resulting in lesions.

B: Parasympathetic nervous system damage starts in the acute phase of the disease and accelerates in the chronic stage, resulting in lesions.

B: Cardiomyocyte toxicity due to *T. cruzi* and/or *T. cruzi*-derived products resulting in host myocytolysis in the acute stage, is thought to start the progress toward Chagas’ heart disease. Due to the finding of myocardial parasite persistence, chronic myocytolysis could aggravate Chagas’ heart disease. However, with the exception of immunosuppressed subjects and the minority of Chagas’ patients with a high parasite titer, the generally low parasite titer in the chronic stage should mean that parasite cytotoxicity and/or the cytotoxicity of parasite-derived products should only be of limited relevance.

C: Parasite-induced microvascular alteration is based on the assumption that parasites interact with essential metabolic reactions in microvascular cells, causing hypoperfusion, subsequent hypoxic/ischemic damage of the cardiomyocytes and inflammatory conditions in the heart. All together could drive Chagas’ heart disease.

D: Polyclonal B cell activation following the disruption of normal immune regulation and leading to immunosuppression and autoimmune processes could support pathogenetic events.

E: Persistent *T. cruzi* antigens might trigger T cell-mediated responses of the delayed-type hypersensitivity cells or cytotoxic cells, leading to damage of the host’s infected and/or bystander cells.

F: Autoimmunity induced by *T. cruzi*-specific antigens or by host antigens might result from *T. cruzi* antigen-associated molecular mimicry, as well as from bystander activation.

Although none of these hypotheses claim exclusivity, autoimmunity is being increasingly accepted as a pathogenetic driver of Chagas’ heart disease. The potential cooperation between molecular mimicry and bystander activation for *T. cruzi*-induced “autoimmune” Chagas’ heart disease is demonstrated in Figure 10, which is based on [28].

Bystander activation means that parasites, especially in the case of strong intracellular parasite replication, cause pro-inflammatory conditions (release of cytokines, NO/peroxynitrite formation, etc.).

**Figure 10:** Suggested mechanisms of *T. cruzi* pathogenicity by molecular mimicry and bystander activation adapted from [28]
Chronic Chagas’ heart disease

nitrite, chemokines), which lead to cardiomyocyte damage and the subsequent liberation of autoantigens and cryptic epitopes that normally have no access to the host’s immune system but which are now recognizable by autoreactive T cells. In parallel, T-cells proliferate and are activated in order to perpetuate the immune response against the heart structures.

In molecular mimicry, *T. cruzi* proteins cross-react with proteins of the host’s heart, which now are recognized by the host’s activated immune system. Molecular mimicry is increasingly being suggested as being the first driver of autoimmunity in chagasic patients. This is supported by the finding of even more cross-reactions between *T. cruzi* and host antigens. A number of such cross-reactions – without claiming completeness – are listed in Table 2, which was adapted from Cunha-Neto et al. [64]. It has been clearly indicated in animal experiments that immunization with *T. cruzi* antigens, as well as the transfer of *T. cruzi*-activated T cells, produces myocardial alterations, mainly focal myocarditis, demyelination, and conduction system defects. Additionally, autoantibodies were found that affected the essential structures and functions of the heart.

Antigens, T cell clones and autoantibodies found after infection with *T. cruzi* are long-persisting, which supports the autoimmunity theory in Chagas’ heart disease [65].

4.1 G-protein-coupled receptors as autoantigen

In view of the autoimmunity story of Chagas’ heart disease, G-protein-coupled receptors (GPCRs) are receiving more and more attention as human antigens that show molecular mimicry of *T. cruzi* antigens. A variety of GPCRs for which autoantibodies (AABs) have been identified in patients with diseases of the heart and circulatory systems are shown in Table 3, adapted from Wallukat et al. [66].

The GPCRs are members of the large superfamily of heptahelical transmembrane proteins, to which about 80% of all receptors belong. The GPCRs are highly important control elements for the regulation of metabolism. The GPCRs sense signal molecules out-

### Table 2: Cross reactivity of *Trypanosoma cruzi* and human antigens favoring molecular mimicry (adapted from [64])

<table>
<thead>
<tr>
<th>Human antigens</th>
<th><em>T. cruzi</em> antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons</td>
<td>Sulphated glycolipid</td>
</tr>
<tr>
<td>Neuronal 47kD protein</td>
<td>FL-160</td>
</tr>
<tr>
<td>Heart and skeletal muscle</td>
<td>Microsomal fraction</td>
</tr>
<tr>
<td>Smooth and striated muscle</td>
<td>150 kDa protein</td>
</tr>
<tr>
<td>Cardiac myosin heavy chain</td>
<td>B13 protein</td>
</tr>
<tr>
<td>Sacroplasmatic reticulum antigen (SRA)</td>
<td>SRA</td>
</tr>
<tr>
<td>Glycosphingolipids</td>
<td>Glycosphingolipids</td>
</tr>
<tr>
<td>Microtuboli-associated protein (MAP) (brain)</td>
<td>MAP</td>
</tr>
<tr>
<td>28 kD lymphocyte membrane protein</td>
<td>55kD membrane protein</td>
</tr>
<tr>
<td>23 kD ribosomal protein</td>
<td>23 kD ribosomal protein</td>
</tr>
<tr>
<td>Beta 1-adrenoreceptor, M2 muscarinic receptor, M2 cholinergic receptor, cardiac</td>
<td>Ribosomal P0, P2ß and 150 kD protein</td>
</tr>
<tr>
<td>Cardiac muscarinic acetylcholine receptor</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
side the cell and change their conformation to activate G-proteins, which – depending on their type – modulate the cAMP or phosphoinositol pathways to translate the outside signal into an internal cellular response. They are involved in sensory perception, inflammatory processes, chemotaxis, endo- and exocytosis, cell growth, and differentiation. The effects of glandular hormones, tissue hormones and neurotransmitters, such as catecholamines, glucagon, endothelin, angiotensin, acetylcholine, and serotonin, amongst others, are mediated via the GPCRs.

Due to the central role of GPCRs in the regulation of metabolism, it is not surprising that altering GPCR-dependent signal transduction results in a wide variety of pathogenetic consequences.

4.1.1 Autoantibodies against G-protein coupled receptors in Chagas’ heart disease

4.1.1.1 Basic remarks

With respect to the AABs against GPCRs found in Chagas’ patients, those directed to the beta-1-adrenergic receptor (beta1-AABs), the beta-2-adrenergic receptor (beta2-AABs), and the muscarinic-2 receptor (M2-AABs) have received particular attention. The AABs target the negatively-charged region of the second extracellular receptor loop of GPCRs and the binding of AABs enables receptor dimerization, stabilizing active receptor conformation [67,68]. Figure 11 shows the schematic structure of the beta-1-adrenergic receptor in relation to beta1-AABs. Only monovalent Fab fragments did not stabilize the receptors.
There is now growing evidence to show that AABs are important pathogenetic substrates of Chagas’ heart disease, particularly beta1-AABs. This is not least because of the similarities between Chagas’ heart disease and dilated cardiomyopathy (DCM). Both patient groups carry a high percentage of beta1-AABs. Consequently, a “cross-talk” [69] was suggested between the beta1-AABs found in Chagas’ heart disease and the beta1-AABs found in DCM, which means that the results from in vitro and animal experiments, as well as from human studies, which focused on the latter case, can consolidate the beta1-AAB-associated history of the former. The first indication for beta1-AABs in the serum of patients with DCM was – although often ignored – supplied in 1987 by Wallukat and Wollenberger [70], followed in 1989 by Limas et al. [71]. Already years before (1976), Sterin-Borda et al. [72] described – in our view for the first time – an antibody isolated from chagasic sera which interacted with beating rat atrial preparations. The agonistic effect of this antibody was prevented in the presence of the beta-receptor blockers. In 1984, the specificity of this antibody to the beta1-receptor was suggested [73].

A variety of functional responses induced by beta1-AABs and thought to drive cardiomyopathy were recently summarized by Yoshikawa et al. [74]. Most important were the experiments that showed heart alterations typical of cardiomyopathy [75,76] fol-

Figure 11: The human beta-adrenergic receptor as a model of G-protein coupled receptors targeted by the corresponding autoantibody. The N-terminal domain usually contains less than 50 amino acids and is located in the extracellular space, whereas the C-terminal part of the protein varies from 23 (muscarinic2 receptor) to about 100 amino acids (beta2-adrenergic receptor). The transmembrane regions usually contain between 23 and 24 amino acids, limited by the helical secondary structure and the thickness of the hydrophobic lipid bilayer. The homology varies throughout the whole superfamly. However, the homology is higher (35%; 90%) in the transmembrane helices and functionally important side chains in the transmembrane helices, and the loops are strongly conserved between different vertebrate species. Agonists bind to a hydrophobic cave formed by the transmembrane helices. Indicated epitopes on the second extracellular loop are the targets of beta1-adrenergic receptor autoantibodies present in patients with cardiomyopathies.
lowing the transfer of beta1-AAB to animals. At cellular and subcellular levels, changes in the action potential duration and contractility of cardiomyocytes have been observed following the addition of beta1-AAB and also M2-AAB [77,78].

Beta1-, beta2-, and M2-AABs which were found in patients with Chagas’ heart disease are agonistic. However, depending on the different downstream effects – the activation of adenylate cyclase by beta1-AAB and beta2-AAB and its inhibition by M2-AAB – positive or negative chronotropy was evident. In agreement with this, IgGs prepared from the blood of Chagas’ patients, which preferentially contained beta1-AAB and beta2-AAB, activated adenylate cyclase and increased cAMP formation in heart cell membranes and cardiomyocytes. This occurred in parallel with increased contractility [79,80].

Activation of the L-type calcium canal was a further characteristic reaction in the presence of especially beta1-AAB. This might result from the protein kinase A-dependent phosphorylation of the canal protein, but it could also result from a direct interaction between the activated G-protein subunits and the canal proteins [81,82]. It has generally been accepted that changes in the calcium flow caused by AABs are an essential component in the pathogenesis of myocarditis and dilated cardiomyopathy [83].

Apoptosis induced by beta1-AAB could be another key event in the pathogenesis of cardiomyopathy [84].

As previously mentioned, alterations of the parasympathetic nervous system could be one of the key events in the pathogenesis of both Chagas’ heart disease and gastrointestinal disease. M2-AABs reduced the contractility of the rat atrium, increasing cGMP and reducing cAMP formation [85]. However, it has been suggested that M2-receptor-dependent pathogenetic mechanisms could be more profound in gastrointestinal manifestations. M2-AABs increased the tonus (basal) at the colon and esophageal strips and reduced the concentration of cAMP [86,87]. It has been assumed that the binding of M2-AAB activates pertussis-sensitive G proteins that inhibit adenylate cyclase. Additionally, it has also been suggested that M2-AAB induces receptor desensitization and sequestering, which would support dysautonomia of the colon and esophagus [88]. However, with respect to the high prevalence of M2-AAB in Chagas’ heart disease, these AABs should not be underestimated as drivers of Chagas’ cardiomyopathy.

4.1.1.2 Autoantibody pattern and frequency

Positivity for beta1-, beta2-, and M2-AABs was frequently found in a distinct percentage of chronically infected Chagas’ patients [89,90]. As indicated in Table 4, we found positivity for beta1-AAB, beta2-AAB, and M2-AAB in nearly one third of all asymptomatic patients [91]. Chagas’ heart patients presented with nearly 100% positivity for beta1-AAB and M2-AAB but only 38% of the heart patients had beta2-AAB positivity. Of the megacolon patients, 90% were positive for all three receptors. Patients who were suffering from cardiomyopathy and megacolon were found to carry all three receptors with a frequency of nearly 100%.

The autoantibody frequency (ca. 30%) of the asymptomatic Chagas’ patients clearly parallels the epidemiological data on the incidence (ca. 30%) of Chagas’ heart disease in chronically infected patients. Consequently, we suggested that autoantibody detection in asymptomatic Chagas’ patients could indicate the patients’ risk for symptomatic Chagas’ disease, especially for Chagas’ heart disease, even before traditional diagnostic tools diagnose Chagas’ heart disease. Furthermore, beta1-AAB together with M2-AAB should be indicative of Chagas’ heart disease risk, whereas beta2- and M2-AAB positivity should be indicative of the risk of gastrointestinal disease.

Recently, we presented the first indications to support this hypothesis [92]. Thirty-
one (27-47) months (median, range) after the primary classification of chronic Chagas’ patients as being asymptomatic and with or without autoantibody positivity, we contacted a sub-cohort of 36 of these patients, of whom 15 were autoantibody negative and 21 autoantibody positive. None of the AAB-negative patients reported clinical symptoms or ECG characteristics indicative of progression to symptomatic Chagas’ disease. Among the group of AAB-positive patients, one patient presented with cardiomyopathy diagnosed by ECG, demonstrating the progression to symptomatic chronic Chagas’ disease. In the primary study, this woman had had the typical cardiomyopathic AAB composition of beta1-AAB combined with M2-AAB. A second patient, a 31-year-old woman, presented with clinically diagnosed megacolon. This woman was burdened in the primary investigation with M2-AAB positivity and borderline positivity for beta2-AAB.

| Table 4: Basic data and percentages of positivity for autoantibodies (AAB) against beta 1-adrenergic, beta 2-adrenergic, and muscarinergic 2 receptors (beta1-AAB, beta2-AAB, M2-AAB) of 228 Chagas’ disease patients and 29 healthy subjects [1]. |
|-----------------|---------|---------|---------|---------|
| n (male/female) | C       | I       | CM      | CM+MC   |
| Age (yrs), median (min/max) | 30 (19/61) | 30 (18/82) | 47 (18/82) | 46 (19/78) | 54 (29/81) |
| AAB positivity (%) |         |         |         |         |
| Beta1-AAB | 0 | 34 | 100 | 38 | 96 |
| Beta2-AAB | 3 | 33 | 89 | 97 | 98 |
| M2-AAB | 0 | 42 | 98 | 100 | 100 |
| Beta1-/M2-AAB | 0 | 29 | 98 | 38 | 96 |
| Beta2-/M2-AAB | 0 | 24 | 89 | 97 | 98 |
| Beta1-/M2-AAB or beta2-/M2-AAB | 0 | 34 | 98 | 99 | 96 |

C = healthy control subjects; CM = patients with chronic Chagas’ disease manifested as cardiomyopathy; CM+MC = patients with chronic Chagas’ disease manifested as cardiomyopathy combined with megacolon; I = patients with Chagas’ disease in the indeterminate (asymptomatic) state; MC = patients with chronic Chagas’ disease manifested as megacolon only. CM > MC, p ≤ 0.001.

5. Therapy

5.1 Acute Chagas’ disease

Therapy in the acute stage concentrates on the elimination of the parasites, preferably by using Nifurtimox or Benznidazol [4,15,93]. While Nifurtimox has to be taken for between 50 and 120 days, Benznidazol is administered for up to 60 days and, due to its safety and efficacy profile, is the first-line treatment option [94]. However, some T. cruzi strains can develop resistance against these drugs. Consequently, only 50% of treated patients are drug responders. Furthermore, the existing drugs show enormous po-
potential for toxic side effects, which manifest in the liver and as an allergic reaction, mainly after long-term administration. Side effects are inversely related to the age of patients. As reviewed recently by Rassi et al. [95], there are clear recommendations for the use of anti-T. cruzi treatment in cases of acute and reactivated infection. Anti-T. cruzi treatment can be offered to children and adults who do not have advanced Chagas heart disease, but it is contraindicated in pregnant women and subjects who suffer from kidney and liver insufficiency. Regardless of the PCR-detected myocardial parasite persistence, its suggested role in the pathogenesis of chronic Chagas’ heart disease, and animal experiments demonstrating the prevention of cardiomyopathy development following parasite reduction [96], a clear benefit of anti-T. cruzi treatment in the transition from the asymptomatic stage to the cardiomyopathic stage and further to severe cardiomyopathy still remains to be found. “BENEFIT”, which is an under-study multicenter trial on 3,000 Latin Americans with mild to moderate Chagas’ heart disease, could clarify the relationship between trypanosomiasis interruption and cardiac disease progression and death [97].

The whole-genome sequencing of T. Cruzi in 2005 revealed kinomes, which are known to contain a large and diverse set of protein kinases and phosphatases, and which could be used as targets in the future to stimulate the development of highly specific anti-T. Cruzi drugs [98].

5.2 Chronic Chagas’ heart disease

5.2.1 Drug treatment

In contrast as reported in several recent reviews [95,99,100], a wide range of therapeutic possibilities are available after the manifestation of Chagas’ heart disease, which, in general, do not differ much from the treatment of cardiomyopathies of other etiologies. The drug treatment of heart failure, arrhythmias and thromboembolism, as well as pacemaker and cardioverter-defibrillator implantation and resynchronization therapy are clearly indicated.

Although beta-blocker treatment in chronic Chagas’ cardiomyopathy has been critically viewed due to bradyarrhythmia and conduction defects, today there is also evidence of its beneficial effects in Chagas’ cardiomyopathy patients [101,102]. After the establishment of a cardioembolic risk score for chagasic patients, anticoagulation was recommended in particular for cardioembolic stroke prophylaxis [103].

Irrespective of the continuous optimization of drug therapy, the outcome of chronic Chagas’ patients with severe heart failure is very poor, as seen for patients with heart failure due to other etiologies. One-year survival rates from 20% to 70% were documented [104,105].

5.2.2 Heart transplantation and assist device and cell-based therapy

Heart transplantation is one of the options for Chagas’ patients with refractory heart failure. In Brazil, Chagas’ cardiomyopathy is, following idiopathic dilated cardiomyopathy and ischemic cardiomyopathy, the third indication for heart transplantation [106,107]. The survival of chagasic heart recipients was found to be better compared to heart recipients of other etiologies. In 27% to 39% of the recipients, reactivation of T. cruzi was seen, which could have been favored by the immunosuppressive protocol required for preventing graft rejection [108].

Whereas heart transplantation could become a viable option for chagasic patients with severe heart failure who live in developed countries, unfortunately, in the endemic area of Latin America, this therapeutic option will not be available to many due to the high costs of heart transplantation and the restricted numbers of heart donors. The same is true for assist device treatment for bridging to heart transplantation, but also sometimes for destination therapy or bridging to recovery. However, assist devices for bridging to
heart transplantation have only been tested in a low number of chagasic patients [109].

As is the case for cardiomyopathy in general, cell-based therapy is being increasingly discussed for Chagas’ cardiomyopathy [56]. Although cell-based therapy is only in its early stages and the number of patients treated so far is very low, the first promising results demonstrating the benefits, for example an improvement in NYHA class, have been published [110]. At present, we are awaiting the results of a clinical trial conducted between 2006 and 2010 in Brazil to evaluate the efficacy of cell-based therapy in Chagas’ cardiomyopathy [111].

5.2.3 Autoimmunity-targeted therapy

Considering the increasing acceptance of an autoimmune background in the pathogenesis of life-threatening complications of chronic Chagas’ disease in general but particularly for Chagas’ heart disease, new treatment regimes similar to those generally used in autoimmune diseases, such as treatment with anti-inflammatory or immunosuppressive drugs, could become increasingly important. Another promising option could be the elimination or neutralization of the high percentage of pathogenetic AABs found in chronic Chagas’ heart disease patients.

5.2.3.1 Autoantibody immunoapheresis

Lobovsky et al. [112] demonstrated for the first time the possibility of clearing serum from chagasic patients positive for beta1-AABs using an apheresis technique. In this in vitro experiment, beta1-AABs of chagasic sera was immunoadsorbed using a commercially available Coraffin matrix (Fresenius Medical Care, Bad Homburg, Germany) containing a peptide representing the second extracellular loop of the beta1-receptor. At the end of this procedure, the beta1-AAB activity of the sera was clearly abolished, which was tested using ELISA and a bioassay that measured the chronotropic response of neonatal rat cardiomyocytes. Consequently, Lobovsky stated that “further experiments are needed to confirm that this procedure may be an efficient therapeutic tool for chronic Chagas patients with cardiac complex and measurable levels of circulating beta1-AABs.” Lobovsky’s idea to use immunoadsorption for the treatment of Chagas’ heart disease had come from the well-documented benefits of beta1-AAB immunoadsorption in patients with DCM.

The first evidence of the benefit of beta1-AAB clearance in DCM patients was supplied by Wallukat et al. [113], who used the Ig-Therasorb (Baxter Corp., München, Germany), which carries immobilized antibodies against the immunoglobulin kappa and lambda light chains and IgG heavy chains for Ig immunoadsorption in DCM patients. After apheresis, a marked decrease in serum levels of immunoglobulins and – as suggested – the accompanying beta1-AABs was observed. This reduction in beta1-AABs was accompanied by an improvement in heart function and a shift to a lower NYHA class. Subsequent studies using adequate protocols replicated these results [114]. An improvement in cardiovascular function was seen immediately after successful immunoadsorption [113,114] but, even more importantly, the functional and clinical benefits to patients were prolonged up to 3 and 12 months, respectively [115,116]. In parallel, renewed beta1-AAB generation was not seen until the end of these studies. With respect to the 12 month lack in the beta1-AAB re-increase, the authors speculated that the ex juvantibus taking of antioxidants prevented the renewed formation of beta1-AAB. Indeed, reduction of oxidative stress was demonstrated after immunoadsorption of beta1-AAB positive DCM patients [117].

Besides the apheresis techniques, which focused on whole immunoglobulin adsorption for beta1-AAB elimination and therefore needed subsequent immunoglobulin substitution, there is also a technique for selective
beta1-AAB removal that has the advantage of not requiring immunoglobulin substitution. Using the Coraffin column apheresis technique in DCM patients - in the same way that Lobovsky introduced the clearance of beta1-AAB from chagasic sera - the outcome indicated by clinical, functional and biochemical markers was comparable to whole immunoglobulin adsorption [118, 119]. Now we know that the benefits of immunoadsorption in DCM patients continued for 3 and 5 years, respectively [120, 121] and, based on preliminary data [122], even for 14.5 years. For the last cohort, this was most impressively demonstrated by long-term cardiac stability in end-stage DCM, which in turn could spare many patients from heart transplantation or delayed heart transplantation listing for many years. The authors suggested that in beta1-AAB-positive patients the benefits of both specific and unspecific immunoadsorption are comparable, and that consequently the possible removal of other AABs during unspecific immunoadsorption appears to have no relevant influence on the therapeutic results.

Besides Ig-Terasorb and Coraffin columns, columns with other matrices such as protein A (Immunosorba) and Peptid-GAM (Globaffin), both from Fresenius Medical Care, Bad Homburg, Germany and Tryptophan (Immunosorba TR from Asahi Kuraray Medical, Tokyo, Japan) are also currently used [123,124]; apheresis with the Tryptophan column was reported to not require immunoglobulin substitution.

Recently, we supplied basic results for the introduction of aptamers in the apheresis technique for cardiomyopathy patients. Aptamers are synthetic, highly-structured single- or double-stranded oligonucleotide ligands which, if well selected, bind to their corresponding target molecules with high specificity but possess low immunogenicity and toxicity in their applications [125]. Consequently, some aptamers have already entered the clinical pipeline, including specific aptamers designed to protect the cardiovascular system from coagulation and thrombosis [126-128]. Recently, we selected and patented an aptamer with highly specific targeting of the beta1-AABs which are directed to the second loop of the human beta1-receptor. Consequently, this aptamer targeted beta1-AABs found in DCM, Chagas’ cardiomyopathy and peripartum cardiomyopathy. We suggested the use of this aptamer as an adsorber for beta1-AAB apheresis in patients suffering from the above mentioned cardiomyopathies [129,130].

However, at present, it is not known which of the AABs (beta1-, beta2- or M2-AAB) found in Chagas’ cardiomyopathy is the leading pathogenic driver. Therefore, in our view, apheresis techniques that remove whole immunoglobulins should be the first-line option but, as already indicated, this procedure requires post-apheresis immunoglobulin substitution. Concepts to find aptamers that target the whole class of AABs directed to the G-protein coupled receptors in Chagas patients could complement or substitute whole immunoglobulin apheresis, and this could possibly open new windows in the apheresis of Chagas’ heart disease.

The apheresis procedure is initially cost-intensive but the significantly better survival rates lead to reasonable costs per life-year gained [121]. However, for Chagas’ patients, mainly those living in endemic areas, the cost factors and logistical problems could be so important that they prevent the wide use of apheresis as a treatment option for many chronic Chagas’ heart patients.

5.2.3.2 In vivo autoantibody neutralization

A new treatment strategy that focuses on the in vivo blockade of AABs against the GPCRs could possibly overcome the problems associated with the apheresis technique. Two main concepts are being studied. The first concept is still exclusively focused on the neutralization of beta1-AAB found in DCM patients and was introduced and patented for the first time in 1999 [131]. For this
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Peptides which reflect the second extracellular loop of the beta-1-receptor are able to abolish the chronotropic response of neonatal rat cardiomyocytes on beta1-AABs isolated from DCM patient sera. Recently, this concept was expanded to include cyclic peptides, which also contain sequences that correspond to the second extracellular loop of the beta-1-receptor. After the application of these peptides, abolishment of the beta1-AAB response was shown in vitro and in vivo [132]. There is no information on whether or not the peptide concept for beta1-AAB neutralization can be used for the blockage of the beta1-AABs of patients with Chagas’ heart disease.

The second concept, based on the selected aptamer [129,130] which was already introduced in 5.2.3.1. This aptamer was not only shown to neutralize beta1-AABs from DCM patients, but it was also found to neutralize those in patients with Chagas’ cardiomyopathy. This finding could possibly open the door to a totally new treatment strategy in both DCM and Chagas’ heart disease.

Although highly speculative, our present activities in expanding the aptamer concept to aptamers that target the whole class of cardiotoxic AABs directed to GPCRs could strongly promote the use of aptamers in the treatment of Chagas’ heart disease.

Whatever strategy of autoantibody-directed blocking therapy is administered to patients with Chagas’ heart disease in the future, asymptomatic but autoantibody-positive patients should be included in the treatment for cardiomyopathy risk reduction.

6. Autoantibody detection and monitoring

Last but not least, 1) assessment of patients risk for Chagas heart disease, 2) guidance of autoantibody-targeted treatment regimes, and 3) patient after treatment control and follow up require the detection and monitoring of the respective AABs.

Well-established tools are available to diagnose infected subjects and to monitor Chagas’ heart disease progress, as summarized by Munoz Saravia et al. [133]. Situations where AABs must be measured in order to estimate patient positivity and to monitor AAB-directed treatments are more critical.

Currently, a bioassay is mostly used for AAB measurement [70, 91,134]. In this assay, cultured neonatal rat cardiomyocytes are the substrate for AABs. The AABs are quantified by monitoring the chronotropic effects induced by the AAB-containing IgG fraction prepared from the patient’s blood. The addition of specific antagonists enables the differentiation between beta1-, beta2-, and M2-AABs. The beating frequency measured in this bioassay – as an integral parameter of cell functionality – is an advantage as it corresponds to the concentration of AABs. Moreover, the bioassay measures beta1-AAB, beta2-AAB, and M2-AAB in parallel. However, the limitations of this bioassay include the sophisticated assay standardization, a lack of adequate control materials, and extensive turnaround times.

There are other cell-based AAB quantification methods. In particular, the quantification of beta1-AABs was performed via cAMP formation in cultured cells that were engineered to express the recombinant receptor protein for beta1-AABs. The quantification of cAMP is then possible using RIA or ELISA technology. Quantifying the formation of cAMP using FRET technology might become important in the future [135].

Considering the time, cost, and capacity of cell-based assays, the development of less costly but well-standardized and more universally available ELISAs seems to be an essential prerequisite for AAB measurement. Whereas the bioassay quantifies AABs by measuring their functionality, ELISA and RIA exclusively use AAB binding to small peptides that are homologous to extracellular receptor epitopes recognized by the AABs. The ELISA and RIA tests do not indicate AAB
functionality. This might be one of the reasons for previous discrepancies in the results between bioassays and ELISA tests [136]. Nevertheless, ELISA tests specific for beta1-AAB, beta2-AAB, and M2-AAB have frequently been used in different studies [137,138]. However, to the best of our knowledge, no RIA or ELISA tests for Chagas’ disease-relevant AABs against GPCRs are presently commercially available. Whether aptamer-based assays (ALISA) will complement or substitute the presently available tools for the quantification of AABs directed to GPCRs remains to be seen.

7. Summary

Chagas’ disease is the most serious parasitic disease in Latin America and has the potential to become a worldwide problem. Its manifestation as Chagas’ heart disease places an enormous burden on economic resources, mainly in Latin America. In order to counteract the negative effects of Chagas’ heart disease, detailed knowledge of its epidemiology and pathogenesis is crucial. In this context, Chagas’ heart disease is increasingly considered as an autoimmune disease where AABs against GPCRs such as beta1-AABs, beta2-AABs, and M2-AABs drive the pathogenesis. Consequently, in order to complement or replace the conventional therapies for Chagas’ heart disease, treatment strategies for the removal (apheresis) or neutralization of the cardiotoxic AABs should be introduced. Such strategies could be based on the already suggested treatment concepts for AAB-positive DCM patients. Besides peptides for binding and blockade, aptamers that specifically target AABs against GPCRs are, in our view, promising for such treatment.

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