Available online at www.sciencedirect.com
ScienceDirect
Microbes and Infection 8 (2006) 2459–2464
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Levels of anti-M_2 and anti-β_1 autoantibodies do not correlate with the degree of heart dysfunction in Chagas' heart disease

Andre Talvani [a,1], Manoel O.C. Rocha [b], Antonio L. Ribeiro [b], Enri Borda [c], Leonor Sterin-Borda [c], Mauro M. Teixeira [a,*]

[a] *Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Av. Antônio Carlos 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil*
[b] *Pós-Graduação em Medicina Tropical, Departamento de Clínica Médica, Faculdade de Medicina, UFMG, Belo Horizonte, Brazil*
[c] *Pharmacology Unit, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina*

Received 3 April 2006; accepted 2 June 2006
Available online 18 July 2006

Abstract

Chronic chagasic cardiomyopathy (CCC) is characterized mainly by a dilated cardiomyopathy complicated by frequent and complex ventricular arrhythmias and/or conduction defects. The aim of the present study was to evaluate functional implications of neurotransmitter receptor autoantibodies in vivo. Sera from chagasic patients were used to measure the level of autoantibodies to peptide fragments from the M_2 cholinergic and β_1 adrenergic receptors. Optical density values and the frequency of anti-M_2 and anti-β_1 antibodies were significantly higher in the indeterminate form and in CCC patients than in normal individuals. There was no correlation between levels of autoantibodies and clinical parameters of ventricular dysfunction, as assessed by echocardiography. Patients presenting with chronotropic insufficiency in exercise test had higher levels of anti-M_2 but not anti-β_1 autoantibodies. Although anti-M_2 and anti-β_1 antibodies do not appear to play a role in the pathophysiology of the heart failure that accompanies severe CCC, anti-M_2 cholinergic autoantibodies may contribute to the pathogenesis of Chagas' disease dysautonomia.

Keywords: Chagas' disease; Cardiomyopathy; Neurotransmitter receptor; Autoantibody

1. Introduction

In Latin America, chronic chagasic cardiomyopathy (CCC) affects around 30% of individuals infected with the protozoan parasite *Trypanosoma cruzi*. In a significant proportion of the latter, severe heart disease occurs and is frequently the cause of death. There are several hypotheses to explain the pathogenesis of severe heart disease in infected individuals, including the role of parasite persistence [1,2], autoimmune events [3–6] and microvascular dysfunction [7].

CCC is characterized mainly by a dilated cardiomyopathy complicated by frequent and complex ventricular arrhythmias and/or conduction defects [8]. Autonomic dysfunction occurs early in the course of the disease and may be associated with poor prognosis [9–12]. It has been argued that the fixation of neurotransmitter receptors by anti-receptor antibodies contributes to the autonomic dysfunction and poor clinical evolution [12–14]. Although a strong association between circulating antipeptide M_2 muscarinic acetylcholine receptor (mAChR) autoantibodies and the presence of low heart rate variability index, bradycardia and cardiac or esophageal autonomic dysfunction in chronic chagasic patients was verified [15], it is not known whether the presence and/or titer of anti-receptor antibodies correlates with CCC severity.

Here, the presence and levels of autoantibodies against peptide sequences belonging to adrenergic (β_1) and muscarinic

* Corresponding author. Tel./fax: +55 31 3499 2651.
E-mail address: mmtex@icb.ufmg.br (M.M. Teixeira).
[1] Present address: Universidade Federal de Ouro Preto (UFOP), Ouro Preto, MG, Brazil.

1286-4579/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2006.06.006

(M_2) receptors were evaluated in sera of a group of 58 individuals. We also investigated the correlation between the titers of autoantibodies and the following aspects of clinical CCC manifestations: left ventricular systolic function and response to effort.

2. Methods

2.1. Study population and subject evaluation

We performed this study with 6 healthy individuals and 52 chagasic patients with different clinical forms of the disease. All individuals were recruited at the Referral Center for Training in Infectious and Parasitic Diseases (CTR-DIP) at the Hospital das Clinicas, Universidade Federal de Minas Gerais (UFMG) and underwent a complete clinical examination and the following laboratory workup: full blood count, free T4, thyroid-stimulating hormone, glucose, potassium, creatinine, blood urea nitrogen, electrocardiogram (ECG), chest X-ray, a 24-h Holter examination, echo Doppler cardiography (ECHO) and a treadmill exercise test. Patients with hypertension, diabetes, thyroid or renal disturbances or any other cardiac or systemic diseases and those using steroidal drugs were excluded from this study, as these conditions could prevent adequate interpretation of cardiac disease severity and immune parameters. The study received ethical clearance from the Ethics Review Board of Universidade Federal de Minas Gerais. Informed consent was obtained from all patients and non-infected individuals. Human experimental guidelines of the Brazilian Ministry of Health were followed in the performance of the experiments described here.

Chagasic patients were also categorized into groups according to the degree of heart dysfunction, as previously described [8]. Briefly, patients with an indeterminate form (IND) ($n = 8$) or chronic chagasic cardiomyopathy grade I (CCC I) ($n = 8$) were those with normal ECG and radiological studies or with only minor alterations in their ECHO (e.g. regional contraction defects), respectively. Patients classified as CCC II/III ($n = 7$) were those with minor or moderate ECG alterations, including block of the anterosuperior division of the left branch, right bundle branch block or uniform ventricular premature contractions. Patients classified as CCC IV ($n = 15$) were those manifesting severe conduction defects (e.g. left bundle branch block, left anterior divisional block with right bundle branch block or total atrioventricular block) or complex ventricular arrhythmias (complex ventricular premature beats, non-sustained or sustained ventricular tachycardia). Finally, patients classified as CCC V were those with ventricular enlargement, as observed on the ECHO, irrespective of the presence of arrhythmias or conduction defects [8]. The control group was made up of non-infected (NI) healthy individuals (Table 1).

A maximal stress test was performed according to the standard Bruce protocol. Chronotropic insufficiency was arbitrarily defined as the inability to achieve at least 85% of the predicted heart rate according to Astrand's formula (220-age) at peak exercise [16]. Patients underwent echocardiography with color flow using an ATL Philips HDI 5000 apparatus operated by an experienced echocardiographer, blinded to the clinical status of the patients. The left ventricular ejection fraction (LVEF) was obtained by Simpson's method using the software provided with the equipment [17].

2.2. Measurement of antibodies against anti-M_2 cholinergic and anti-β_1 adrenergic receptors

Serum samples were obtained by conventional venipuncture, centrifuged and stored at −80 °C until use in an immunoassay (ELISA) with M_2 synthetic cholinergic peptide [18] and β_1 adrenergic synthetic peptide [14] as coating antigens, as previously described [19]. The sequence H-W-W-R-A-E-S-D-E-A-R-R-C-Y-N-D-P-K-C-C-D-F-V-T-N-R-C (20 μg/ml), corresponding to the second extracellular loop of the human β_1-adrenergic receptor, and the sequence V-R-T-V-E-D-G-E-C-Y-I-Q-F-FS-N-A-A-V-T-F-G-T-A (10 μg/ml), corresponding to the second extracellular loop of the human M_2 cholinergic receptor, were used in the present studies. The samples were assayed in parallel at a 1/50 dilution, and optical density (OD) values were measured with an ELISA reader (Uniskan Laboratory System). This antibody dilution was found to be optimal to separate CCC patients from the control indeterminate form of Chagas' heart disease (data not shown).

2.3. Statistical analysis

Data are expressed as means ± S.E.M. or median and interquartile range. Analysis was performed using the computer program GraphPrism (GraphPad, San Diego, CA, USA).

Table 1
Clinical characteristics of non-infected individuals and patients classified with different levels of CCC

	NI ($n = 6$)	IND ($n = 8$)	I ($n = 8$)	II/III ($n = 7$)	IV ($n = 15$)	V ($n = 14$)
Age	38 ± 6.9	47 ± 3.4	48 ± 8.2	47 ± 4.5	44 ± 2.0	43 ± 2.3
Gender (% male)	67	38	50	29	47	79
LVEF (%)	68 ± 6	64 ± 4	66 ± 2	60 ± 3	61 ± 2	45 ± 3[#]
LVDD (mm)	48 ± 1	48 ± 1	49 ± 1	47 ± 2	50 ± 1	62 ± 1[#]
NVPB in 24 h	ND	1 [0–5]	3 [0–258]	725 [399–5676]	86 [18–2863]	840 [192–3002]

Values are shown as mean ± S.E.M., except for number of ventricular premature beats in 24 h that are shown as median [25–75% percentile]. NI, non-infected; IND, indeterminate form; LVEF, left ventricle ejection fraction; LVDD, left ventricle diastolic diameter; NVPB, number of ventricular premature beats. [#]$P < 0.01$ when compared to non-infected and chagasic individuals. Non-normally distributed data were transformed before performing ANOVA and means comparisons.

Comparison between groups was carried out by using analysis of variance (ANOVA) followed by Student–Newman–Keuls post test (parametric distribution). Probability values were considered significant when $P < 0.05$.

3. Results

There was no significant difference in the age distribution between non-infected individuals and chagasic patients (Table 1). In agreement with the clinical parameters used to classify the group, patients with CCC V had lower left ventricle ejection fraction and greater diastolic diameter than patients with the other degrees of CCC. There was great variation in the number of ventricular premature beats over 24 h, and patients with CCC II/III or worse had greater numbers of premature beats than those with the IND form or CCC 1 (Table 1).

The distribution of anti-M_2 cholinergic and anti-β_1 adrenergic autoantibodies detected by ELISA is shown in Table 2. It can be seen that the frequency of anti-M_2 cholinergic autoantibody was higher in CCC patients (mean 86%) than in subjects with the IND form (mean 38%) of Chagas' disease. The non-infected individuals were negative in the study system. When the distribution of anti-β_1 adrenergic autoantibodies was evaluated, no differences in the frequency between CCC patients (mean 67%) and IND (mean 77%) were observed. Table 2 also shows the distribution of both autoantibodies in the different degrees of CCC, and it can be observed that no differences existed between the different degrees of CCC.

Fig. 1 shows the levels of cholinergic (M_2) and adrenergic (β_1) autoantibodies in serum from non-chagasic and chagasic individuals. It can be seen that chagasic patients presented greater levels of both autoantibodies. However, when chagasic patients were grouped according to disease severity [8], there was no difference in the OD values of both M_2 and β_1 autoantibodies among the different clinical groups (Fig. 1). The lack of correlation between levels of autoantibodies and disease severity was further reinforced when the levels of antibodies and clinical parameters of ventricular dysfunction were studied (Table 3); there was clearly no correlation with the degree of heart dysfunction, as assessed by the left ventricular diameter and ejection fraction and the number of ventricular premature beats (Table 3).

The levels of anti-M_2 antibodies were higher in patients with than in those without chronotropic insufficiency (Fig. 2). The levels of anti-β_1 antibodies were similar in patients with or without chronotropic incompetence (Fig. 2).

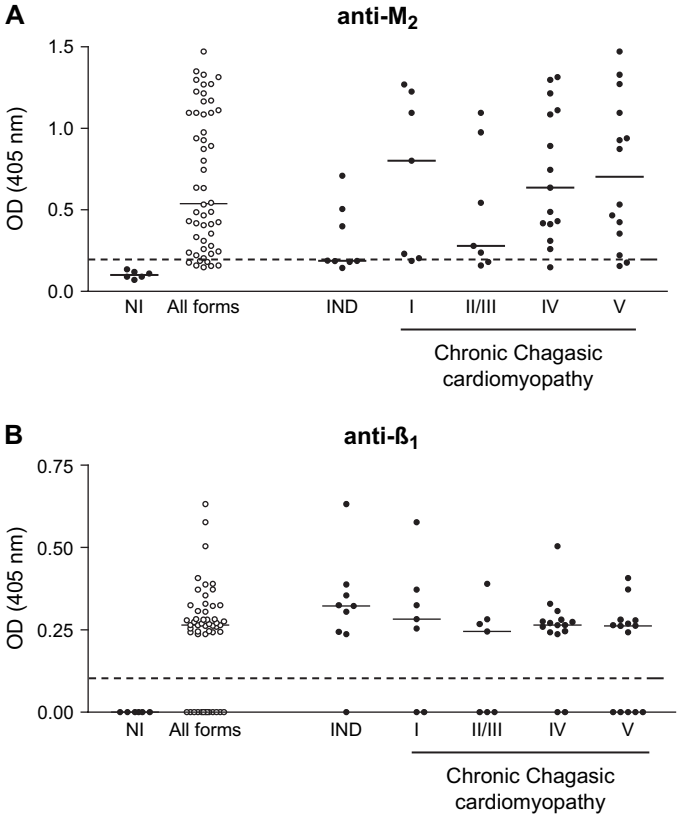

Fig. 1. Scattergram showing immunoreactivity of serum anti-M_2 cholinergic (A) and anti-β_1 adrenergic (B) autoantibodies tested by ELISA. Dots represent the individual OD values for each serum sample at 1/50 dilution from 6 non-infected (NI), 8 infected patients in indeterminate form (IND) and 52 chronic chagasic cardiomyopathy (CCC) patients. Dotted line shows cut-off values (mean OD values ± 2 SD from NI group): anti-M_2 cholinergic, 0.200, and anti-β_1 adrenergic, 0.100. Horizontal lines show median OD values. *$P < 0.0005$ all forms versus NI for the graphics (A) and (B).

Table 2
Distribution of anti-M_2 cholinergic and anti-β_1 adrenergic autoantibodies in non-infected individuals and infected patients

Groups	Anti-M_2 cholinergic		Anti-β_1 adrenergic	
	No. positive/total	Percentage	No. positive/total	Percentage
NI	0/6	0	0/6	0
IND	3/8	37.5*	6/8	77.1
CCC I	7/8	87.5	5/8	62.5
CCC II/III	5/7	71.4	4/7	57.1
CCC IV	14/15	93.3	13/15	86.6
CCC V	12/14	85.7	9/14	64.3

Microtiter wells were coated with 1 μg peptides (anti-M_2 and anti-β_1) and ELISA was carried out in the presence of sera from non-infected individuals (NI), indeterminate form (IND) and infected patients with different degrees of chronic chagasic cardiomyopathy (CCC I to V). OD values more than 2 SD above normal mean were considered positive. Cut-off values for anti-M_2 cholinergic, 0.200, and for anti-β_1, adrenergic, 0.100. Prevalence values of anti-M_2 cholinergic autoantibodies differ with *$P < 0.0005$ versus CCC I to V.

Table 3
Lack of correlation between parameters of ventricular dysfunction and levels of anti-adrenergic or anti-cholinergic receptor antibodies in CCC patients

	LVEF (%)	LVEDD (mm)	NVPB (in 24 h)
Anti-adrenergic antibodies	$R = 0.053$	$R = 0.052$	$R = 0.027$
	$P = 0.725$	$P = 0.733$	$P = 0.858$
Anti-cholinergic antibodies	$R = 0.005$	$R = 0.120$	$R = 0.167$
	$P = 0.973$	$P = 0.406$	$P = 0.270$

LVEF, left ventricle ejection fraction; LVEDD, left ventricle end-diastolic diameter; NVPB, number of ventricular premature beats.

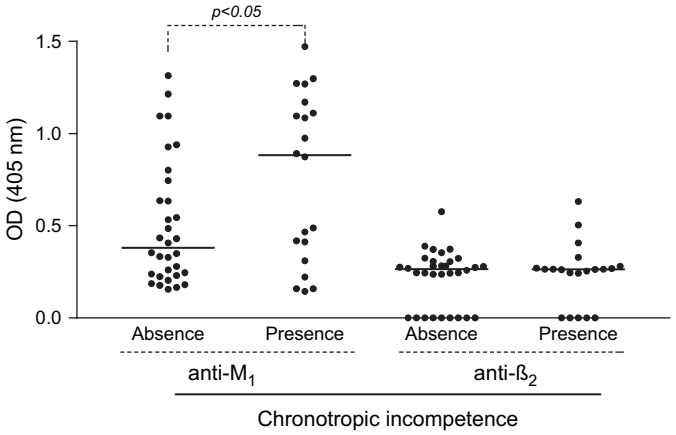

Fig. 2. Distribution of anti-M_2 cholinergic and anti-β_1 adrenergic autoantibodies in sera of chagasic patients with chronotropic incompetence. Chagasic patients were subjected to treadmill exercise, and the presence or absence of chronotropic insufficiency evaluated. Dots represent the individual OD values for each serum sample at 1/50 dilution. Horizontal lines show median OD values.

4. Discussion

Global systolic left ventricular dysfunction is the strongest predictor of morbidity and mortality during Chagas' heart disease [20,21]. It has been argued that an autoimmune response against antigens present in heart tissue may favor the development of the more severe forms of Chagas' cardiomyopathy. Antibodies against adrenergic and cholinergic receptors are among the many autoantibodies that have been described in Chagas' disease. For example, antibodies against β_1-adrenoceptores and M_2 mAChR have been found in the sera of patients and experimental animals with Chagas' disease [5,12,14]. Anti-M_2 and anti-β_1 antibodies are also found in sera of patients with non-chagasic forms of heart disease [22–25]. In Chagas' disease, these antibodies may induce acute functional alterations of isolated hearts from experimental animals (e.g. enhance or decrease contractility) and may also interact with the respective receptor and induce sequestration and endocytosis of the receptor [26–29]. Thus, it is clear that chagasic patients have anti-M_2 or anti-β_1 receptor antibodies and that the binding of these autoantibodies to the receptors may have functional consequences. In non-chagasic individuals, anti-β_1 receptor antibodies were previously found in association with failure of left ventricle function, serious ventricular arrhythmias and elevated incidence of sudden death [22–25]. However, it is unclear whether individuals with heart failure caused by *T. cruzi* infection or by idiopathic factors are able to develop these heart alterations because they possess autoantibodies or whether they develop these autoantibodies as a consequence of chronic cardiac tissue injury.

Our results showed that individuals with CCC had elevated levels of antibodies against peptide sequences of both adrenergic and cholinergic receptors when compared with non-infected controls. The frequency of anti-M_2 autoantibody was higher in CCC than in the IND form of the disease, suggesting that the anti-M_2 autoantibody could be used as an early marker of evolution in Chagas' cardiomyopathy. However, levels of this autoantibody were not able to differentiate the various forms of Chagas' heart disease. This was reflected in the lack of correlation between levels of autoantibodies and the left ventricular ejection fraction or the left ventricular end-diastolic diameter, both important parameters of left ventricular dysfunction. Thus, although the presence of autoantibodies is associated with the presence of Chagas' heart disease, there seems to be no association between the levels of autoantibodies and the degree of left ventricular function. The latter results suggest that left ventricular dysfunction appears not to be the cause of augmented serum levels of anti-M_2 and anti-β_1 autoantibodies in Chagas' disease and, on the other hand, the autoantibodies may not have a direct role in the pathogenesis of the left ventricular dysfunction that accompanies the most severe cases of Chagas' disease. One limitation of the present study is that we measured levels of the antibodies against peptide sequences present in the relevant M_2 and β_1 receptors and not the function of the antibodies present in serum of patients. One could argue that it is the function and not the levels of antibodies that determine their function and putative relevance in causing or worsening disease. Most studies to date have evaluated the function of these receptors using isolated cells or organs from animals, a clearly artificial situation (see for example refs. [13] and [28]). We are currently trying to address this situation by evaluating the function of these receptors using tests of autonomic function, including heart rate variability analysis, best suited for the study of vagal influences, and muscle sympathetic nerve activity (RMSA), a procedure that uses microneurography to directly record sympathetic nerve activity to muscle.

Despite the apparent lack of association between left ventricular function and the level of autoantibodies, there were significant associations between the level of anti-M_2 autoantibodies and the chronotropic response to exercise. Indeed, the level of anti-M_2, but not anti-β_1, receptor peptide autoantibodies was greater in patients in whom the presence of chronotropic incompetence during exercise testing was detected. One possibility to explain the latter findings could be that a partial agonist action of the antibodies could potentially increase parasympathetic tone during exercise. There is experimental evidence to support the latter possibility [26]. On the other hand, chronic activation of muscarinic receptors by anti-receptor antibodies may induce their internalization and, consequently, loss of parasympathetic function [27]. Heart rate increment during exercise is dependent on both vagal withdraw and sympathetic activation [30] and baroreflex impairment and/or parasympathetic dysfunction may be responsible for chronotropic incompetence observed in Chagas' disease [30,31] and other cardiopathies [32]. Indeed, we have shown that, in Chagas' disease, reduced exercise-induced heart rate increase is clearly associated with reduced vagal modulation evaluated by heart rate and heart rate variability parameters [31]. Moreover, we have also observed an association between higher anti-M2 receptor autoantibody levels and reduced HF power density obtained using HRV frequency-domain analysis, an almost pure vagal index [33].

So, anti-M2 receptor autoantibodies, reduced vagal modulation and chronotropic incompetence are closely related phenomena in Chagas' disease [33]. An alternative possibility is that there is loss of parasympathetic tonus that could be accompanied by unopposed adrenergic stimulation, leading to a loss of sympathetic tonus secondary to chronic stimulation. These hypotheses were not tested in the present study, and future studies should attempt to evaluate the expression (and perhaps function) of these receptors in the heart of patients with varying degrees of Chagas' disease. Regardless of the mechanism, our results do suggest that the presence of anti-M2 autoantibodies may play a role in the dysautonomia frequently observed in chagasic patients [8,11].

In conclusion, we showed that the levels of anti-M_2 or anti-β_1 receptor autoantibodies in sera of patients with Chagas' disease seemed not to correlate with the severity of left ventricular dysfunction. Nevertheless, the levels of anti-M_2 autoantibodies were greater in patients with chronotropic incompetence. Overall, these results point to an important role of anti-M_2 receptor autoantibodies in the pathogenesis of the dysautonomia but question the relevance of autoantibodies in the cascade of events leading to heart failure in Chagas' disease.

Acknowledgments

This investigation received financial support from FAPEMIG, the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (970728), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil), National Research Council (CONICET) of Argentina, TDR-WHO A20771, and the University of Buenos Aires (UBACYT), Buenos Aires, Argentina.

References

[1] R.-L. Tarleton, Parasite persistence in the aetiology of Chagas disease, Int. J. Parasitol. 31 (2001) 550–554.
[2] M.-M. Teixeira, R.T. Gazzinelli, J.S. Silva, Chemokines, inflammation and *Trypanosoma cruzi* infection, Trends Parasitol. 18 (2002) 262–265.
[3] M.-B. Soares, L. Pontes-De-Carvalho, R. Ribeiro-Dos-Santos, The pathogenesis of Chagas disease: when autoimmune and parasite-specific immune responses meet, An. Acad. Bras. Cienc. 73 (2001) 547–559.
[4] L.-C. Abel, L.V. Rizzo, B. Ianni, F. Albuquerque, F. Bacal, D. Carrara, E.A. Bocchi, H.C. Teixeira, C. Mady, J. Kalil, E. Cunha-Neto, Chronic Chagas disease cardiomyopathy patients display an increased IFN-g response to *Trypanosoma cruzi* infection, J. Autoimmun. 17 (2001) 99–108.
[5] E.-S. Borda, P. Cossio, M. de la Vega, R. Arana, L. Sterin-Borda, A circulating IgG in Chagas' disease which binds to beta-adrenoceptors of myocardium and modulates their activity, Clin. Exp. Immunol. 57 (1984) 670–686.
[6] J.-S. Leon, D.M. Engman, The significance of autoimmunity in the pathogenesis of Chagas heart disease, Front. Biosci. 8 (2003) 315–322.
[7] S.-B. Petkova, H. Huang, S.M. Factor, R.G. Pestell, B. Bouzahzah, L.A. Jelicks, L.M. Weiss, S.A. Douglas, M. Wittner, H.B. Tanowitz, The role of endothelin in the pathogenesis of Chagas disease, Int. J. Parasitol. 31 (2001) 499–511.
[8] M.O.-C. Rocha, A.L. Ribeiro, M.M. Teixeira, Clinical Management of chronic Chagas cardiomyopathy, Front. Biosci. 8 (2003) E44–E54.
[9] D. Iosa, V. Dequatro, D. De-Ping-Lee, U. Elkayam, H. Palmero, Plasma norepinephrine in chagasic cardiomyopathy: a marker of progressive dysautonomia, Am. Heart J. 117 (1989) 882–887.
[10] D. Iosa, V. Dequatro, D. De-Ping-Lee, U. Elkayam, T. Caiero, H. Palmero, Pathogenesis of cardiac neuropathy in Chagas Disease and the role of autonomic nervous system, J. Auton. Nerv. Syst. 30 (1990) 583–588.
[11] A.-L. Ribeiro, R.S. Moraes, J.P. Ribeiro, E.L. Ferlin, R.M. Torres, E. Oliveira, M.O. Rocha, Parasympathetic dysautonomia precedes left ventricular systolic dysfunction in Chagas disease, Am. Heart J. 141 (2001) 260–265.
[12] E.-S. Borda, L. Sterin-Borda, Antiadrenergic and muscarinic receptor antibodies in Chagas cardiomyopathy, Int. J. Cardiol. 54 (1996) 149–156.
[13] L. Joensen, E. Borda, T. Kaohout, S. Perry, G. Garcia, L. Sterin-Borda, *Trypanosoma cruzi* antigen that interacts with the beta 1 adrenergic receptor and modifies myocardial contractile activity, Mol. Biochem. Parasitol. 127 (2003) 169–177.
[14] L. Sterin-Borda, G. Gorelik, M. Postan, S. Gonzales Cappa, E. Borda, Alterations in cardiac beta-adrenergic receptors in chagasic mice and their association with circulating beta-adrenoceptor- related autoantibodies, Cardiovasc. Res. 41 (1999) 116–125.
[15] L. Sterin-Borda, E. Borda, Role of neurotransmitter autoantibodies in the pathogenesis of chagasic peripheral dysautonomia, Ann. N.Y. Acad. Sci. 917 (2000) 273–280.
[16] L. Fei, P.J. Keeling, N. Sadoul, X. Copie, M. Malik, W.J. McKenna, A.J. Camm, Decreased heart rate variability in patients with congestive heart failure and chronotropic incompetence, Pacing Clin. Electrophysiol. 19 (1996) 477–483.
[17] N.-B. Schiller, P.M. Shah, M. Crawford, A. DeMaria, R. Devereux, H. Feigenbaum, H. Gutgesell, N. Reickek, D. Sahn, I. Schnittger, Recommendations for quantitation of the left ventricle by two-dimensional echocardiography, American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms, J. Am. Soc. Echocardiogr. 2 (1989) 358–367.
[18] L. Sterin-Borda, L. Joensen, C. Bayo Hanza, M. Esteva, E. Borda, Therapeutic used of muscarinic acetylcholine receptor peptide to prevent mice chagasic cardiac dysfunction, J. Mol. Cell. Cardiol. 34 (2002) 1645–1654.
[19] L. Sterin-Borda, J.C. Goin, C.R. Bilder, G. Iantorno, A.C. Hermano, E. Borda, Interaction of human chagasic IgG with human colon muscarinic acetylcholine receptor: molecular and functional evidence, Gut 49 (2001) 699–705.
[20] C. Mady, R.H.A. Cardoso, A.C.P. Barreto, P.L. da Luz, G. Bellotti, F. Pileggi, Survival and predictors of survival in patients with congestive heart failure due to Chagas cardiomyopathy, Circulation 90 (1994) 3098–3102.
[21] R.-B. Bestetti, Predictors of unfavourable prognosis in chronic Chagas disease, Trop. Med. Int. Health. 6 (2001) 476–483.
[22] R. Jahns, V. Boivin, L. Hein, S. Triebel, C.E. Angermann, G. Ertl, M.J. Lohse, Direct evidence for a beta-1 adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy, J. Clin. Invest. 113 (2004) 1419–1429.
[23] N.-J. Freedman, R.J. Lefkowitz, Anti-beta1-adrenergic receptor antibodies and heart failure: causation, not just correlation, J. Clin. Invest. 113 (2004) 1379–1382.
[24] C.-J. Limas, C. Limas, Beta-receptor antibodies and genetics in dilated cardiomyopathy. An overview and review, Eur. Heart J. 12 (1991) 175–177.
[25] A.-L. Caforio, N.J. Mahon, F. Tona, W.J. McKenna, Circulating cardiac autoantibodies in dilated cardiomyopathy and myocarditis: pathogenetic and clinical significance, Eur. J. Heart Fail. 4 (2002) 411–417.
[26] S.-F. Oliveira, R.C. Pedrosa, J.H. Nascimento, A.C. Campos de Carvalho, M.O. Masuda, Sera from chronic chagasic patients with complex cardiac arrhythmias depress electrogenesis and conduction in isolated rabbit hearts, Circulation 96 (1997) 2031–2037.
[27] C.-J. Leiros, L. Sterin-Borda, E. Borda, J.C. Goin, M.M. Hosey, Desensitization and sequestration of human M2 muscarinic acetylcholine receptors by autoantibodies from patients with Chagas disease, J. Biol. Chem. 272 (1997) 12989–12993.

[28] M.-O. Masuda, M. Levin, S.F. De Oliveira, P.C. Dos Santos Costa, P.L. Bergami, N.A. Dos Santos Almeida, R.C. Pedrosa, I. Ferrari, J. Hoebeke, A.C. Campos de Carvalho, Functionally active cardiac antibodies in chronic Chagas disease are specifically blocked by *Trypanosoma cruzi* antigens, FASEB J. 12 (1998) 1551–1558.

[29] C. Perez Leiros, N. Goren, L. Sterin-Borda, L. Lustig, E. Borda, Alterations in cardiac muscarinic acetylcholine receptors in mice with autoimmune myocarditis and association with circulating muscarinic receptor-related autoantibodies, Clin. Auton. Res. 4 (1994) 249–255.

[30] L. Gallo Junior, J. Morelo Filho, B.C. Maciel, J.A. Marin Neto, L.E. Martins, E.C. Lima Filho, Functional evaluation of sympathetic and parasympathetic system in Chagas' disease using dynamic exercise, Cardiovasc. Res. 21 (1987) 922–927.

[31] A.L.-L. Rocha, F. Lombardi, M.O.C. Rocha, M.V.L. Barros, V.C.V. Barros, A.M. Reis, A.L. Ribeiro, Chronotropic incompetence and abnormal autonomic modulation in ambulatory Chagas disease patients, Ann. Noninvas. Electrocardiol. 11 (2006) 3–11.

[32] N. Fukuma, K. Oikawa, N. Aisu, K. Kato, Y.K. Kimura-Kato, T. Tuchida, K. Mabuchi, T. Takano, Impaired baroreflex as a cause of chronotropic incompetence during exercise via autonomic mechanism in patients with heart disease, Int. J. Cardiol. 97 (2004) 503–508.

[33] A.-L. Ribeiro, L.E. Díaz Gimenez, C.C. Quintero Hernández, A.C. Carvalho, M.M Teixeira, V.C. Guedes, M.V.L. Barros, F. Lombardi, M.O.C. Rocha, Early occurrence of anti-muscarinic autoantibodies and abnormal vagal modulation in Chagas disease, Int. J. Cardiol., in press.