Brazilian Journal of Medical and Biological Research (2008) 41: 796-803
ISSN 0100-879X

Trypanosoma cruzi infection induces up-regulation of cardiac muscarinic acetylcholine receptors *in vivo* and *in vitro*

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The pathogenesis of chagasic cardiomyopathy is not completely understood, but it has been correlated with parasympathetic denervation (neurogenic theory) and inflammatory activity (immunogenic theory) that could affect heart muscarinic acetylcholine receptor (mAChR) expression. In order to further understand whether neurogenic and/or immunogenic alterations are related to changes in mAChR expression, we studied two models of *Trypanosoma cruzi* infection: 1) in 3-week-old male Sprague Dawley rats chronically infected with *T. cruzi* and 2) isolated primary cardiomyocytes co-cultured with *T. cruzi* and peripheral blood mononuclear cells (PBMC). Using [^{3}H]-quinuclidinylbenzilate ([^{3}H]-QNB) binding assays, we evaluated mAChR expression in homogenates from selected cardiac regions, PBMC, and cultured cardiomyocytes. We also determined *in vitro* protein expression and pro-inflammatory cytokine expression in serum and cell culture medium by ELISA. Our results showed that: 1) mAChR were significantly ($P < 0.05$) up-regulated in right ventricular myocardium (means ± SEM; control: 58.69 ± 5.54, N = 29; Chagas: 72.29 ± 5.79 fmol/mg, N = 34) and PBMC (control: 12.88 ± 2.45, N = 18; Chagas: 20.22 ± 1.82 fmol/mg, N = 19), as well as in cardiomyocyte transmembranes cultured with either PBMC/*T. cruzi* co-cultures (control: 24.33 ± 3.83; Chagas: 43.62 ± 5.08 fmol/mg, N = 7 for both) or their conditioned medium (control: 37.84 ± 3.84, N = 4; Chagas: 54.38 ± 6.28 fmol/mg, N = 20); 2) [^{3}H]-leucine uptake was increased in cardiomyocytes co-cultured with PBMC/*T. cruzi*-conditioned medium (Chagas: 21,030 ± 2321; control 10,940 ± 2385 dpm, N = 7 for both; $P < 0.05$); 3) plasma IL-6 was increased in chagasic rats, IL-1β was increased in both plasma of chagasic rats and in the culture medium, and TNF-α level was decreased in the culture medium. In conclusion, our results suggest that cytokines are involved in the up-regulation of mAChR in chronic Chagas disease.

Key words: Chagas disease; Muscarinic acetylcholine receptor; Super-sensitivity; *Trypanosoma cruzi*; Immunogenic theory; Neurogenic theory

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Research supported by the National Grants for Science and Technology (Fonacit, Caracas, Venezuela, #S1-2001001134) and the Scientific, Humanistic, and Technology Developing Council (CDCHT-UCLA, Barquisimeto, Venezuela, #021-ME-2002 and #006-ME-2008).

Received October 18, 2007. Accepted August 27, 2008

Introduction

Chagas disease is caused by the parasitic protozoan *Trypanosoma cruzi*, which is transmitted to humans by various species of the bloodsucking insects of the Reduvidae family. Chagas disease is endemic in 21 countries of the Americas and currently there are more than 17 million infected people with 100 million more expected to become infected in the next decade. It is estimated that 1 million new cases are developed per year with 45,000 deaths per year due to advanced disease (1).

In Venezuela, Chagas disease is a public health problem, affecting mainly people living in rural areas under poor sanitary conditions (2). The heart is one of the organs most frequently affected in Chagas disease; 25 to 30% of the infected cases develop chagasic myocarditis that, if left untreated, leads to heart failure and sudden death (3). Chagasic myocarditis is characterized by dilated myocardiopathy and subsequent alterations of cardiac functions. At the tissue level, it is characterized by myocardial remodeling and associated mononuclear cell infiltration (3). It is intriguing, however, that there is no clear correlation between the presence of *T. cruzi* in the cardiac tissue and the development of chagasic myocardiopathy (4).

The origin of chagasic myocardiopathy has been addressed by two theories: the neurogenic theory postulates that myocardiopathy emerges as the result of an autonomic unbalance. In this scenario, the sympathetic activity predominance is a direct consequence of the loss of parasympathetic input, due to selective and irreversible destruction of the postganglionic vagal neurons, a phenomenon that occurs during the acute phase of infection (5,6). The immunogenic theory postulates that the onset of chagasic myocardiopathy is the direct consequence of an inflammatory response. The myocardial inflammatory infiltrate is the source of a variety of immune mediators, such as neutralizing antibodies and pro-inflammatory cytokines. Immunologic insults can modulate cardiac function by causing direct muscle remodeling associated with modulation of the β-adrenergic receptor-associated adenylylcyclase activity (7-10). Similarities between *T. cruzi* epitopes and human cardiomyocyte receptor epitopes such as β-adrenergic receptors, M_2-subclass muscarinic acetylcholine receptor (mAChR), nicotinic cholinergic receptors, and certain voltage-dependent channels have been described (11-15).

Antibodies directed at *T. cruzi* antigens, especially those of the IgG class, can act as cholinergic mAChR agonists, reducing atrial contractility and triggering classic mAChR signal transduction outcomes (i.e., cGMP stimulation or cAMP inhibition). Atropine neutralizes these effects by direct competition for the mAChR binding sites, while pertussis toxin acts by preventing the inhibition of cAMP (16). It has been reported that serum from chronic chagasic patients with complex cardiac arrhythmias is able to reduce cardiac rate (17) and induce auricular-ventricular conduction blockades, in isolated beating heart preparations, which is partially antagonized by atropine (18). Furthermore, the relationship between the immune system and the muscarinic cholinergic system is suggested by the report that activation of mAChR expressed on T cell receptor-activated T cells increases the production of IL-2 (19).

The neurogenic theory predicts that the consequences of parasympathetic denervation should be a muscarinic cholinergic receptor population up-regulation; however, according to the immunogenic theory, putative agonistic activity of the autoantibodies should induce mAChR subsensitivity and desensitization (20). Even though there is strong evidence in favor of both neurogenic and immunogenic theories, some investigators have failed to demonstrate mAChR up- or down-regulation. Torres et al. (21) observed that the mAChR function is neither depressed nor enhanced during the acute stage of Chagas disease in rats. Similarly, Tanowitz et al. (22) did not observe changes in the mAChR number or function in the heart of *T. cruzi*-infected mice.

In the present investigation, we analyzed the status of mAChR in a rat model of chronic Chagas disease. We evaluated 1) mAChR *ex vivo* expression in heart muscle and in peripheral blood mononuclear cells (PBMC), and 2) serum levels of pro-inflammatory cytokines in infected animals. Also, using primary cardiomyocytes co-cultured with autologous PBMC and *T. cruzi* we evaluated 1) mAChR expression *in vitro*, and 2) the levels of pro-inflammatory cytokines in the culture medium.

Material and Methods

Animal model

Three-week-old male Sprague Dawley rats, obtained from the Instituto Venezolano de Investigaciones Científicas (IVIC) animal facilities, were divided into experimental (N = 29) and control groups (N = 36). The experimental animals were inoculated intradermally with metacyclic trypomastigotes suspended in 0.9% NaCl, at a dose of 1000 trypomastigotes per gram of body weight. The *T. cruzi* MHOM/VE/92/2-92-YBM strain (23,24) used in these experiments was isolated from the dejections of laboratory-infected *Rhodnius prolixus*. The control animals were inoculated with a suspension of dejections of healthy *R. prolixus* diluted in 0.9% NaCl achieving the same concentration as the inoculum of laboratory-infected *R. prolixus* dejections. All animals were tested for parasitemia levels biweekly and

those animals belonging to experimental group that tested negative were excluded. After 6 months of infection and under general anesthesia (40 mg/kg pentobarbital), 8-12 mL blood was collected from the abdominal aorta of each animal and the heart was removed for dissection of selected regions (i.e., atria, septum, and both ventricles). All animal studies were carried out in conformity with the American Physiological Society Guiding Principles in the Care and Use of Laboratory Animals (25).

Serum and PBMC isolation

Non-heparinized blood (4 mL) was allowed to clot at room temperature and the resulting serum was inactivated at 60°C for 30 min. For PBMC isolation, 4 mL heparinized whole blood was diluted 1:1 (v/v) in cold balanced saline solution (BSS: 5 µM $CaCl_2$, 0.98 µM $MgCl_2$, 5.4 mM KCl, 14.5 mM Tris buffer, 0.126 M NaCl, and 5.56 mM D-glucose), loaded onto a 4-mL Ficoll-Paque® cushion (Amersham Pharmacia Biotech, UK) and centrifuged at 1000 *g* for 1 h at 5°C. The PBMC layer (buffy coat) was carefully recovered, diluted in 5 mL cold BSS, and centrifuged at 1000 *g* for 30 min at 5°C. The resulting pellet was resuspended in RPMI medium supplemented with 10% homologous rat serum, 100 IU/mL penicillin and 0.1 mg/mL streptomycin. Cell number and viability was determined by Trypan blue exclusion.

Cardiomyocyte cultures

Hearts were removed from 1-day-old Sprague Dawley rats under general anesthesia (40 mg/kg pentobarbital) and antiseptic conditions. They were rinsed in cold Ca^{2+}/Mg^{2+} free Hank's balanced saline solution, minced and dissociated with 1 mg/mL collagenase (Worthington Biochemical Corporation, USA) at 37°C for 40 min. Collagenase activity was terminated by adding fetal bovine serum (FBS; 20% final concentration). Dissociated cardiomyocytes were seeded at a density of 1.5-2 x 10^5 cells/mL on laminin-coated 35 x 10 mm dishes in complete basal medium Eagle (BME; Gibco Invitrogen Corporation, USA) supplemented with 10% FBS, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin, and incubated at 37°C in a 5% CO_2 incubator. After 24 h, the culture medium was totally replaced with fresh complete BME and after that only 50% of the medium was replaced twice a week. Cardiomyocyte cultures were assayed on the 4th or 7th days of culture.

Transmembrane cultures of cardiomyocytes and *T. cruzi*/PBMC co-cultures

Parasites (2 x 10^5) and PBMC (5 x 10^5) were co-seeded in an upper transmembrane Millicell® chamber (Millipore Corporation, USA) and co-cultured with 4-day-old cardiomyocytes seeded on the bottom of the main chamber. After 48 h, co-culture media were collected and stored at -70°C. The cardiomyocyte layer was scraped, homogenized, and stored at -70°C until further use. Protein content was determined using a modification of Lowry's method (26).

Protein synthesis

[^{3}H]-leucine (5 µCi; Amersham Pharmacia Biotech) was added to 1-week-old cardiomyocyte cultures concomitantly with 10 µM cytarabine to inhibit cellular proliferation. After 24-h incubation at 37°C in a 5% CO_2 incubator, cells were washed three times with 2 mL ice-cold BSS. Cell integrity was disrupted by incubating them with 2 mL 10% trichloroacetic acid for 10 min at 4°C following the addition of 2 mL 100% methanol. Preparations were allowed to air-dry before adding 1 mL 0.3 N NaOH, 1% SDS solution and further incubated for 30 min. Solubilized cell preparations (400 µL) were assessed for [^{3}H]-leucine-dependent radioactivity levels in a liquid scintillation counter (57% counting efficiency; Wallac 1410, Pharmacia, Inc., Finland). Total protein levels were also determined as indicated above.

Cytokine determination

IL-6, IL-1β, and TNF-α levels were assessed using commercial ELISA kits, following manufacturer instructions (Biosource Europe S.A., Belgium, http://www.biosource-diagnostics.com/catalog/default.asp?categ=50). Intra-assay coefficients of variation were 5.2% for IL-6, 4.5% for TNF-α and 2.8 for IL-1β. Interassay coefficients of variation were 9% for TNF-α, 9.9% for IL-6, and 4.5% for IL-1β.

[^{3}H]-QNB binding assay

Receptor density was measured in quadruplicate in homogenates from isolated cardiomyocytes, PBMC, or selected cardiac regions (atrium, septum, right and left ventricles), using saturating concentrations of [^{3}H]-quinuclidinylbenzilate ([^{3}H]-QNB) in equilibrium binding assay conditions according to Perez et al. (27).

Data analysis

Data are reported as means ± SEM. Differences between control and experimental subjects were analyzed by the Student *t*-test and $P < 0.05$ was considered to be statistically significant.

Results

mAChR expression in *T. cruzi*-infected rat cardiomyocytes

Using saturating concentrations of [^{3}H]-QNB (500 pM), we determined the number of mAChR (specific binding:

moles of bound [^{3}H]-QNB per mg of protein) in atrium, inter-ventricular septum, and ventricle homogenates from both chagasic and control rats, which displayed similar mAChR density (Table 1). However, the mAChR density was 23% higher in the right ventricle of *T. cruzi*-infected rats (72.29 ± 5.79 fmol/mg) compared with control (58.69 ± 5.54 fmol/mg; P < 0.05; Table 1).

mAChR expression in cultured cardiomyocytes

Cardiomyocytes co-cultured with chagasic rat PBMC and *T. cruzi* trypomastigotes displayed a significantly higher (P < 0.05) mAChR density (43.62 ± 5.08 fmol/mg; N = 7) than those cardiomyocytes co-cultured with healthy rat PBMC and *T. cruzi* trypomastigotes (24.33 ± 3.83 fmol/mg; N = 7) (Table 2). In order to show that these results are due to soluble molecules secreted by the parasites or by the PBMC as a consequence of cell-parasite interactions, we prepared cardiomyocyte cultures using conditioned medium from 2-day-old chagasic or healthy PBMC/*T. cruzi* co-cultures and tested the cardiomyocyte mAChR density. Cardiomyocytes cultured with conditioned medium from chagasic PBMC/*T. cruzi* showed increased (P < 0.05) mAChR density (54.38 ± 6.28 fmol/mg; N = 20) compared with the receptor density detected in cardiomyocytes cultured in conditioned medium derived from non-chagasic PBMC/*T. cruzi* co-cultures (37.84 ± 3.84 fmol/mg; N = 4).

Protein expression in cardiomyocytes and PBMC

First, we determined the effect of chagasic or healthy rat serum on the total protein expression in cardiomyocytes, and we observed increased (P < 0.05) total protein expression in cardiomyocytes cultured with chagasic rat inactivated serum (0.92 ± 0.1 mg/mL; N = 4) compared with control cultures (0.59 ± 0.08 mg/mL; N = 4). We determined the total protein content in cardiomyocytes from transmembrane cultures and we found that cardiomyocytes co-cultured with chagasic rat PBMC/*T. cruzi* trypomastigotes expressed higher protein levels (2.31 ± 0.20 mg/plate; N = 8) compared with the cardiomyocytes co-cultured with healthy rat PBMC and *T. cruzi* trypomastigotes (1.70 ± 0.09 mg/plate; N = 6; P < 0.05; Table 2). The protein synthesis rate, measured by [^{3}H]-leucine uptake, indicated that cardiomyocytes cultured with chagasic-conditioned medium have an elevated protein synthesis rate (21,030 ± 2321 dpm; N = 7) compared with those cardiomyocytes cultured with healthy conditioned medium (10,940 ± 2385 dpm; N = 7; P < 0.05).

Table 1. Muscarinic cholinergic receptor density and distribution in healthy and chagasic rat heart regions.

Cardiac region	Control (fmol/mg protein)	N	Chagasic (fmol/mg protein)	N
A	109.00 ± 9.97	20	112.00 ± 8.37	28
IVS	74.74 ± 4.12	28	75.20 ± 3.76	36
LV	77.37 ± 3.47	22	76.30 ± 3.77	26
RV	58.69 ± 5.54	29	72.29 ± 5.79*	34

Data are reported as means ± SEM. A = atrium; IVS = inter-ventricular septum; LV = left ventricle; RV = right ventricle. *P < 0.05 compared to control (Student *t*-test).

Table 2. Muscarinic acetylcholine receptor (mAchR) and protein expression in cardiomyocytes and peripheral blood mononuclear cells (PBMC).

	Cardiomyocytes		PBMC	
	Control	Chagas	Control	Chagas
mAChR (fmol/mg)	24.33 ± 3.83	43.62 ± 5.08*	12.88 ± 2.45	20.22 ± 1.82*
Protein (mg/mL)	1.70 ± 0.09	2.31 ± 0.20*	3.31 ± 0.37	2.07 ± 0.32*

Data are reported as means ± SEM. *P < 0.05 compared to control (Student *t*-test).

mAChR density and protein expression in healthy or chagasic rat non-activated PBMC

Total protein levels were significantly different between chagasic PBMC homogenates (2.07 ± 0.32 mg/mL; N = 7) and control PBMC homogenates (3.31 ± 0.37 mg/mL; N = 7; P < 0.05; Table 2). Interestingly, the changes in mAChR density, as demonstrated by binding assays, did not parallel those changes observed in total protein levels. Chagasic PBMC preparations presented higher mAChR density (20.22 ± 1.82 fmol/mg protein; N = 19) than the control (12.88 ± 2.45 fmol/mg protein; N = 18; P < 0.05; Table 2).

Cytokine levels in chagasic rat plasma and PBMC/*T. cruzi* co-culture media

IL-6, IL-1β and TNF-α levels *in vivo* (plasma) and *in vitro* (PBMC/*T. cruzi* co-cultures media) were selectively disregulated. As shown in Table 3, IL-1β serum levels were elevated in chagasic rat plasma compared with control animals (9.15 ± 2.08 and 3.38 ± 0.59 pg/mL, respectively; N = 10; P < 0.05). IL-1β concentration also appeared elevated in the PBMC-*T. cruzi* culture media (16.54 ± 4.42 *vs* 6.76 ± 1.34 pg/mL, respectively; N = 10, P < 0.05). IL-6 levels were significantly elevated in chagasic PBMC/*T. cruzi* co-culture media (21.86

± 3.15 *vs* 11.64 ± 1.06 pg/mL, respectively; N = 10; P < 0.05), but there were no significant changes in chagasic animal serum *in vivo*. TNF-α levels were significantly lower in the chagasic PBMC/*T. cruzi* cultures (59.39 ± 3.56 *vs* 71.47 ± 2.77 pg/mL, respectively; N = 10; P < 0.05) but no changes were detected in chagasic animal serum *in vivo* (Table 3).

Discussion

The main objective of this study was to determine whether there are changes in the expression of cardiac mAChR in *in vitro* and *in vivo* Chagas disease models. Our results demonstrate the up-regulation of cardiomyocyte muscarinic receptors when these cells were cultured with PBMC/*T. cruzi* co-cultures. There was also an increase of IL-6 and IL-1β levels in the culture media. However, in our *in vivo* model, the up-regulation of mAChR was partially observed only in the right ventricle.

The participation of the muscarinic cholinergic system in the development of chagasic cardiomyopathy is supported by both the neurogenic (6) and the autoimmune theories (11). During the acute phase of Chagas disease, the post-ganglionic vagal neurons of the intra-cardiac plexus are selectively destroyed by either *T. cruzi* cell invasion or by auto-antibodies directed to membrane structures (5,6). The result of either insult should promote the development of functional supersensitivity and/or up-regulation of post-synaptic mAChR in the cardiac muscle, due to a denervation phenomenon. Rocha et al. (28) observed that C57BL/6 mice that have been inoculated intraperitoneally with *T. cruzi* trypomastigotes have an increased cardiac muscarinic receptor density 8 months after infection. In our *in vivo* chagasic rat model, the up-regulation of mAChR was restricted to the right ventricle from chronic chagasic adult rat heart, which suggests a selective denervation of the right ventricle vagal fibers. However, this explanation is not sufficient to explain the mAChR supersensitivity observed in the cardiomyocytes/PBMC-*T. cruzi* co-cultures where nervous input is absent.

The possible agonistic activity of some anti-muscarinic and/or anti-nicotinic cholinergic receptor antibodies (11, 12,17) could induce receptor functional subsensitivity and down-regulation of the post-synaptic cardiac muscle mAChR, due to an agonist-induced receptor desensitization phenomenon (20); however, this event in cardiac tissues of chagasic animals has not been observed or reported. In the scenario in which both phenomena (vagal denervation and muscarinic receptor modulation by auto-antibodies) are supposed to occur simultaneously, each phenomenon could neutralize the other with no overall changes in mAChR expression. This explains the absence of mAChR supersensitivity in the other cardiac regions (atrium, septum, and left ventricle) observed in the present investigation in our *in vivo* rat model. Our observations are consistent with Tanowitz and colleagues (22), who reported no significant changes in the number and kinetics properties (Kd and Bmax) of the mAChR[22] of chagasic mice on the acute phase of the disease. Similarly, Torres et al. (21), working on chagasic Wistar rats in the acute or subacute phase of the disease, also failed to demonstrate any significant cardiac mAChR supersensitivity.

We observed that cardiomyocytes cultured *in vitro* with PBMC and *T. cruzi,* in a trans-well system, exhibited enhanced protein synthesis and up-regulation of their mAChR population. These enhanced parameters could be the result of soluble factors produced by *T. cruzi*-activated PBMC, which are able to modulate mAChR and other protein expression, as was suggested in the present study. Previously, it has been reported that pro-inflammatory cytokines are able to regulate mAChR expression, i.e., in human embryonic lung fibroblasts, transforming growth factor β1 (TGF-β1), TNF-α, and IL-1β have been associated with M_2-subclass mAChR desensitization (29). Similarly, using HEL 299 cells it has been reported that TNF-α and IL-1β synergistically down-regulate M_2-subclass mAChR at the protein and mRNA levels (30). Moreover, incubation of embryonic chicken heart cells with TGF-β1

Table 3. Cytokine levels in plasma and culture medium.

Group	IL-6 (pg/mL)		IL-1β (pg/mL)		TNF-α (pg/mL)	
	Plasma	Culture medium	Plasma	Culture medium	Plasma	Culture medium
Control	11.43 ± 0.98	11.64 ± 1.06	3.38 ± 0.59	6.76 ± 1.34	78.05 ± 3.34	71.47 ± 2.77*
Chagas	10.26 ± 2.95	21.86 ± 3.15*	9.15 ± 2.08*	16.54 ± 4.42*	68.26 ± 5.57	59.39 ± 3.56

Data are reported as means ± SEM for N = 10. Culture medium was taken from transmembrane cultures of cardiomyocytes and peripheral blood mononuclear cell/*Trypanosoma cruzi* co-cultures. *P < 0.05 compared to control (Student *t*-test).

resulted in a concentration- and time-dependent down-regulation of the mAChR transcriptional and translational products, explaining the observed refractory response to carbachol-mediated inhibition of adenylylcyclase activity (31). Also, up-regulation of the M_3-subclass mAChR and CCK-A receptor expression, concomitantly with the elevation of plasma IL-6 levels, was described in a bile-pancreatic juice exclusion from constriction-induced acute pancreatitis in rats (32). Our finding that up-regulation of the cardiac mAChR population could be the consequence of increased local levels of pro-inflammatory cytokines such as IL-6 is consistent with these observations.

Our results demonstrated a significant up-regulation on the right ventricle mAChR population, suggesting that mAChR supersensitivity is a response to cytokines released by inflammatory cells involved at the myocarditis sites. This idea is supported by our *in vitro* model of cardiomyocytes trans-well cultured with PBMC-*T. cruzi* co-cultures. In this *in vitro* system the up-regulation of the muscarinic receptors was parallel to high IL-6 and IL-1β, but low TNF-α levels detected in the culture-conditioned media, suggesting a modulator effect of these cytokines on the cardiac tissue muscarinic receptor population.

The importance of the integrity of the cholinergic system in the development of Chagas disease myocardiopathy is still controversial. The development of alterations in mAChR expression and/or function and their role in the pathogenesis of the chagasic cardiac dysfunction could be related to the presence of circulating antibodies against these receptors. It has been reported that patients in the early stages of Chagas disease have circulating auto-antibodies to the muscarinic receptors, associated with abnormal modulation of vagal activity (33). Furthermore, serum from chagasic patients is reported to have a muscarinic agonistic activity, evoking strong ventricular re-polarization rhythm disorders (34). Administration of the anti-parasitic drug benznidazole during the chronic phase of Chagas infection prevents the development of a more severe form of chronic cardiomyopathy. The amelioration of the cardiac pathology occurs concomitantly with a decrease in the levels of antibodies to the cardiac M_2-muscarinic receptors (35). Likewise, heart dysfunction is prevented in mice infected with Tulahuen *T. cruzi* trypomastigotes if they are treated with an M_2 mAChR immunogenic peptide (36). However, there is no clear correlation between the detectable auto-antibody levels and the clinical parameters of ventricular dysfunction (37).

Mononuclear cell-derived cytokines have also been involved in the development of cardiac tissue remodeling and heart failure (7,8). Studies on the participation of cytokines in Chagas disease evolution suggest that while IL-2 and IFN-γ have protective effects during the acute phase of the disease by decreasing the parasite load, TNF-α and IL-10 are associated with an exacerbation of the cardiac damage (38). Studies conducted on Chagas disease patients revealed a correlation between increased serum levels of TNF-α (39) or IL-6 (40) with progression of the disease to more severe clinical stages.

Increased plasma levels of IL-6 must be a consequence of increased heart inflammatory foci indicative of severe damage of postganglionic vagal neurons, which allows adrenergic hyperactivity and muscle mAChR supersensitivity. According to the neurogenic theory, the cardiac autonomic nervous system falls off balance with sustained sympathetic activity, which is the major contributing factor for the progressive deterioration of cardiac function (5).

Our results provide arguments favoring the concept that during Chagas disease evolution, PBMC supply high levels of pro-inflammatory cytokines, such as IL-6 and IL-1β, which could modulate cardiomyocyte mAChR expression. Increased pro-inflammatory cytokine plasma levels could have origin in diffused heart inflammatory foci, associated or not with postganglionic vagal neuron damage and thus, adrenergic hyperactivity and muscle mAChR supersensitivity.

Acknowledgments

We express our gratefulness to TSU Carmen Alvarez for her excellent technical assistance.

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