Letter to the Editor

Anti-adrenergic and muscarinic receptor autoantibodies in a canine model of Chagas disease and their modulation by benznidazole ☆

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ARTICLE INFO

Chronic chagasic cardiomyopathy (CCC) is a public health problem still without a defined physiopathology. It results from a chronic infection by Trypanosoma cruzi and is characterized by irreversible lesions to the heart. The contribution of autoimmune processes to cardiac dysfunctions observed in CCC has been hypothesized by several authors [1–3]. Those studies demonstrated the presence of IgG components in sera of patients with CCC and dilated cardiomyopathy interacting with cardiac β1-adrenergic (anti-β1-AR) and muscarinic membrane receptors (anti-M$_2$-CR) [3]. Of note, sustained activation of β-adrenergic receptors, particularly of the β1-subtype, promotes contractile dysfunction, ventricular arrhythmias and congestive heart failure with cardiomyocyte hypertrophy and interstitial fibrosis [3].

In the present work we investigated the existence and time-dependent appearance of anti-β1-AR and anti-M$_2$-CR AAbs in sera from dogs infected by three different T. cruzi strains (VL10, AAS and Y) and further, under modulation by benznidazole (Bz) (Roche, Rio de Janeiro, Brazil) treatment. To our knowledge, this is the first time that a defined time-course for antibody appearance is investigated in the canine model, which has a greater translational potential when compared

to other animal models [4]. Furthermore, most of the published data with humans are based on patients in the indeterminate and/or chronic phase of Chagas disease, not answering the question of when those AAbs were produced and what is their fate during the infection with T. cruzi.

Sixty mongrel (all 4 months old) dogs were inoculated with three different strains of T. cruzi (VL10, AAS and Y) by intraperitoneal route with 4.0×10^3 bloodstream trypomastigotes per kg of body weight. The infected animals with each of the strains were divided into two experimental groups: (i) 10 dogs were treated with Bz at 7.0 mg/kg twice daily for 60 days after infection confirmation by parasite detection; and (ii) 10 dogs were maintained as non-treated controls [5]. Additional 10 animals were maintained as a non-infected and untreated control group. Anti-β1-AR and anti-M$_2$-CR AAbs were evaluated at 30 (acute phase), 90 (early chronic phase) and 270 (late chronic phase) days post-infection in sera samples by enzyme-linked immunosorbent assay (ELISA). For AAb detection by ELISA synthetic peptides comprising the second extracellular loops of the β1 or M$_2$ cardiac receptors were used [6]. All animals were submitted to electrocardiographic (ECG) exams before infection and at 9 months post-infection. All procedures were conducted in accordance with the guidelines issued by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee in Animal Research at UFOP (protocol 98 number 2008/08).

Concerning the anti-β1-AR AAb titers, a similar pattern during the course of infection with the different parasite strains was observed. They appeared in all infected dogs at 30 dpi and were reduced at 90 dpi, recovering the acute phase levels at the late chronic phase (Table 1). With respect to anti-M$_2$-CR, they also appeared during the acute phase of the disease however, the levels during progression of the infection were highly variable, depending on parasite strain. Once increased, the anti-M$_2$-CR AAb levels for VL10 strain were maintained elevated during the whole course of infection (Table 1). On the other hand, the anti-M$_2$-CR AAb levels for AAS strain had a clear peak at 30 dpi reducing at 90 days, and remained at similar levels at 270 dpi (Table 1). Curiously, for the Y strain the anti-M$_2$-CR AAb levels were lower when compared to the other T. cruzi strains, especially at 30 dpi, and only 44% of the dogs were positive for this AAb (Table 1). At 90 dpi the AAb titers were similar to 30 dpi, however increased significantly in the chronic phase (Table 1). The fact that both AAbs appeared in the sera at an early stage of the infection with all the strains

Table 1

Autoantibody titers assessed by ELISA in dogs infected with three different strains of *Trypanosoma cruzi* and their modulation by benznidazole treatment.

	T. cruzi strain	R (% of positivity)		
		30 dpi Acute phase	90 dpi Early chronic phase	270 dpi Late chronic phase
Anti-β1-AR	VL10	2.3 ± 0.2[a] (100)	1.0 ± 0.2[b] (0)	2.7 ± 0.9[a,c] (100)
	AAS	3.0 ± 0.9[a] (100)	0.9 ± 0.2[b] (14)	1.9 ± 1.2[a,d,e] (75)
	Y	2.3 ± 0.7[a,§] (100)	0.7 ± 0.2[b] (22)	2.0 ± 0.8[a,c,§§] (89)
Anti-M$_2$-CR	VL10	1.7 ± 0.6[a] (100)	1.6 ± 0.6[a] (33)	2.3 ± 0.6[a] (100)
	AAS	3.1 ± 0.4[a,§] (100)	1.4 ± 0.9[a,b] (50)	1.8 ± 1.1[a,b] (78)
	Y	1.2 ± 0.3[f] (44)	0.8 ± 0.3[g] (33)	1.7 ± 0.7[a,c,h] (78)

ELISA values were expressed as the ratio (R) between the optical densities (OD) determined for each sample and cut-off values. Cut-off was the mean OD of non-infected animals plus 2 standard deviations (SD). Data are expressed as mean ± SD. Positivity was defined as R > 1.2.

[a] P < 0.001 *versus* non-infected control group.
[b] P < 0.001 *versus* 30 dpi.
[c] P < 0.001 *versus* 90 dpi.
[d] P < 0.01 *versus* 30 dpi.
[e] P < 0.01 *versus* 90 dpi.
[f] P < 0.01 *versus* non-infected control group.
[g] P < 0.05 *versus* non-infected control group.
[h] P < 0.05 *versus* 30 dpi.
§ Bz modulation P < 0.001.
§§ Bz modulation P < 0.05.

Table 2

Electrocardiographic findings at 270 days post-infection (dpi) of *Trypanosoma cruzi*-infected dogs with VL10, AAS and Y strains treated (T) or not treated (NT) with benznidazole.

	VL10 NT	VL10 T	AAS NT	AAS T	Y NT	Y T
Chamber overload	3/5	3/4	1/5	1/5	3/4	None
First degree atrioventricular block	1/5	2/4	1/5	None	None	None
Other arrhythmias[a]	None	None	3/5	None	2/4	None
Percentage of dogs with ECG alterations	80	100	100	20	100	0

[a] Other arrhythmias include: supraventricular extrasystole, first degree sinoatrial block, bradycardia–tachycardia syndrome, atrial arrhythmia, incomplete right bundle branch block, anterosuperior divisional block of the left bundle branch, complete right bundle branch block, and premature ventricular contractions.

studied and that there was a strain-specific modulation of anti-M$_2$-AAb titers reinforces the hypothesis that those AAbs are produced as a response to the parasite rather than a consequence of heart injury [7,8]. This hypothesis proposes that AAbs are produced against the parasites and are able to cross-react with selective host antigens leading to autoimmunity [7,8]. Similar to what was found by Wallukat [3] in chronic chagasic patients, the majority of the infected animals are positive for both anti-β1-AR and anti-M$_2$-CR AAbs in the late chronic phase (75 to 100%, depending on the strain) (Table 1).

Bz treatment in dogs infected with VL10 strain did not change the anti-β1-AR and anti-M$_2$-CR AAb titers at all time-points evaluated, and showed no improvement in ECG alterations (Table 1 and 2). On the other hand, in dogs infected with AAS strain, Bz although not altering anti-β1-AR AAb titers caused a 48% reduction in anti-M$_2$-CR AAb titers at 30 dpi (Table 1) and an 80% reduction in ECG abnormalities (Table 2). Bz treatment in dogs infected with Y strain led to no modulation of anti-M$_2$-CR AAb titers, a 35 and 30% reduction in anti-β1-AR AAb titers at 30 and 270 dpi, respectively (Table 1) and 100% reduction of ECG alterations (Table 2). Altogether, the absence of alterations in AAb titers by Bz treatment was accompanied by a corresponding absence of ECG modulation, while decreases in at least one of the AAb levels, anti-β1-AR or anti-M$_2$-CR, were always associated to ECG improvement. We previously reported that Bz treatment reduced fibrosis in dogs infected with AAS and Y strains, while this drug did not alter collagen deposition in dogs infected with VL10 strain [9]. Since increased fibrosis is a trigger for the development of arrhythmias, our findings are consistent with the effects of Bz treatment in collagen deposition previously reported. Recently, Cutrullis et al. [10] reported a significant reduction of serum anti-M$_2$-CR titers in children in the indeterminate form of Chagas disease when submitted to Bz treatment, however they could not associate this finding with heart dysfunction as the patients did not show any ECG or ECHO alterations.

Although IgGs with anti-β1-AR or anti-M$_2$-CR reactivity are sometimes associated with cardiac function worsening in CCC, others have not found such association. Our study indicates that AAb levels are increased early throughout the course of infection suggesting that they are produced in response to parasite presence. Additionally, the modulation of AAb levels by Bz treatment is dependent on *T. cruzi* strain and associated to ECG abnormality modulation.

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