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In vivo inhibitory effect of anti-muscarinic autoantibodies on the parasympathetic function in Chagas disease

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Dear Sir,

In 2007, we described in this *Journal* [1] that higher levels of anti-muscarinic (anti-M2) autoantibodies correlated with reduced indexes of heart rate variability (HRV) in Chagas disease (ChD), suggesting that their inhibitory effect might play a role in the genesis of impaired heart vagal modulation [2,3]. This publication was followed by several letters [4–8] that discussed many aspects of the pathogenesis and the physiopathology of ChD. A major issue in this controversy is related to the possible existence of enhanced parasympathetic activity in ChD.

Benchimol-Barbosa hypothesized that the continuous effect of the anti-M2 antibody acting on muscarinic receptors on the surface of sinus node cells in ChD is expected to both steadily reduce resting heat rate (HR) and further impair vagal indexes of heart rate variability (HRV) [6]. In our response, we pointed out that our published data [1] showed that reduced HRV was not accompanied by reduced heart rate in ChD patients, and that anti-M2 titers did not correlate with basal HR. In this context and after other considerations, we concluded that the occurrence of vagal enhancement remains a theoretical hypothesis that still begs demonstration [7]. In a later text, Benchimol-Barbosa enumerated reasons that might explain the apparent paradoxical maintenance of the HR associated with both elevated anti-M2 levels and abnormal HRV indexes under the hypothesis of occurrence of tonic parasympathetic stimulation due to partially agonistic anti-M2 activity in chronic ChD [8]. We would like to praise the author for the insightful comments and to answer, point-by-point, all the issues raised in his letter.

(1) In Schwartz and De Ferrari and colleagues' first-in-man experience of electrical chronic vagal stimulation in advanced heart failure [9,10], HR was significantly reduced after chronic vagal stimulation and presented only a partial return after six months (basal: 87 ± 13 bpm, 1-month: 78 ± 13, 3-months: 79 ± 13, 6-months: 83 ± 12, $p < 0.01$); no HRV index was recorded. This experiment cannot be compared with our study, since it was performed (a) in a different population: class III heart failure versus ChD patients with mainly normal left ventricular (LV) function, and (b) with a different kind of stimulus: electrical versus immunological. Moreover, Schwartz et al. [9] demonstrated a marked improvement in heart failure symptoms and a reduction of LV volumes during treatment, making it impossible to know the concomitant influence of other neurohumoral factors, or the effects of the drugs used.

(2) In a previous study [11], we demonstrated that optical density values and the frequency of anti-Beta1 adrenoceptor antibodies were significantly higher in the indeterminate form and in Chagas cardiopathy patients than in normal individuals. In this sample, we

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also measured anti-Beta1 antibodies (unpublished data) [1], but we could not find a significant correlation between heart rate and anti-beta1 optical density values ($rs = 0.03$, $p = 0.84$), suggesting that this is not a main counterbalancing mechanism in this ChD population.

(3) In our original study [1], ChD patients without LV dysfunction (group 1) had similar median values and frequencies of BNP values, diastolic dysfunction, age, and gender, when compared to the control group (group 0). Thus, differences in anti-M2 optical density values and heart rate variability indexes between these two groups could not be ascribed to the influence of these variables.

(4) The study of the effect of immunologic and inflammatory influences on the autonomic cardiac modulation is an attractive and promising new field [12,13], but there is not a convincing body of knowledge that supports the putative role of these factors in our findings.

(5) An experiment very similar to that proposed by Benchimol-Barbosa was conducted by Peter et al. in 2005 [14]. Mice immunized with a peptide (M2-G19K) derived from the second extracellular loop of the M2 achetylcholine receptor (M2ACh-R) were compared to controls immunized with an irrelevant peptide. M2-G19K immunized mice developed anti-M2 antibodies detectable by immunoblot test and able to behave as positive allosteric modulators of M2ACh-R *in vitro*. However, no *in vivo* difference was found in resting HR or HRV between the two groups of mice [14]. In another study in mice immunized with plasmid DNA coding for the human M2ACh-R, Gimenez et al. [15] observed that antibodies against M2ACh-R did not change the HR after 42 weeks of follow-up. Thus, in this experimental model of pure anti-M2 agonistic stimulation, no reduction was observed in resting HR and HRV indexes, contrasting with our findings in ChD patients, whose increased anti-M2 levels were correlated with lower resting HRV indexes without any HR reduction.

None of these considerations changed our original observation that anti-M2 antibodies had an inhibitory effect in parasympathetic heart modulation, which was manifested by a reduced high frequency component of HRV analysis in patients with chronic ChD; no functional evidence of enhanced parasympathetic activity could be demonstrated in this study [1]. How anti-M2 antibodies can compromise vagal response is still uncertain. Receptor desensitization following chronic stimulation by the antibodies has been demonstrated and it is logical to consider this as the main mechanism involved in this phenomenon [5,16]. Nonetheless, positive allosteric modulation, as suggested by Benchimol-Barbosa [8], may also play a role. Indeed, in the same experiment quoted above, Peter et al. [14] found that M2-G19K immunized mice showed blunted response to beta-adrenoceptor agonist isoproterenol, indicating that anti-M2 antibodies have positive allosteric modulation when the sympathetic tone is high. This is a very reasonable explanation for the blunted chronotropic response during stress testing in ChD patients with high levels of anti-M2 antibodies [11], a process that is also related to reduced heart rate variability indexes [17].

In conclusion, many aspects of ChD pathogenesis remain rather obscure, like the above mentioned mechanisms involved in vagal dysfunction. Since this gap is an obstacle to advancement in the management of ChD, further research including experimental, epidemiological, and clinical studies is needed.

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