Internal Medicine Review

What we could learn about Chagas´ disease from our experimental therapeutic trials

January 2020

What we could learn about Chagas´ disease from our experimental therapeutic trials

Authors

Dr. Sadí Cossy Isasi
Cátedra de Bioquímica y Biología Molecular, Escuela de Medicina, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Pabellón Argentina 2° piso, Haya de La Torre s/n, Ciudad Universitaria, Córdoba, Argentina.
E-mail scossy04101962@yahoo.com.ar.
T.E. 54 351 4333024 int 102

Dr. Juan Carlos Muiño
Director del Centro Formador de la Especialidad en Alergia e Inmunología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Ambrosio Olmos 820.
Hospital Ntra. Señora de la Misericordia, Servicio de Clínica Médica, Belgrano 1502 Córdoba Argentina.
T.E. +54 351 434 4110
Hospital San Roque, Servicio de Clínica Médica, Bajada Pucará 1900 Córdoba Argentina. T.E. +54 351 434 8912
E-mail: jcmuino@gmail.com

Abstract

Background: Chagas´ disease is an important endemic in Latin America, caused by the protozoan *Trypanosoma cruzi* (T. cruzi), presents irreversible autoimmune panmyocarditis in 24% of infected patients only in Argentina. We already reported lethally T. cruzi infected mice survival when treated with GM1 bovine brain ganglioside. Healthy IgGs anti-β adrenergic receptors (ABrs) become autoimmune in Chagas´ disease but in GM1 treatment it remains unclear. Since GM1 promotes Th2 response, it would be expected to worsen the fate of infected mice due to increased ABrs.

Methods: We analyze our results and selected literature to improve our understanding going along the "blade" of immunity-behavior. New experimental sets were: 80 mice divided into 2 groups: a) healthy control, b) healthy challenged with serum albumin (A);160 three months old outbreed Albino Swiss mice divided into 4 groups: a) non-infected, b) survivors of infection with T. cruzi, 0.7×10^5 parasites c) infected with T. cruzi, 0.7×10^5 parasites and treated with GM1 0,1 mg /day by intramuscular injection for 30 days d) infected with T. cruzi, 0.7×10^5 parasites and treated with oral fluoxetine (F) 0,08 mg /day. Mice were tested at 120 days post treatment. Another set of experiments comprised equal number of mice but GM1 was administered after F or vice versa. All mice of both sexes were subject of forced swimming test (FST).

Results: healthy mice showed climbing -adrenergic-dominance, chronic mice showed swimming and then floating, signs of progressive depression. "A" mice were swimmers but GM1 treated ones preserved the climber profile.

Conclusion: Considering depression (floating) is associated with inflammation, negotiation (swimming) with IgG production and reaction (climbing) with healing, we propose that the onset of infection shifted the immune metabolic frame from regulation through healthy IgGs ABrs to self-challenged through autoimmune IgGs, and that process was partly reversed by GM1.

1. Introduction

Chagas´ disease is an important endemic in Latin America, caused by Trypanosoma *cruzi* (T. cruzi), a flagellated protozoan that undergoes complex morphological changes throughout its life cycle in the insect vector and the vertebrate host. Two phases can be clearly differentiated regarding clinical aspects and treatment. The acute infection is stated with detectable parasitemia, acheless eye swelling (the "Romaña sign"), and sometimes, with a pertinacious grippe condition: violent headaches, muscles pain, inappetence and ganglion (lymphatic nodes) acheless inflammation [1]. Heart disease is an outstanding point, since irreversible chagasic pan myocarditis is developed in almost 600.000 person out of 2,500,000 infected only in Argentina who progress from an acute tachycardia into a more benign chronic bradycardia and left ventricle remodeling [2,3]. Nifurtimox and benznidazole, antiparasitic drugs recommended in the acute phase and with arguable results in the chronic phase, cause adverse drug-related reactions with frequency and severity that increase with age and weight of the patient, reducing treatment compliance [4-6]. Therefore, there is still need of experimental research to develop alternative drugs and chemotherapeutic programs, but mostly there is need for a more accurate understanding of the biological processes underlying the clinical evolution. We already reported that mice infected with a lethal amount of T. cruzi to simulate an acute phase survived when treated with bovine brain gangliosides [7]. Treated mice diminished parasitemia, reaching undetectable levels by day 30 post infection (d.p.i). Those positive effects were almost exclusively due to GM1 monosialoganglioside present in the total bovine brain gangliosides mixture [8]. Hearts from GM1 treated mice presented only minor mononuclear infiltration and neither amastigotes nests nor fibrosis were observed. Parasite DNA in plasma was undetectable suggesting complete clearance of circulating parasites. GM1 treatment outcome could be characterized by parasitemia decay and myocardial recovery, two aspects that may be explained through different mechanisms. Of course, no parasite, no Chagas´ disease. We mean that although concomitant, parasitemia and cardiac physiology imbalance might not be linearly or directly related in a mechanistic cause-effect way. The analysis and therapy of both factors might be approached in different ways. The considerations presented in this communication are concerned with, and extended beyond, myocardial recovery. Chagasic chronic cardiopathy courses with decreased β adrenergic receptor function (bradycardia) and alterations in pharmacological response. We based our work on an experimental model of post-acute phase with its main outcome dependent on the inoculum size: decreased affinity of β adrenergic receptors when mice were inoculated with few parasites versus diminished receptor density when lethal number of parasites were inoculated [9]. With a sublethal inoculum the fraction of infected cells is small and a reduction in functional receptor number would be

hardly appreciated. The shift in affinity seems to be an organ wide metabolic symptom of the parasite burden. T. cruzi sheds lipidic vesicles as decoys and exposes phosphatidylserine in outer plasma membrane layer, attenuating monocyte inflammatory reaction [10]. In the same direction, since β adrenergic receptor stimulation promotes IL6 expression (an inflammatory cytokine) in muscle and other cell types, a reduction in myocyte β adrenergic receptor affinity would reduce the expression of IL6. Thus, reduction of myocyte β adrenergic receptor affinity contributes to parasite survival. This response to infection is also beneficial for the host because IL6 produces myocardial remodeling in an inflammatory environment like synergy with IL 1 [11] to compensate cell loss. In support of this interpretation, in our experiments, mice with acute lethal infection presented diminished β adrenergic receptor affinity but density was reduced to 60% of adrenergic receptors indicating enormous cell destruction. Mice treated with GM1 on a daily regime from the onset of the infection, survived, and at day 35 post infection, beta adrenergic receptor affinity was higher than in untreated healthy control. The effect of GM1 was on myocyte metabolism since healthy GM1 treated mice presented even higher affinity. Parasitemia at day 35 was almost null, so we might think that conserved or high β adrenergic receptor affinity could favor a better adapted immune response with preserved expression of IL6. Receptor density in GM1 mice (day 35), on the contrary, was lower, even than infected mice. This might be attributed to a new density-affinity equilibrium since healthy (without infection) GM1 treated mice showed the lowest density. At day 120, the rest of surviving (only GM1 treated or healthy) mice presented affinities comparable to healthy untreated control mice. Though this means a reduction in affinity (compared to day 35), it is important to note that GM1 treatment was suspended by day 30 post infection, and surely no GM1 remained in circulation nor in tissues. But receptor density recovered to healthy untreated values or even better, accounting for conduction recovery or perhaps for cell mass recovery also [12]. One aspect that remains to be elucidated is the contribution of anti-β adrenergic receptors antibodies to pathogenesis of Chagas´ disease in the context of our model of acute lethal infection under GM1 treatment. On one side human and experimental models support that immunopathological mechanisms may be involved in heart disease. Antibodies from Chagas disease patients' blood were of IgG class and recognized myocardial β 1 and spleen cell β 2 adrenoreceptors and their interaction with the receptors resembled non-competitive inhibitors. The prevalence of anti- β 1-adrenergic antibody was low in the acute stage, but it increased over time post infection, being higher in the group with more than 15 years of infection [13]. The anti- β 2-adrenoreceptor IgG appears during the acute stage, peaks on the group with less than 10 years of infection, and then decreases. In human dilated cardiomyopathy (DCM), anti-β adrenergic receptors antibodies belong to IgG class also, and contribute to apoptosis signaling in the cardiomyocyte similarly to that seen in cultures treated with the nonselective β 1AR/ β 2AR agonist isoproterenol. Patients with

dilated cardiomyopathy presented anti- β 1 adrenergic receptor antibodies that recognized the extracellular loops of the receptor and, like isoprenaline, induced positive chronotropism in cultured rat cardiomyocytes [14]. Thus, they seem to behave as potent agonists. Medei EH et al 2008[15] observed that sera from chronic Chagas´ disease patients shortened the QT interval in isolated rabbit hearts and the action potential duration (APD) of M cells and increased ICa and IKs. Thus, anti- β 1-adrenergic antibody in vitro could induce arrythmia through altering IKs and ICas. Although it seems a clear mechanism of immune induced damage, it is not so clear how it could operate for long periods. The main antigenic determinant in idiopathic DCM has been shown to be the second extracellular loop of Beta1-adrenergic receptors [16]. The antigen is usually exposed, and it is recognized by IgG anti-β adrenergic receptors of low concentration that are produced in the healthy state. It is not clear whether the antigenic determinant in the disease is the same peptide, in which case there seems to be no reason for it to become pathogenic. Then it could be proposed that a shift to a different metabolic frame determined the reading of previous immune information in terms of antigenic challenge. Since GM1 also acts as immunomodulator shifting the immune response towards Th2 profile, it would be expected to worsen the fate of infected mice through stimulation of IgG production and consequently of anti-β adrenergic receptors antibodies. These antibodies are present in plasma of healthy individuals at very low titles. A modulatory or "buffering" role was proposed for them possibly determined by commensal microorganisms cohabiting in the human body [17]. The classical scheme of receptor and hormone regulation by negative feedback should be modified incorporating the anti-β adrenergic receptors antibodies. But the antibodies do not produce a response with identical mechanism as hormones adding complexity to the system. Although they may behave as agonists they do not produce immediately down regulation. In this respect GM1 treatment strongly improved both, mice β adrenergic receptors affinity, and antibodies production against T. cruzi. Considering the deleterious activity of the immune response, it could be hypothesized that GM1 regulation of β adrenergic receptors mainly through tuning myocyte metabolism or modification of receptor membrane microenvironment (perhaps hiding the receptors) overcomes or prevents the consequences of the augmented production of antibodies (anti-β adrenergic receptors included). If that were the case, GM1 treatment should have reduced the number of accessible receptors or density. Another explanation could be that autoreactive anti β adrenergic receptor IgG triggered by infection come from a "rebel" signaling pathway characteristic of auto immune diseases while GM1 mediated signaling follows a "loyal" pathway that leads to healthy outcomes. We measured receptors density and found GM1 to render lower values even in the absence of infection, supporting the first hypothesis. But we cannot exclude the second since we have not tested the signaling pathway of immune system in GM1 treated infected mice. Anyway, both hypotheses are not mutually exclusive. An important lesson to

understand is that the parasite shifts the production of agonist antibodies from a healthy equilibrium to the synthesis of antibodies that behave in a complex hybrid way. The mechanism for the switch cannot be attributed merely to cardiomyocyte destruction since the binding site for both types of action is on the extracellular domain of the receptor [17]. It seems that a kind of shift on the interpretation of the epitope takes place upon parasite entrance. A cytokine of growing importance in this respect is IL-23. Many autoimmune inflammatory diseases and cancer are related to the activation of IL-23. This interleukin became the target for therapeutic approaches for psoriasis, inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis and cancer [18]. IL-23 strongly drives the synthesis of interleukin-17 (IL-17) by Th17 cells and IL-17-producing γδT (γδT17) cells that has propelled IL-23 to a major therapeutic target [19,20]. A challenging hypothesis could be that the parasite stimulates dendritic cells to produce IL-23 upon entrance and, in our experimental model in which GM1 treatment began the same day of infection, GM1 disrupted IL-23 signaling. This in turn would diminish Th-17 cell population in favor of classical Th2 response.

2. Methods

We analyzed our results and selected literature to improve our understanding going along the "blade" of immunity-behavior. New experimental sets were: 160 three months old outbreed Albino Swiss mice divided into 4 groups: a) non-infected, b) survivors of infection with T. cruzi, 0.7×10^5 parasites c) infected with T. cruzi, 0.7×10^5 parasites and treated with GM1 0,1 mg /day by intramuscular injection for 30 days d) infected with T. cruzi, 0.7×10^5 parasites and treated with oral fluoxetine (F) 0,08 mg /day. Mice were tested at 120 days post treatment. Another set of experiments comprised equal number of mice but GM1 was administered after F or vice versa. All mice of both sexes were subject of forced swimming test (FST).

3. Results

To know whether GM1 enhancement of TH2 response presented similar behavior signature as immunization with albumin, (swimmer, depressive, see Discussion), both coursing with increased IgG production, we measured the extension of the lag to the first floating event in chronic T. cruzi infected mice of both sexes that were treated only in the acute phase with anti-depressant fluoxetine (F, 0.25mg/Kg) or GM1 (1.5mg/Kg) against age matched physiological solution injected controls. The variable was chosen because the greater the lag the more adrenergic the profile. While F treated mice showed no difference, GM1 treated mice showed an increase of ± 40% in the lag (Fig 1). This result indicated that GM1 treated mice were more resistant to depression.

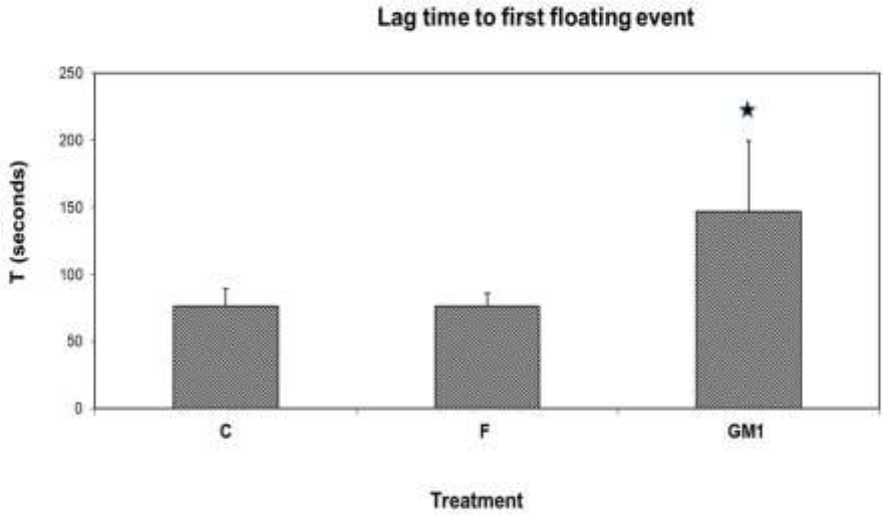

Fig 1: Time lag for mice of both sexes, healthy control (c) and lethally infected with 0.7×10^5 parasites and treated with fluoxetine (F) or GM1. * p> 0.05

When the three types of behavior were considered, control mice were mainly climbers, F treated showed no significative differences among climbing, swimming or floating, but GM1 treated ones preserved the climber profile Fig 2.

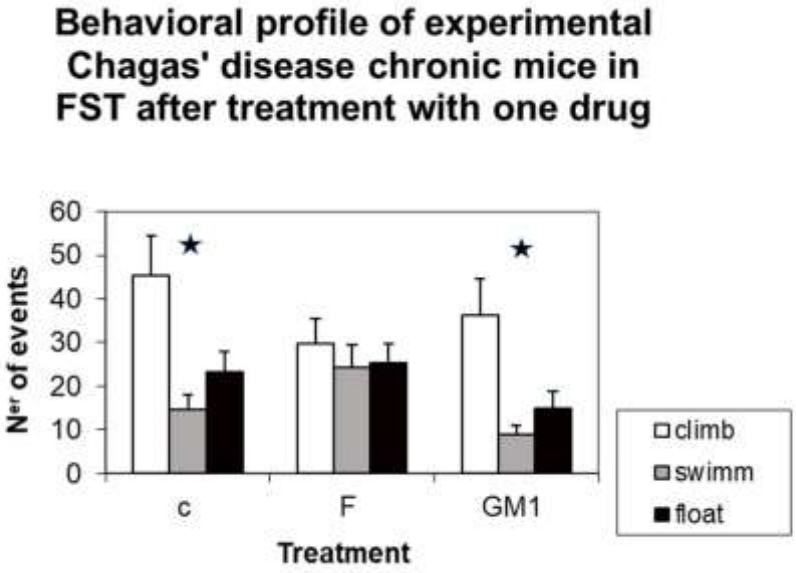

Fig 2 Behavioral profile of control (c) mice and mice with chronic experimental Chagas' disease in FST 120 days after treatment with oral fluoxetine (F) 0,08 mg /day and GM1 0,1 mg /day by intramuscular injection, both for 30 days. * p> 0.05

The question about combining fluoxetine and GM1 emerged spontaneously, so we repeated the experiments with another set of mice that were treated in the acute

phase with fluoxetine and then one dose of GM1 (3mg/Kg) or vice versa. Results are summarized in Fig. 3. GM1 improved F performance decreasing floating behavior with a moderate shift to climber profile. F after GM1 only decreased flotation and preserved the proportion among climbing and swimming.

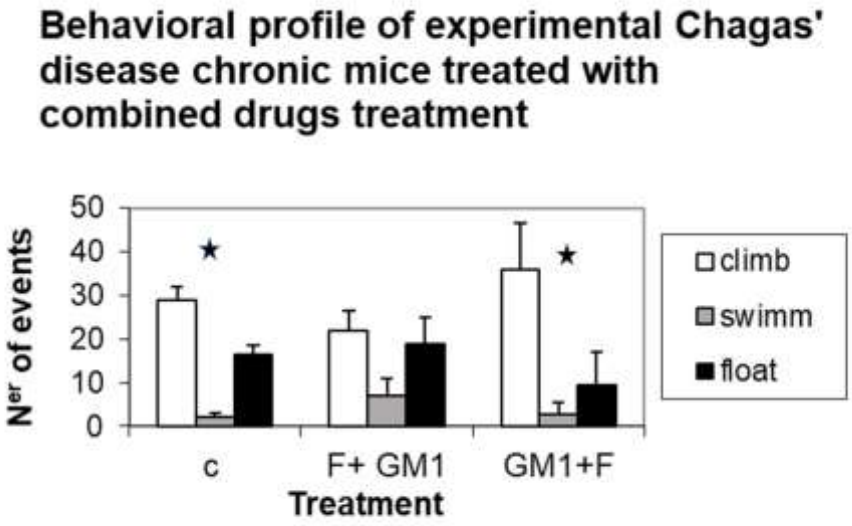

Fig. 3: Behavioral profile of control (c) mice and mice with chronic experimental Chagas' disease in FST 120 days after treatment with oral fluoxetine (F) 0,08 mg /day for 30 days plus one dose GM1 0,3 mg by intramuscular injection and GM1 0,1 mg /day by intramuscular injection for 30 days plus one dose (F) 0,08 mg. Bars represent average number of each type of behavior in FST. * $p > 0.05$

4. Discussion

The rise of antibodies production in GM1 treated mice helped to struggle the infection but those mice looked more active even than healthy uninfected matched mice. GM1 treatment of infected mice stimulated the TH2 immune response to infection with high titers of IgG. Previous studies show that IgE does not change during T. cruzi infection compared to healthy control [20]. In another paper we presented evidence for the influence of immunization on the performance of mice in Forced Swimming Test (FST). Three are the main outcomes of the test: number of times that each mouse climbed, swam, or floated to escape the test. The interpretation of results from a pharmacological scope is that climbing is a reactive profile determined by adrenergic activation, swimming a negotiating profile denotes serotonergic dominance, but depressed in relation to climbing and floating with depression or inactivation of adrenergic and serotonergic pathways. While IgE promoted an initial climber profile, IgG favored a swimmer profile [21]. It is important to note that in preliminary

experiments, sex differences regarding FST performance could be clearly appreciated, males are typically climbers and females tend to be swimmers. Thus, immunization coursing with IgG production seems to favor a kind of improvement with a feminization bias in behavior, a feature that is in good correlation with chagasic adrenergic hypotony. In this regard it should be taken in mind that catecholamines stimulate the production and release of testosterone in males through synergy with LH [22].

Our interpretation was that GM1 although promoting a Th2 response, presented an alternative pathway, since IgG anti-albumin was accompanied by a swimmer profile.

These experiments favored our hypothesis of GM1 stimulating immune response of Th2 type by a signaling pathway different from the one triggered by the antigen.

5. Conclusions

In the analysis of our previous and new results we could argument in favor of the hypothesis that GM1 set the heart of healthy and infected mice in a distinctive metabolic state that favored survival. Modulation of muscle energetic metabolism by exogenous ganglioside has been reported [23] and recently the importance of muscle metabolism in heart disease deserved growing attention [24]. Cardiomyocyte metabolism is also dependent on the activity of lymphoid and granulocytes in healthy state [25], thus the immune function of immune system cells, is surpassed by the metabolic regulation one. With regard to myocardiopathy, auto immune antibodies anti-β1 adrenergic receptors have a pivotal role but GM1 treatment seemed to drive the production of IgG to a protective rather than autoagressive participation. This assumption is supported by recovery of cardiac electrical parameters [12]. Cytokine specialized papers contributed considerable amount of evidence that points to IL-23 as the mediator of the onset of autoimmunity through hard stimulation of Th-17 cells [18,19]. The protective action of GM1 implies some disruption of this signaling pathway. Autoimmunity and inflammation are comorbidities of depression [26,27]. If GM1 disrupted that signal pathway, it would be expected that GM1 treated mice were more resistant to depression. When chronic experimental Chagas disease mice were subjected to FST, they clearly showed a reactive behavior. These results support our hypothesis that GM1 disrupted IL-23 pathway, and in that way, protected infected mice from autoimmunity and helped restore heart frequency and QT interval. We propose this interpretation because there is still need to perform experiments some action of GM1 treatment directly on IL-23 production or signaling.

6. Acknowledgements

This work was supported by Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECyT-UNC) and Fondo Nacional para la Ciencia y la Tecnología (FONCyT).

7. References

1. Tanowitz HB, Kirchhoff LV, Simon D, Morris SA, Weiss LM, Wittner M. Chagas' disease. Clin Microbiol Rev. **1992**; 5(4):400-19.

2. Jörg M, Storino R Consenso de la Enfermedad de Chagas. La enfermedad de Chagas en el siglo XXI. Consenso para una asignatura pendiente. Rev. Argent. Cardiol. 70 (Supl 1) 910

3. Mordini OD; Bavio E; Beloscar J; Tognoni G; Sosa F.J; Reyes O; Pairone E; Lacunza D; Manzur R; Redondo M; Hernández D; Mujica H, Olavegogeoescoechea P. Chagas Disease in Argentina. "National Registry of Chagas Disease of the ‗Federación Argentina de Cardiología'. RENECH study. Revista FAC, 2016, Sumario Vol. 45 (No 2) Abril-Junio.

4. Altcheh J, Moscatelli G, Moroni S, Garcia-Bournissen F, Freilij H. Adverse events after the use of benznidazole in infants and children with Chagas disease. Pediatrics 2011, 127: 212-218.

5. Altcheh J, Moscatelli G, Mastrantonio G, Moroni S, Giglio N, et al. Population pharmacokinetic study of benznidazole in pediatric Chagas disease suggests efficacy despite lower plasma concentrations than in adults. PLoS Negl Trop Dis 2014, 8: 1-9, e2907. doi: 10.1371/journal.pntd.0002907 PMID: 24853169

6. Sartor P, Colaianni I, Cardinal MV, Bua J, Freilij H, Gürtler RE. Improving access to Chagas disease diagnosis and etiologic treatment in remote rural communities of the Argentine Chaco through strengthened primary health care and broad social participation. PLoS Negl Trop Dis, 2017, 11(2): 1-18 e0005336. doi:10.1371/journal.

7. Bronia DH, Pereira BM, Luján H.D, Fretes RE, Fernández A, Paglini PA. Ganglioside treatment of acute Trypanosoma cruzi infection in mice promotes long-term survival and parasitological cure. Ann Trop Med Parasitol. 1999, 93(4):341-50.

8. Cossy Isasi S, Fernandez AR, Paglini P, Bronia DH. GM1 ganglioside induced myocardial restoration and survival of mice with experimental Chagas' disease. Acta Trop. 1999; 73(3):295-302.

9. Fernandez AR, Enders JE, Rivarola W, Paglini P, Palma JA.. Cardiac beta receptors' density or affinity modified by different Trypanosoma cruzi amount. Acta Physiol Pharmacol Ther Latinoam, 1996; 46(2):139-43

10. DaMatta RA, Seabra SH, Deolindo P, Arnholdt ACV, Manhäes L, Goldenberg S & de Souza W. Trypanosoma cruzi exposes phosphatidylserine as an evasion Mechanism. FEMS Microbiol Lett. 2007; 266: 29–33.

11. Szabo-Fresnais N, Lefebvre F, Germain A, Fischmeister R, Pomérance M. A new regulation of IL-6 production in adult cardiomyocytes by β-adrenergic and IL-1β receptors and induction of cellular hypertrophy by IL-6 trans-signalling. Cellular Signalling. 2010; 22:1143–1152.

12. Cossy Isasi S, Muiño JC. Considerations on the mechanisms involved on the benefits of GM1 treatment of mice with experimental Chagas´ disease. Internal Medicine Review. 2017; 3:1-19.

13. Goin JC, Borda E, Segovia A, Sterin-Borda L., Distribution of antibodies against beta-adrenoceptors in the course of human Trypanosoma cruzi infection.

Proc Soc Exp Biol Med., 1991; 197(2):186-92.

14. Jane/wit D, Altuntas CZ, Johnson JM, Yong S, Wickley PJ, Clark P, Wang Q, Popovic ZB, Penn MS, Damron DS, Perez DM, Tuohy VK. β1-Adrenergic Receptor Autoantibodies Mediate Dilated Cardiomyopathy by Agonistically Inducing Cardiomyocyte Apoptosis. Circulation. 2007; 116:399-410.

15. Medei EH, Nascimento JHM, Pedrosa RC, Barcellos L, Masuda MO, Sicouri S, Elizari MV, Campos de Carvalho AC. Antibodies with beta-adrenergic activity from chronic chagasic patients modulate the QT interval and M cell action potential duration. EP Europace, 2008;10 (7): 868–876.

16. Jahns R1, Boivin V, Lohse MJ. Beta 1-adrenergic receptor-directed autoimmunity as a cause of dilated cardiomyopathy in rats. Int J Cardiol. 2006;112(1):7-14.

17. Levin MJ & HOEBEKE J. Cross-talk between anti-β1-adrenoceptor antibodies in dilated cardiomyopathy and Chagas' heart disease. Autoimmunity, 2008; 41(6): 429–433

18. Floss DM, Schröder J, Franke M, Scheller J. Insights into IL-23 biology: From structure to function. Cytokine & Growth Factor Reviews. 2015; 26: 569–578.

19. Bloch Y, Bouchareychas L, Merceron R, Sk1adanowska K, Van den Bossche L, Detry S, Govindarajan S, Elewaut D, Haerynck F, Dullaers M, Adamopoulos IE, and Savvides SN. Structural Activation of Pro-inflammatory Human Cytokine IL-23 by Cognate IL-23 Receptor Enables Recruitment of the Shared Receptor IL-12Rb1. Immunity. 2018; 48: 45–58.

20.Geller M, Geller M, Flaherty DK, Black P and Capanema-Souza AP. Serum IgE levels in Chagas' disease. Clinical Allergy. 1978; 8: 383-385.

21. Cossy Isasi S, Jalil C, Giordano G, Ortiz J, Cosiansi JC and Muiño JC. Immunity and Sex Concerns on Behaviour From IgE vs IgG to sex conditioned socialization? Modern Advances in Pharmaceutical Research. 2018; 1:121-135.

22. Mayerhofer A, Bartke A, and Steger RW. Catecholamine Effects on Testicular Testosterone Production in the Gonadally Active and the Gonadally Regressed Adult Golden Hamster. BIOLOGY OF REPRODUCTION. 1989; 40:752-761.

23. Chan KF. Ganglioside-modulated protein phosphorylation in muscle. Activation of phosphorylase b kinase by gangliosides. J Biol Chem. 1989; 264 (31):18632-7.

24. Guertl B, Noehammer C, Hoefler G. Metabolic cardiomyopathies. Int J Exp Pathol. 2000; 81(6): 349-72.

25. Melo RC. Depletion of immune effector cells induces myocardial damage in the acute experimental Trypanosoma cruzi infection: ultrastructural study in rats. Tissue Cell. 1999;31(3):281-90.

26. Nadeema A, Ahmada SF, Al-Harbia NO, Fardana AS, El-Sherbeenyb AM, Ibrahimc KE, Attiaa SM. IL-17A causes depression-like symptoms via NFκB and p38MAPK signaling pathways in mice: Implications for psoriasis associated depression. Cytokine. 2017; 97:14–24.

27. Dinan TG. Inflammatory markers in depression. Current Opinion in Psychiatry 2008, 22:32–36.