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Pathogenesis of Chagas Cardiomyopathy: Role of Inflammation and Oxidative Stress

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Described by the Brazilian scientist Carlos Chagas more than a century ago, Chagas disease (ChD) currently affects 8 to 10 million persons and causes more than 10 000 deaths each year.[1] Originally confined to Latin American countries, ChD had been considered an exotic disease and received less attention from global health policy-makers and the scientific community than it could expect due to its high morbidity and mortality. Recently, the migration of infected persons to large urban cities and nonendemic countries changed the epidemiological profile of ChD from a disease of poor rural areas to a globalized problem of large cities in Latin America as well as most of the developed world.[1]

Although the recognition of the clinical and epidemiological importance of ChD has improved over the last decade, many gaps in our understanding of the pathogenesis of the disease hampered the development of efficacious treatments. ChD is a life-long infection by the protozoa *Trypanosoma cruzi*, which begins generally in young age and persists until the death of the individual, due to causes related or unrelated with the infection. After a usually mild febrile acute phase, most patients enter a chronic indeterminate form defined by the presence of infection by *T. cruzi* (confirmed by serologic or parasitological tests) and the absence of clinical manifestations, electrocardiographic, or radiological abnormalities of the heart and the digestive tract.[1] Infected persons can remain asymptomatic for decades or an entire lifetime, or evolve into a definite chronic form, with cardiac, digestive, or cardiodigestive complications.[2]

Chagas cardiomyopathy (ChCM) may eventually affect approximately 20% to 40% of chronically infected patients, manifesting as heart failure, arrhythmias, thromboembolism, stroke, and sudden death.[1] Each year, ≈2% of patients in the indeterminate form develop new electrocardiographic or echocardiographic abnormalities,[3] the earliest manifestations of ChCM.[4] Dilated cardiomyopathy is a late and severe manifestation of the chronic phase of ChD, with high mortality.[1,5] Sudden cardiac arrest, the most common mode of death in ChD, can interrupt the disease course at any time, even before the development of clinical symptoms, but is more frequent in those with more severe ChCM.[1]

A large number of longitudinal studies have identified markers of poor prognosis in ChD and clinical prediction parameters are now available to physicians that allow risk stratification of individual patients.[1] However, there is scarce information regarding the mechanisms leading to the development of the cardiopathy and, equally important, to the progression of early ChCM to more severe disease, eventually leading to death.[6]

In recent years, studies have suggested that the pathogenesis of ChD is complex and multifactorial, involving many interactive pathways.[7] Due to the scarcity of parasites in the myocardium of chronically infected patients, several theories were proposed to explain the development of ChCM such as inflammation, autoimmunity, dysautonomia, and microvascular damage.[7] In this context, the participation of eicosanoids, chemokines, cytokines, reactive oxygen species (ROS), and oxidative stress in the development of ChCM have been extensively investigated. An imbalance of both pro- and antiinflammatory factors has profound consequences in ChCM progression.

Experimental models of *T. cruzi* infection have provided critical information regarding mechanisms of cell activation, parasite control, and development of ChCM.[7,8] It is clear that both innate and acquired immune responses triggered during early infection are essential for controlling *T. cruzi* dissemination and host survival. The production of proinflammatory Th1 cytokines, such as IL-12, TNF-α, and IFN-γ, play an important role in parasite control[7,8] by inducing nitric oxide

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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J Am Heart Assoc. 2013;2:e000539 doi: 10.1161/JAHA.113.000539.

(NO) production.[7–9] Immunoregulatory cytokines such as TGF-β and IL-10 increase susceptibility to acute infection.[7] TNF-α, IFN-γ, TGF-β, and IL-10 also regulate chemokine production by *T. cruzi*-infected macrophages and cardiac myocytes. In turn, chemokines stimulate/enhance NO release consequently killing parasites,[7] and contributing to the pathogenesis of *T. cruzi* infection due to their effects on leukocyte migration and activation.[7,10] The circulating and tissue levels of TNF-α and IFN-γ are elevated in chagasic patients and considered to reflect the severity of heart failure. In fact, elevated plasma levels of TNF-α, presence of Th1 type cytokine profile (mainly IFN-γ), and suppression of Th2 type cytokines (mainly IL-4 and IL-10) are described in chronic patients.[8,11,12] The enhanced expression of cytokines and chemokines such as IL-6, TNF-α, IL-1β, CCL2, and CCL5 has also been noted in the myocardium of experimental models of infection. Gene expression profiling of myocardial tissue from chagasic experimental animals and humans revealed that 15% of genes known to be selectively up-regulated are IFN-γ-inducible, demonstrating the significant participation of IFN-γ and TNF-α in ChCM.[8]

Notably, the inflammatory milieu, mainly IFN-γ, TNF-α, and IL-1β, enhance mitochondrial ROS release by *T. cruzi*-infected cardiac myocytes, resulting in increased levels of ROS in the myocardium of chagasic individuals.[8]Dr Nisha Garg's group from The University of Texas Medical Branch demonstrate that oxidative stress in the myocardium induced by *T. cruzi* infection can be monitored by measurements of oxidative stress markers (malonylaldehyde, glutathione disulfide [GSSG], lipid hydroperoxides) and antioxidant defenses (superoxide dismutase, MnSOD, catalase) in the peripheral blood.[13]

ROS, including superoxide ($O_2^{\bullet-}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), are derivatives of molecular oxygen and its site and extent of production could determine the ultimate cell/tissue outcome. ROS can be formed in splenocytes, blood leukocytes, and vascular and heart tissue through the action of specific oxidases and oxygenases (eg, xanthine oxidase, NOX2), peroxidases (eg, myeloperoxidase, MPO), the Fenton reaction, and as by-products of the electron transport chain of mitochondria.[14] Pathways triggered by cytochrome P-450, lipooxygenase, and cyclooxygenase enzymes (through arachidonic acid metabolism) also contribute to enhanced ROS production.[15]

In experimental ChD, the 2 major ROS producers are NADPH oxidase (gp91phox, renamed NOX2) and mitochondria.[8] The first line of host defense against microbes is associated with rapid ROS production by the one-electron reduction of O_2 by NOX2 activity, known as "a respiratory burst". During *T. cruzi* infection, NOX2 was detected at the macrophage plasma membrane, resulting in activation and increased levels of $O_2^{\bullet-}$ formation, suggesting a NOX-dependent oxidative burst. In fact, ROS are also important regulators of parasite growth/dissemination by triggering the production of TNF-α and IL-1β by dendritic cells and macrophages, and act as a critical signaling intermediating/linking the innate and adaptive immune systems.

ROS production found in hearts in the acute phase of *T. cruzi* infection is primarily associated with infiltrating activated neutrophils and macrophages. In addition, mitochondrial release of electrons and superoxide production in infected cardiac myocytes are also demonstrated.[8] Dr Garg's group has also demonstrated that ROS of mitochondrial origin elicits cytokine gene expression in infected cardiac myocytes by activating NF-κB-dependent gene expression of TNF-α, IFN-γ, and IL-1β and by causing 8-hydroxyguanine lesions and DNA fragmentation in infected cardiac myocytes.[8] Thus, ROS hyperactivation could contribute to the development of ChCM inducing depletion of NAD^+, catalytic activation of inflammatory, and hypertrophic gene expression and cell death.[8]

During the acute phase of *T. cruzi* infection, it was demonstrated that up-regulation of glutathione peroxidase (GPx), glutathione reductase (GSR), and glutathione (GSH), the glutathione antioxidant defense contributes to host's ability to control oxidative stress. Nevertheless, in chronic experimental ChCM, a prooxidant profile was observed, including increased ROS levels, decreased activity of MnSOD, insensitivity of glutathione defense to oxidative stress, increased GSSG, and lipid and protein oxidation products.[8] Likewise, seropositive humans displayed a prooxidant profile, including increased GSSG and malondialdehyde (MDA) contents, decreased MnSOD, GPX activity, and GSH contents, and inhibition of CIII complex activity.[8] Treatment of *T. cruzi*-infected rodents with phenyl-α-tert-butyl nitrone, a spin-trapping antioxidant, improved cardiac hemodynamics, whereas treatment with benznidazole (antiparasitic) alone was not effective in improving cardiac function.[16,17] Studies also demonstrated that human chagasic patients treated with vitamin A demonstrated a decline in oxidative stress.[6] These studies suggest that an antioxidant depletion and inefficient scavenging of ROS could result in sustained oxidative stress followed by human chagasic cardiomyopathy progression.

In the current issue of *JAHA*, an important study by Dr Garg's group describes a potential new target molecule/pathway, which is active against *T. cruzi* experimental model in vivo.[18] The authors demonstrated that enhancing the mitochondrial ROS scavenging capacity is beneficial in controlling the inflammatory and oxidative pathology, and avoiding cardiac remodeling responses in chronic ChD. In this novel study, Dhiman et al have utilized MnSODtg mice overexpressing the MnSOD in the myocardium and GPx1 deficient mice (without a critical cellular antioxidant that

utilizes GSH to reduce H_2O_2 and lipid peroxides) in highlighting the role of ROS in ChCM.

Trypanosoma cruzi-infected wild-type (WT), $MnSOD^{tg}$, and $GPx1^{-/-}$ mice, irrespective of the antioxidant status, exhibited similar levels of blood and tissue parasite burden at chronic phase. The authors suggested that neutrophil (MPO) and macrophage (NOS_2/NO) activation contributed to chronic inflammatory state in WT-infected mice, and these responses along with tissue injury were minimal or absent in chronically infected MnSODtg mice. Interestingly, MnSOD overexpression resulted in: (1) reduced tissue infiltrate, (2) a balanced type 1/type 2 cytokine responses, (3) prevention of myocardial oxidative damage by enhancing mitochondrial capacity to scavenge ROS, and (4) partial control of disease-associated myofibrillar and mitochondrial losses, all in chronically infected mice. $MnSOD^{tg}$ mice with enhanced ability to scavenge cardiac mitochondrial ROS were capable of achieving a significant control of myocardial oxidative adducts, and subsequently able to preserve the mitochondrial and myofibrillar structure and arrangement, and prevent myocardial collagen deposition. The present study is the first that provides conclusive evidence that scavenging the mitochondrial ROS was beneficial in preventing oxidative damage and cardiac remodeling in ChD.

Dhirman et al also utilized P47 deficient mice (unable to mobilize NADP oxidase-mediated oxidative burst) and found that *T. cruzi* infection increased susceptibility as evidenced by higher level of blood and cardiac parasite burden, compared to that noted in WT-infected controls. The authors suggested that phagocytes' oxidative burst and inflammatory activation is required for parasite control and that mitochondrial oxidative stress was the main cause for cardiac remodeling in $p47^{phox-/-}$ mice.

In the past, Garg's group has suggested that ROS-PARP-1-RelA signaling pathway contributes to *T. cruzi*-induced inflammatory cytokine production. Herein, they validate these findings in an in vivo model and provide evidence for the mitochondrial ROS to serve as an activator of myocardial inflammatory responses during ChD.

Although the authors demonstrated that the increased activity of MnSOD in transgenic mice resulted in a reduction in myocardial remodeling and inflammatory infiltrates, hallmarks of ChD, there remain several issues to be resolved before concluding that increasing MnSOD levels would be beneficial in ChD. Besides the SylvioX10/4 strain, the authors have not examined the efficacy of MnSOD overexpression during infection with other *T. cruzi* strains and animal models. This is relevant because the development and severity of ChD was described as strain-specific and differs in different animal models. Not all strains of *T. cruzi* induce the same degree of cardiomyopathy which appears to be a consequence of the combination of (1) course of infection, (2) parasite strain, (3) metabolic and environment conditions, (4) host genetics and age, and (5) levels of inflammatory mediators.

Despite these concerns, this study opens a new avenue for therapeutic studies of compounds to target the MnSOD pathway, mainly modulating its expression, especially to be used in prevention of *T. cruzi*-induced cardiomyopathy. There is no treatment that definitiveely interferes with disease progression in ChD and chronic patients are still treated only by the symptomatic management of the clinical manifestations.

Sources of Funding

This study was partly supported by grants from Conselho Nacional do Desenvolvimento Cientőfico e Tecnológico (CNPq), Brasőlia, Brazil; and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Belo Horizonte, Brazil.

Disclosures

None.

References

1. Ribeiro AL, Nunes MP, Teixeira MM, Rocha MO. Diagnosis and management of Chagas disease and cardiomyopathy. *Nat Rev Cardiol*. 2012;9:576–589.
2. Nunes MC, Dones W, Morillo CA, Encina JJ, Ribeiro AL. Chagas disease: an overview of clinical and epidemiological aspects. *J Am Coll Cardiol*. 2013;62:767–776.
3. Sabino EC, Ribeiro AL, Salemi VM, Di Lorenzo OC, Antunes AP, Menezes MM, Ianni BM, Nastari L, Fernandes F, Patavino GM, Sachdev V, Capuani L, de Almeida-Neto C, Carrick DM, Wright D, Kavounis K, Goncalez TT, Carneiro-Proietti AB, Custer B, Busch MP, Murphy EL. Ten-year incidence of Chagas cardiomyopathy among asymptomatic *Trypanosoma cruzi*-seropositive former blood donors. *Circulation*. 2013;127:1105–1115.
4. Ribeiro AL, Sabino EC, Marcolino MS, Salemi VM, Ianni BM, Fernandes F, Nastari L, Antunes A, Menezes M, Oliveira CD, Sachdev V, Carrick DM, Busch MP, Murphy EL. Electrocardiographic abnormalities in *Trypanosoma cruzi* seropositive and seronegative former blood donors. *PLoS Negl Trop Dis*. 2013;7:e2078.
5. Nunes MD, Barbosa MM, Ribeiro AL, Amorim Fenelon LM, Rocha MO. Predictors of mortality in patients with dilated cardiomyopathy: relevance of Chagas disease as an etiological factor. *Rev Esp Cardiol*. 2010;63:788–797.
6. Nunes MC, Carmo AA, Rocha MO, Ribeiro AL. Mortality prediction in Chagas heart disease. *Expert Rev Cardiovasc Ther*. 2012;10:1173–1184.
7. Machado FS, Jelicks LA, Kirchhoff LV, Shirani J, Nagajyothi F, Mukherjee S, Nelson R, Coyle CM, Spray DC, de Carvalho AC, Guan F, Prado CM, Lisanti MP, Weiss LM, Montgomery SP, Tanowitz HB. Chagas heart disease: report on recent developments. *Cardiol Rev*. 2012;20:53–65.
8. Machado FS, Dutra WO, Esper L, Gollob KJ, Teixeira MM, Factor SM, Weiss LM, Nagajyothi F, Tanowitz HB, Garg NJ. Current understanding of immunity to *Trypanosoma cruzi* infection and pathogenesis of Chagas disease. *Semin Immunopathol*. 2012;34:753–770.
9. Machado FS, Martins GA, Aliberti JC, Mestriner FL, Cunha FQ, Silva JS. *Trypanosoma cruzi*-infected cardiomyocytes produce chemokines and cytokines that trigger potent nitric oxide-dependent trypanocidal activity. *Circulation*. 2000;102:3003–3008.
10. Machado FS, Koyama NS, Carregaro V, Ferreira BR, Milanezi CM, Teixeira MM, Rossi MA, Silva JS. CCR5 plays a critical role in the development of myocarditis and host protection in mice infected with *Trypanosoma cruzi*. *J Infect Dis*. 2005;191:627–636.
11. Cunha-Neto E, Teixeira PC, Fonseca SG, Bilate AM, Kalil J. Myocardial gene and protein expression profiles after autoimmune injury in Chagas' disease cardiomyopathy. *Autoimmun Rev*. 2011;10:163–165.

12. Higuchi ML, Benvenuti LA, Martins RM, Metzger M. Pathophysiology of the heart in Chagas' disease: current status and new developments. *Cardiovasc Res*. 2003;60:96–107.
13. Dhiman M, Coronado YA, Vallejo CK, Petersen JR, Ejilemele A, Nunez S, Zago MP, Spratt H, Garg NJ. Innate immune responses and antioxidant/oxidant imbalance are major determinants of human Chagas disease. *PLoS Negl Trop Dis*. 2013;7:e2364.
14. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003;552:335–344.
15. Cohen G. Enzymatic/nonenzymatic sources of oxyradicals and regulation of antioxidant defenses. *Ann N Y Acad Sci*. 1994;738:8–14.
16. Wen JJ, Bhatia V, Popov VL, Garg NJ. Phenyl-alpha-tert-butyl nitrone reverses mitochondrial decay in acute Chagas' disease. *Am J Pathol*. 2006;169:1953–1964.
17. Wen JJ, Gupta S, Guan Z, Dhiman M, Condon D, Lui C, Garg NJ. Phenyl-alpha-tert-butyl-nitrone and benzonidazole treatment controlled the mitochondrial oxidative stress and evolution of cardiomyopathy in chronic Chagasic rats. *J Am Coll Cardiol*. 2010;55:2499–2508.
18. Dhiman M, Wan X, Popov VL, Vargas G, Garg NJ. MnSODtg mice control myocardial inflammatory and oxidative stress and remodeling responses elicited in chronic Chagas disease. *J Am Heart Assoc*. 2013;2:e000302 doi:10.1161/JAHA.113.000302.

Key Words: Editorials • cardiomyopathy • Chagas disease • inflammation • oxidative stress • reactive oxygen species