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PARASITOLOGY (N KUMAR, SECTION EDITOR)

Pathogenesis of Chronic Chagas Disease: Macrophages, Mitochondria, and Oxidative Stress

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Abstract

Purpose of Review *Trypanosoma cruzi* is the causative agent of Chagas disease. Decades after initial infection, ~30% of individuals can develop chronic chagasic cardiomyopathy. There are several proposed mechanisms for pathogenesis of Chagas disease, including parasite persistence, immune responses against parasite or self that continue in the heart, vascular compromise, and involvement of autonomic and central nervous system. Herein, we will focus on the significance of macrophages, mitochondrial dysfunction, and oxidative stress in progression of chagasic cardiomyopathy.

Recent Findings The current literature suggests that *T. cruzi* prevents cytotoxic activities of the innate immune cells and persists in the host, contributing to mitochondrial oxidative stress. We discuss how the neoantigens generated due to cellular oxidative damage contribute to chronic inflammatory stress in chagasic disease.

Summary We propose that metabolic regulators, PARP-1/SIRT1, determine the disease outcome by modulating the mitochondrial and macrophage stress and antioxidant/oxidant imbalance and offer a potential new therapy against chronic Chagas disease.

Keywords *Trypanosoma cruzi* · Reactive oxygen species · Mitochondrial dysfunction · Innate immunity · Oxidative stress · Chagas disease

Introduction

Chagas disease, or American trypanosomiasis, is a zoonotic disease caused by infection with the parasite, *Trypanosoma cruzi*. It is endemic in Mexico, Central, and South America, where transmission of *T. cruzi* is maintained by insect vectors (triatomines) and domestic and wild mammals that serve as reservoirs [1]. Other routes of transmission of *T. cruzi* include blood transfusion or transplantation of organs from infected donors, maternal-fetal transmission, and acquisition of infection via the oral route [2]. Due to increased immigration, individuals with Chagas disease have been identified in the USA, Canada, Europe, Australia, and Japan [3]. It is estimated that 300,000 persons living in the USA are chronically infected with *T. cruzi* [4]. In recent years, vectorial transmission of *T. cruzi* and autochthonous cases of Chagas disease has also been reported in the southern states of the USA [5].

The clinical course of Chagas disease is divided into the acute and chronic phases. The acute infection is usually mildly symptomatic and often misdiagnosed as febrile illness of childhood. Approximately 1% of the acutely infected persons manifest lymphadenopathy, hepatosplenomegaly, myocarditis, pericardial effusion, and heart failure or meningoencephalitis. Parasitemia is evident and lasts for 2 to 4 months, and then infected individuals evolve into a chronic phase. While many remain in an indeterminate phase without any clinical symptoms, but having a positive serology, approximately 30% of the infected individuals progress into clinically relevant Chagas disease. Chronic cardiomyopathy is the most important clinical manifestation of Chagas disease because of its frequency, severity, and effects on morbidity and mortality. It is a complex disease that includes a wide spectrum of

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manifestations, ranging from minor myocardium involvement to left ventricular systolic dysfunction, dilated cardiomyopathy, arrhythmias, thromboembolic events, and terminal cardiac failure [6••]. Gastrointestinal (GI) manifestations, such as mega-syndromes involving tubular structures of the GI tract, though not commonly recorded, are frequent in certain geographic areas [7].

The virtual absence of parasites in the heart of chronically infected individuals has stimulated a discussion in the literature regarding the etiology of chronic Chagas disease. Different strains of the parasite have been associated with distinct clinical outcomes of infection in experimental models [8] and human disease [9]. Host factors, such as genetic background of the host, role of B and T cell immunity in control of *T. cruzi* and pathogenesis of chronic disease, autoimmunity, vascular compromise, and involvement of autonomic and central nervous system have also been associated with distinct clinical outcomes of Chagas disease and are discussed elsewhere. Herein, we briefly discuss the role of macrophages, mitochondrial dysfunction, and oxidative stress in *T. cruzi* infection and chronic Chagas disease.

Innate Immunity against *T. cruzi* Infection

The significance of innate immune responses to *T. cruzi* infection has primarily been studied by using experimental models and has provided important information regarding mechanisms of parasite control and disease processes.

Cytokine and Chemokine Response

As universal cells of innate immunity, the epithelium, macrophages, dendritic cells, and NK cells deserve attention in Chagas disease. Parasites enter their host through a skin lesion. *T. cruzi* infection of epithelial cells have been shown to enhance the expression of proinflammatory genes associated with toll-like receptor (TLR) pathway and TNF-α and TGF-β signaling pathways. The frequently upregulated chemokines in infected epithelial cells, e.g., CXCL1, CXCL2, CXCL3, CCL8, CCL20, and IL8, participate in the recruitment of professional phagocytic cells [10].

The interaction of *T. cruzi* with macrophages and other innate immune cells induces a substantial increase in the expression and secretion of proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) at a level similar to that seen in IFN-γ/LPS-induced proinflammatory macrophages ([11] and references therein). Toll-like receptors recognize the pathogen-associated molecular patterns and transmit a signal via cytoplasmic Toll/IL-1R domains for the recruitment of cytosolic adaptor molecules, including myeloid differentiation primary-response protein 88 (MyD88), and subsequently induce nuclear factor-κB (NF-κB) activation, leading to the production of inflammatory cytokines and linking the innate to the adaptive immune responses [12, 13]. *T. cruzi*-derived glycosylphosphatidylinositol (GPIs) and GPI-anchored mucin-like glycoproteins have been demonstrated to stimulate the synthesis of IL-12, TNF-α, and nitric oxide (NO) by innate immune cells [14•]. The mucin-linked GPI anchors induced TLR2-dependent leukocyte recruitment via CCL2 [15]. The parasite expresses cruzipain, a kinin-releasing cysteine protease, which induces dendritic cell maturation via the activation of bradykinin (BK) B_2 receptors (B_2R) [16, 17]. TLR2 activation by *T. cruzi* also signals dendritic cell-driven mechanisms that stimulate Th1 responses via the cruzipain/kinin/B_2R pathway [18]. TLR4 and TLR9 likely recognize parasite-derived GIPLs and DNA, respectively, and cooperate in the activation of host innate immune response against *T. cruzi* infection [19]. $TLR2^{-/-}$, $TLR9^{-/-}$, and $MyD88^{-/-}$ mice exhibited increased susceptibility to *T. cruzi* infection, and macrophages of these mice were defective in eliciting proinflammatory response [20•, 21•], thus, suggesting that TLR2 and TLR9 are the major TLRs involved in *T. cruzi* recognition by innate immune cells. In addition to MyD88, TRIF-dependent induction of type 1 IFNs (especially IFN-β) has also been documented to contribute to resistance to *T. cruzi* infection in dendritic cells and macrophages [22]. The $Myd88^{-/-}Trif^{-/-}$ mice exhibited TLR-independent, NFATc1-dependent Th1 responses and dendritic cell maturation after *T. cruzi* infection [23], thus suggesting that NFATc1 complements TLR-dependent innate immune responses in *T. cruzi* infection.

T. cruzi-infected macrophages produce IL-12, a key mediator of IFN-γ production through activation of NK cells and induction of Th1 cell development. IFN-γ is required to activate the macrophage expression and activation of inducible nitric oxide synthase (iNOS) and NADPH oxidase (NOX2) and production of nitric oxide (NO) and reactive oxygen species (ROS), respectively [24••]. TNF-α may also provide a second signal stimulating NO/ROS production in IFN-γ-activated macrophages, as well as in infected cardiac myocytes [25, 26], and, thus, enhance the trypanocidal function. In the absence of IFN-γ, mouse and human macrophages produced insufficient amounts of ROS and NO and failed to clear the parasite [11]. Others have shown the splenic enrichment of $Ly6C^+$ dendritic cells—like inflammatory cells that produced TNF-α and NO to kill parasite but also produced IL-10 that negatively affected the development of anti-parasite T cell response in infected mice [27]. Ponce et al. [28] suggested that a transient increase in CD39/CD73 enzyme pair (hydrolyzes extracellular adenosine) attenuated the inflammatory macrophage response to *T. cruzi*, and inhibition of CD73 was beneficial in establishing the proinflammatory macrophages' predominance and reduce parasite load in the myocardium of acutely infected mice.

Nucleotide-binding oligomerization domain-like receptors (NLRs) are characterized by the presence of a central NACHT domain and a C-terminal leucine-rich repeats (LRRs) domain of variable length (20–29 amino acids). The N-terminal effector binding region consists of a protein-to-protein interaction domain, i.e., pyrin domain (PYD), a caspase recruitment domain (CARD), and a baculovirus inhibitor of an apoptosis protein repeat (BIR) domain, and based on this, NLRs are classified as NLRP, NLRC, and NAIP, respectively. The multimeric protein macromolecules formed by NLRs are named inflammasomes [29]. The most studied NLRP1 and NLRP3 inflammasomes recruit ASC (apoptosis-associated, speck-like protein containing a CARD domain) and caspase-1 proteins. The ASC-dependent activation of caspase-1 is essential for the cleavage of pro-IL-1β and pro-IL-18 into their functional form and initiation of the inflammatory cytokine response [30]. Recent studies showed that a deficiency of caspase-1/ASC and NLRC1 (also called NOD1) inflammasomes attenuated the activation of IL-1β/ROS and NF-κB-dependent cytokine gene expression for *T. cruzi* control in human and mouse macrophages [31–33]. However, NLRP3-mediated IL-1β/NF-κB activation was dispensable because NLRP3$^{-/-}$ macrophages produced high amount of ROS that provided efficient control of *T. cruzi* replication and survival in macrophages [33].

The cytokines synthesized during *T. cruzi* infection are capable of inducing or regulating the production of chemokines in macrophages and cardiac myocytes both in vitro and in vivo. The enhanced expression and release of chemokines and their receptors affected T cell proliferation, Th1/Th2 differentiation, and resistance to infection in mice [25, 34]. The chemokine receptors, CCR5 and CXC3, are immunological preferential markers of Th1 response, and CCR3 and CCR4 are preferentially associated with Th2 response [35]. CCR5 recognizes CCL3, CXCL10, and CCL5 chemokines. Chagasic patients with a point mutation in CCR5 promoter resulting in low levels of CCR5 expression in leukocytes exhibited attenuated heart disease [36]. Treatment of mice with CCR5 antagonist (Met-RANTES) decreased the tissue infiltration of CD4$^+$ or CD8$^+$ T cells and chronic myocarditis [37]; however, CCR5-deficient mice were susceptible to acute infection [38]. These results indicated an important role for CCR5 in the control of acute infection as well as in chronic immunopathology (reviewed in [39]).

In addition to innate immune cells, endothelial cells, cardiac myocytes, and vascular smooth muscle cells (VSMCs) can also sense and respond to pathogens (or PAMPs) [40–43]. *T. cruzi* induced the expression and release of IL-1β, TNF-α, and IL-6 in endothelial cells and cardiac myocytes [41, 44]. Trans-sialidase, a released surface protein of *T. cruzi*, induced IL-6 production in isolated endothelial cells [45]. VSMCs exhibited increased proliferation and upregulation of ERK-cyclin D1-endothelin-1 pathway in response to *T. cruzi* infection [46]. Others have shown that TGF-β plays a role in parasite invasion. Treatment of cardiac myocytes with SB-431542, which inhibits the TGF-β type 1 receptor, impaired the parasite invasion and replication and prevented heart damage in acute Chagas disease [47]. Thus, depending on the cell type, cytokine and signaling responses by the non-immune cells serve as a component of innate defense, a bystander effect to *T. cruzi* infection, or a mechanism exploited by parasite for invasion of a variety of cells.

Lipid Mediators

The precursor arachidonic acid (AA) is metabolized by a series of enzymes into a variety of biologically and clinical relevant eicosanoids and their metabolites. AA is metabolized by 5-lipoxygenase (5-LO) enzyme for the synthesis of leukotrienes (LTA4, LTB4, LTC4, LTD4, LTE4). 5-LO is primarily expressed in macrophages, granulocytes, and mast and dendritic cells. Leukotrienes are produced during experimental *T. cruzi* infection, by tissue-resident and recruited leukocytes, and LTB4 synthesis activates intracellular killing of *T. cruzi* in macrophages [47]. 5-LO deficiency significantly increases acute parasitemia; however, 5-LO knockout mice were still able to control tissue parasites and exhibited decreased mortality and cardiac damage [48, 49]. The absence of LXA4 in 5-LO null mice modulated the expression of suppressor of cytokine signaling (SOCS2) in spleen and heart of infected mice. The SOCS2 deficiency enhanced the number of T regulatory cells and decreased the levels of proinflammatory cytokines; however, SOCS2-deficient macrophages were hyper-responsive to IFN-γ, produced increased levels of NO, and dealt with infection efficiently [50].

The cyclooxygenases COX-1/COX-2 convert AA to prostaglandin H2 (PGH2) that is further metabolized by thromboxane A_2 (TXA_2) synthase into TXA_2. It has been demonstrated that the parasite also has a synthase capable of producing TXA_2. The Tanowitz and Ashton laboratories found that *T. cruzi* likely utilizes host-derived PGH2 to produce TXA_2 and that *T. cruzi*-derived TXA_2 is the major source of TXA_2 detected in serum of infected mice [48, 51]. TXA_2-regulated vasospasm, thrombosis, vascular permeability, and endothelial cell dysfunction are observed in acute infection [44]. Interestingly, TXA_2 receptor knockout mice displayed increased mortality, tissue parasitism, and myocardial inflammation upon infection [51] leading to suggestions that autocrine/paracrine TXA_2 receptor activation provides a quorum sensor that regulates intracellular amastigote proliferation, providing opportunities to survive from infection. TXA_2 and its receptor contribute to innate immunity by virtue of the fact that it mobilizes inflammatory cells and results in the release of proinflammatory cytokines. Furthermore, activation of TXA_2 receptors on naïve T cells enhances chemokinesis, prevents adhesion of antigen presenting cells such as dendritic cells, and inhibits T cell proliferation to negatively modulate acquired immunity [49]. As such, TXA_2

release by the parasite likely prevents the full development of host immunity, choosing short-term over long-term responses, and may contribute to the transition to the chronic state and the persistence of the infection.

Reactive Oxygen Species

In addition to cytokines/chemokines, activated macrophages exert cytotoxic effects against microbes by production of reactive oxygen (ROS) and nitrogen species. NADPH oxidase (NOX2), a multimeric complex, utilizes NADPH as substrate and reduces O_2 to produce superoxide ($O_2^{\cdot -}$) that is then further dismutated into stable and diffusible H_2O_2 pro-oxidant. The plasma membrane-associated components gp91^{phox} and p22^{phox} together form flavocytochrome-b558 that is responsible for enzymatic stability and activity of the NADPH oxidase. Phosphorylation of cytosolic components (p47^{phox}, p67^{phox}, and p40^{phox}), and small Rho GTPases, in response to exogenous or endogenous stimuli initiates their translocation to the cell membrane, and NOX2 activation [50]. *T. cruzi*-generated stimuli that initiate translocation of cytosolic factors and NOX2 assembly in infected macrophages are not identified. However, cytochemical detection of NOX2 components at the plasma membrane of peritoneal mouse macrophages exposed to *T. cruzi* is noted [51, 52]. Others have used an in vitro assay system or animal models and shown that NOX2-dependent $O_2^{\bullet -}$ formation is required for parasite control in macrophages and splenocytes [53, 54]. In addition to direct killing, NOX2/ROS also signaled the development of antigen-specific CD8$^+$ T cell response that was required for control of tissue parasites in infected mice [55].

Inducible nitric oxide synthase (iNOS or NOS2) is induced by immunological stimuli in a Ca^{+2}-dependent manner, and it utilizes L-arginine and O_2 for the synthesis of L-citrulline and nitric oxide (NO) in a complex oxidoreductase reaction [56]. NF-κB and ISGF3 transcription factors act sequentially and cooperatively at the Nos2 promoter to signal iNOS expression and NO production in macrophages. The reaction of NO with $O_2^{\cdot -}$ produces peroxynitrite that is a strong cytotoxic oxidant shown to promote killing of *T. cruzi* in macrophages [57, 58]. However, the extent of NOX2-dependent ROS response and iNOS-dependent NO response in human and mouse macrophages infected with *T. cruzi* is significantly lower than that observed in LPS/IFN-γ-induced proinflammatory macrophages [11], suggesting a potential mechanism for survival and dissemination of parasite by macrophages.

Notably, trypanosomes have evolved an elaborate antioxidant system to prevent ROS/NO-mediated killing. Trypanosome antioxidant defense utilizes trypanothione ($T(SH)_2$) that shuttles the reducing equivalents to peroxidases through tryparedoxin intermediate [59]. Of the five tryparedoxin peroxidases (TXNPxs) identified in *T. cruzi*, cytosolic and mitochondrial TXNPxs were shown to increase during differentiation from the non-infective to the infective forms of the parasite and were found to be present at higher levels in the virulent isolates compared with the attenuated strains [59, 60•]. Parasite isolates overexpressing cytosolic and mitochondrial TXNPxs were able to infect and multiply more efficiently in macrophages, thus suggesting that TXNPxs provide at least one mechanism to provide survival benefits to parasite in macrophages and other cells [58, 61]. Importantly, trypanothione synthase is unique to parasites and rated as the most promising target to achieve selective inhibition of parasite [62].

At low levels, ROS are critical signaling intermediates involved in NF-κB-dependent expression of proinflammatory cytokines (e.g., TNF-α, IL-1β) by macrophages and dendritic cells (DCs). Low levels of ROS are produced during *T. cruzi* infection, and if scavenged, resulted in inhibition of inflammatory cytokines' production in macrophages. The in vitro observations were confirmed by studies in mice. Garg and group initially utilized chemical antagonists of NOX2 and ROS scavenging antioxidants to demonstrate that blocking NOX2/ROS arrested the activation and proliferation of splenic phagocytes and production of inflammatory cytokines (e.g., IL-1, IL-6, IFN-γ, TNF-α) in infected mice [53]. These findings were also confirmed in p47phox$^{-/-}$ mice that also exhibited increased susceptibility to *T. cruzi* and succumbed to infection [55]. Whether *T. cruzi*-induced NOX2/ROS in macrophages signal the nuclear transport or assembly of transcription factors (e.g., NF-κB and AP-1) for promoting cytokine gene expression is not fully delineated. However, NF-κB activation has been described in a number of other cell types, including epithelial cells, endothelial cells, myocytes, and fibroblasts infected with *T. cruzi* (or *T. cruzi*-derived proteins, e.g. trans-sialidase) [63–66, 67••]. NF-κB activation increased the resistance to infection in many of these cell types. A majority of these studies, however, did not attempt to determine the source of ROS and its role in signaling NF-κB-dependent cytokine gene expression in non-phagocytic cells invaded by *T. cruzi*.

Interestingly, in cardiac myocytes, *T. cruzi* signals ROS production through mitochondria. It was found that *T. cruzi* invasion of cardiac myocytes disturbed the mitochondrial membrane potential and enhanced the release of electrons to O_2 resulting in $O_2^{\cdot -}$ production at the complex I and complex III of the respiratory chain [68]. The *T. cruzi*-induced mitochondrial ROS (mtROS), like NOX2, induced ROS in macrophages and signaled nuclear translocation of Rel A (p65) and activation of NF-κB-dependent cytokine gene expression in infected cardiac myocytes [41, 68]. The mtROS also provided secondary signal for cytokine gene expression; Garg and coworkers showed that ROS-induced DNA damage (e.g., 8-hydroxyguanine (8-oxoG) lesions) enhanced the expression and activation of a DNA repair enzyme polyadenosine ribose polymerase 1 (PARP-1) in infected cardiac myocytes. PARP-1 functions by poly ADP-ribosylation of nuclear proteins [69]. However, in the context of *T. cruzi* infection, PARP-1 had pathophysiological effects. This was evidenced by the observation that inhibition of PARP-1 by using RNAi or a chemical inhibitor (PJ34) was beneficial in blocking mtROS formation, DNA damage, and cytokine gene expression [41]. How PARP-1/PAR contribute to mitochondrial disturbance is not known, though PARP-1's role in regulating cytokine gene expression in infected cardiac myocytes was described. PARP-1 does not directly interact with p65, and it does not signal RelA (p65) translocation to nuclei in infected cardiac myocytes. Instead, PARP-1 contributes to PAR modification of RelA (p65)-interacting nuclear proteins and assembly of an NF-κB transcription complex. Sirtuin 1, a highly conserved member of the family of NAD^+-dependent Sir2 histone deacetylases, competes with PARP-1 for NAD^+ substrate and integrates mitochondrial metabolism and inflammation. In a recent study, Wan et al. demonstrated that treatment with SIRT1 agonist (SRT1720) had no effect on parasite burden, but it suppressed the NF-κB transcriptional activity and reduced the oxidative and inflammatory stress in infected cells and mice [67••]. These studies point to the possibility that the ROS-PARP-1/PAR-RelA contribute to inflammatory pathology in *T. cruzi* infection, and it can be controlled by enhancing the SIRT1 activity.

Macrophages and Chronic Inflammation

The New York Heart Association (NYHA) functional classification of heart failure places patients in one of the four categories according to the severity of their symptoms. Chagasic patients with NYHA class III-IV display a proinflammatory transcriptomic and proteomic profile in peripheral blood mononuclear cells [70–73] and increased levels of TNFα^+ monocytes [74–76] in the circulation. In comparison, $IL10^+$ monocytes with an anti-inflammatory transcriptome were detected in peripheral blood of infected humans classified in NYHA class I-II with none-to-minimal left ventricular dysfunction [74–76]. The factors that drive macrophage phenotype in chronic infection are not described; however, these observations suggest that proinflammatory (vs. pro-healing) response of macrophages is a contributing factor in clinical evolution of Chagas disease.

Cells release diverse types of membrane vesicles into extracellular environment. These extracellular vesicles (EVs) selectively sort the biological information and carry out an important mode of intercellular communication in health and disease [77]. Recently, Garg and colleagues demonstrated that plasma EVs of NYHA class III-IV (vs. NYHA class I-II) patients elicited proinflammatory macrophage responses with upregulation of $CD14^+/CD16^+$ surface markers and inflammatory gene expression profile and cytokine release (IL-2 + IFN-γ > GCSF) [78••]. Similarly, sera components or plasma EVs of chronically infected (vs. control) mice elicited a strong ROS/NO response and proinflammatory cytokine profile in murine macrophages. In comparison, mice given a *T. cruzi* vaccine followed by a *T. cruzi* challenge elicited an M2-like macrophage phenotype [79]. Compositional analysis revealed that EVs of chronically infected mice and patients were composed of membrane vesicles of cardiac myocyte, macrophage, and leukocyte origin [78••]. In another study, Dhiman et al. demonstrated that cardiac proteins oxidized during *T. cruzi* infection serve as antigens, and treatment of infected rodents with phenyl-α-tert-butyl nitrone (antioxidant) resulted in normalized immune detection of cardiac proteins associated with control of cardiac pathology and preservation of heart contractile function in chagasic rats [80]. Though molecular markers on EVs are yet to be identified, these results strongly suggest that peripheral EVs consisting oxidized proteins of the cardiac and other cellular origin contribute to inflammatory state of macrophages in the setting of Chagas disease. Further studies may delineate the extracellular and intracellular immune receptors engaged by EVs in signaling inflammatory macrophages and further evaluate the potential benefits of PARP-1/SIRT1 balance in establishing resting homeostasis in peripheral and tissue macrophages in Chagas disease.

Mitochondrial Dysfunction and Oxidative Stress in Chronic Chagas Disease

Garg and colleagues were the first to report that *T. cruzi* invasion elicits Ca^{+2} overload, mitochondrial membrane potential transition [41, 68], and O_2•− production in cardiac myocytes [81, 82]. In vivo studies showed that mitochondrial defects continue beyond the acute infection phase with consistently high levels of mtROS and oxidative adducts (e.g., protein carbonyls, lipid hydroperoxides) and a decline in oxidative phosphorylation capacity in the myocardium of chronically infected mice [81, 83]. A similar pro-oxidant milieu evidenced by a decline in the activities of the respiratory complex III and antioxidant enzymes (MnSOD and GPX) as well as in GSH contents and an increase in oxidative adducts has been reported in humans chronically infected with *T. cruzi* [84–87]. Moreover, treatment of *T. cruzi*-infected mice and rats with phenyl-α-tert-butyl nitrone, a spin-trapping antioxidant, tipped the balance in favor of preserving mitochondrial and left ventricular function associated with a significant decline in the myocardial oxidative adducts [86, 88]. Likewise, treatment with sildenafil, an inhibitor of phosphodiesterase 5, provided cardioprotection through preservation of cGMP/PKG activity and antioxidant/oxidant balance in chronically infected mice [89]. Others have shown a decline in oxidative stress in human chagasic patients given vitamin A [90]. Finally, Garg and colleagues demonstrated a significant control of myocardial oxidative adducts, preservation of mitochondrial and myofibrillar structure and arrangement, and improved mitochondrial and left ventricular function in MnSOD^{tg} mice equipped with an extra copy of MnSOD to scavenge cardiac mtROS [91••], thus conclusively establishing the pathological significance of mtROS and chronic oxidative stress in Chagas disease.

The re-expression of fetal cardiac genes (ANP, BNP, αsk-Actin, and β-MHC) is a hallmark of hypertrophic remodeling. Recent data suggest the involvement of ERK-1/2, small GTPase Ras, and NF-κB/ASK-1 in response to α-adrenergic agonist or angiotensin II stimulation in signaling cardiac remodeling [92]. Mice and cultured myoblasts infected with *T. cruzi* display increased expression of ERK, cyclin D1, and AP-1 whereas the expression of caveolins (Cavs), which negatively regulate ERK and cyclin D1, was decreased [93–96]. In that regard, Cav-1 and Cav-3 null mice as well as Cav-1/Cav-3 double-knockout mice display cardiac hypertrophy and interstitial fibrosis [97–99]. These observations, along with the finding that scavenging of ROS suppressed the hypertrophic gene expression and collagen content in chagasic mice, imply that ROS contributes to cardiac remodeling in chagasic disease. Further, the mtROS was found to be of pathological significance. The authors noted that treatment with benznidazole (anti-parasite drug) suppressed the classical mediators of inflammatory ROS (e.g., NOX2 and myeloperoxidase) but not the hypertrophic response in chagasic rodents, while the hypertrophic phenotype was depressed when mice or rats were treated with an antioxidant as well as in MnSOD^{tg} mice with enhanced mitochondrial antioxidant capacity [88, 91••].

Why antioxidant response is not triggered in the presence of continued oxidative stress in the chagasic myocardium was not understood until recently. NFE2L2 (also called Nrf2) is a transcription factor that regulates the expression of antioxidant proteins. Several drugs that stimulate the NFE2L2 pathway are being evaluated for treatment of diseases that are caused by oxidative stress. Garg and colleagues showed that NFE2L2 expression, nuclear translocation, and binding to cis-acting DNA regulatory antioxidant response elements (AREs) were significantly decreased and associated with a decline in antioxidants' (e.g. γGCS, HO1, GCLM) expression in cardiac myocytes and myocardium of mice infected with *T. cruzi*. Importantly, inhibiting the *T. cruzi*-induced mtROS by overexpression of MnSOD in cardiac myocytes preserved the NFE2L2 transcriptional activity and antioxidant/oxidant balance, and MnSOD^{tg} mice also preserved the cardiac structure and function [91••]. This study provides evidence that mtROS inhibition of NFE2L2/ARE pathway constitutes a key mechanism in signaling the fibrotic gene expression and evolution of chronic chagasic cardiomyopathy.

Summary and Future Perspectives

The Benznidazole Evaluation for Interrupting Trypanosomiasis (BENEFIT) trial was designed to evaluate the efficacy and safety of benznidazole in reducing the clinical outcomes among patients with established chagasic cardiomyopathy [100••]. Unfortunately, benznidazole treatment reduced the serum parasite detection but did not significantly reduce cardiac clinical deterioration through 5 years of follow-up. These disappointing results indicate that anti-parasite drugs are not effective in chronic Chagas disease, and new strategies are required in the treatment of this disease.

The current literature suggest that the inability of macrophages to elicit strong ROS/NO response, coupled with parasites' ability to scavenge oxidants, contribute to long-term parasite persistence and mitochondrial oxidative stress in the heart. The cellular oxidative damage provides stimulus to macrophage activation and chronic inflammatory stress in chagasic disease. We propose that metabolic regulators, PARP-1/SIRT1, determine the disease outcome by modulating the mitochondrial and macrophage stress and antioxidant/oxidant imbalance and offer a potential new therapy against chronic Chagas disease.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. World Health Organization (2014) Chagas disease (American Trypanosomiasis), Technical, UNDP/World Bank/WHO. http://www.who.int/mediacentre/factsheets/fs340/en/index.html.
2. Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. Clin Infect Dis. 2012;54(6):845–52. https://doi.org/10.1093/cid/cir956.
3. Tanowitz HB, Weiss LM, Montgomery SP. Chagas disease has now gone global. PLoS Negl Trop Dis. 2011;5(4):e1136. https://doi.org/10.1371/journal.pntd.0001136.
4. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis. 2009;49(5):e52–4. https://doi.org/10.1086/605091.

5. Garcia MN, Aguilar D, Gorchakov R, Rossmann SN, Montgomery SP, Rivera H, et al. Evidence of autochthonous Chagas disease in southeastern Texas. Am J Trop Med Hyg. 2015;92(2):325–30. https://doi.org/10.4269/ajtmh.14-0238.
6.•• Tanowitz HB, Machado FS, Spray DC, Friedman JM, Weiss OS, Lora J, et al. Developments in the management of Chagasic cardiomyopathy. Expert Rev Cardiovasc Ther. 2016;13:1393–409. **This study provides a thorough review of treatment of Chagas disease**
7. Stanaway JD, Roth G. The burden of Chagas disease: estimates and challenges. Glob Heart. 2015;10(3):139–44. https://doi.org/10.1016/j.gheart.2015.06.001.
8. dos Santos DM, Talvani A, Guedes PM, Machado-Coelho GL, de Lana M, Bahia MT. *Trypanosoma cruzi*: genetic diversity influences the profile of immunoglobulins during experimental infection. Exp Parasitol. 2009;121(1):8–14. https://doi.org/10.1016/j.exppara.2008.09.012.
9. Vago AR, Andrade LO, Leite AA, d'Avila Reis D, Macedo AM, Adad SJ, et al. Genetic characterization of *Trypanosoma cruzi* directly from tissues of patients with chronic Chagas disease: differential distribution of genetic types into diverse organs. Am J Pathol. 2000;156(5):1805–9. https://doi.org/10.1016/S0002-9440(10)65052-3.
10. Chiribao ML, Libisch G, Parodi-Talice A, Robello C. Early *Trypanosoma cruzi* infection reprograms human epithelial cells. Biomed Res Int. 2014;2014:439501.
11. Koo SJ, Chowdhury IH, Szczesny B, Wan X, Garg NJ. Macrophages promote oxidative metabolism to drive nitric oxide generation in response to *Trypanosoma cruzi*. Infect Immun. 2016;84(12):3527–41. https://doi.org/10.1128/IAI.00809-16.
12. Machado FS, Tyler KM, Brant F, Esper L, Teixeira MM, Tanowitz HB. Pathogenesis of Chagas disease: time to move on. Front Biosci (Elite Ed). 2012;4:1743–58.
13. Dos-Santos AL, Carvalho-Kelly LF, Dick CF, Meyer-Fernandes JR. Innate immunomodulation to trypanosomatid parasite infections. Exp Parasitol. 2016;167:67–75. https://doi.org/10.1016/j.exppara.2016.05.005.
14.• Abel LC, Ferreira LR, Cunha Navarro I, Baron MA, Kalil J, Gazzinelli RT, et al. Induction of IL-12 production in human peripheral monocytes by *Trypanosoma cruzi* is mediated by glycosylphosphatidylinositol-anchored mucin-like glycoproteins and potentiated by IFN-gamma and CD40-CD40L interactions. Mediat Inflamm. 2014;2014:345659. **This study identified the signaling molecules of *T. cruzi* that contribute to macrophage activation**
15. Coelho PS, Klein A, Talvani A, Coutinho SF, Takeuchi O, Akira S, et al. Glycosylphosphatidylinositol-anchored mucin-like glycoproteins isolated from *Trypanosoma cruzi* trypomastigotes induce in vivo leukocyte recruitment dependent on MCP-1 production by IFN-gamma-primed-macrophages. J Leukoc Biol. 2002;71(5):837–44.
16. Monteiro AC, Schmitz V, Morrot A, de Arruda LB, Nagajyothi F, Granato A, et al. Bradykinin B2 receptors of dendritic cells, acting as sensors of kinins proteolytically released by *Trypanosoma cruzi*, are critical for the development of protective type-1 responses. PLoS Pathog. 2007;3(11):e185. https://doi.org/10.1371/journal.ppat.0030185.
17. Schmitz V, Svensjo E, Serra RR, Teixeira MM, Scharfstein J. Proteolytic generation of kinins in tissues infected by *Trypanosoma cruzi* depends on CXC chemokine secretion by macrophages activated via Toll-like 2 receptors. J Leukoc Biol. 2009;85(6):1005–14. https://doi.org/10.1189/jlb.1108693.
18. Monteiro AC, Schmitz V, Svensjo E, Gazzinelli RT, Almeida IC, Todorov A, et al. Cooperative activation of TLR2 and bradykinin B2 receptor is required for induction of type 1 immunity in a mouse model of subcutaneous infection by *Trypanosoma cruzi*. J Immunol. 2006;177(9):6325–35. https://doi.org/10.4049/jimmunol.177.9.6325.
19. Oliveira AC, de Alencar BC, Tzelepis F, Klezewsky W, da Silva RN, Neves FS, et al. Impaired innate immunity in Tlr4(−/−) mice but preserved CD8+ T cell responses against *Trypanosoma cruzi* in Tlr4-, Tlr2-, Tlr9- or Myd88-deficient mice. PLoS Pathog. 2010;6(4):e1000870. https://doi.org/10.1371/journal.ppat.1000870.
20.• Bafica A, Santiago HC, Goldszmid R, Ropert C, Gazzinelli RT, Sher A. Cutting edge: TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia in *Trypanosoma cruzi* infection. J Immunol. 2006;177(6):3515–3519. **This study identified the role of TLRs in parasite control**. https://doi.org/10.4049/jimmunol.177.6.3515.
21.• Caetano BC, Carmo BB, Melo MB, Cerny A, dos Santos SL, Bartholomeu DC, et al. Requirement of UNC93B1 reveals a critical role for TLR7 in host resistance to primary infection with *Trypanosoma cruzi*. J Immunol. 2011;187(**4**):1903–1911. **This study identified the role of TLRs in parasite control.** https://doi.org/10.4049/jimmunol.1003911.
22. Koga R, Hamano S, Kuwata H, Atarashi K, Ogawa M, Hisaeda H, et al. TLR-dependent induction of IFN-beta mediates host defense against *Trypanosoma cruzi*. J Immunol. 2006;177(10):7059–66. https://doi.org/10.4049/jimmunol.177.10.7059.
23. Kayama H, Koga R, Atarashi K, Okuyama M, Kimura T, Mak TW, et al. NFATc1 mediates Toll-like receptor-independent innate immune responses during *Trypanosoma cruzi* infection. PLoS Pathog. 2009;5(7):e1000514. https://doi.org/10.1371/journal.ppat.1000514.
24.•• Tanowitz HB, Wen JJ, Machado FS, Desruisseaux MS, Robello C, Garg NJ. Trypanosoma cruzi and Chagas disease: innate immunity, ROS, and cardiovascular system. Waltham: Academic Press; 2016. **This article provides first summary of the role of ROS and innate immunity in Chagas disease.**
25. 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(24):3003–8. https://doi.org/10.1161/01.CIR.102.24.3003.
26. Silva JS, Vespa GN, Cardoso MA, Aliberti JC, Cunha FQ. Tumor necrosis factor alpha mediates resistance to *Trypanosoma cruzi* infection in mice by inducing nitric oxide production in infected gamma interferon-activated macrophages. Infect Immun. 1995;63(12):4862–7.
27. Poncini CV, Gonzalez-Cappa SM. Dual role of monocyte-derived dendritic cells in Trypanosoma cruzi infection. Eur J Immunol. 2017;47(11):1936–48. https://doi.org/10.1002/eji.201646830.
28. Ponce NE, Sanmarco LM, Eberhardt N, Garcia MC, Rivarola HW, Cano RC, et al. CD73 inhibition shifts cardiac macrophage polarization toward a microbicidal phenotype and ameliorates the outcome of experimental Chagas cardiomyopathy. J Immunol. 2016;197(3):814–23. https://doi.org/10.4049/jimmunol.1600371.
29. Garg NJ. Inflammasomes in cardiovascular diseases. Am J Cardiovasc Dis. 2011;1:244–54.
30. van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LA. Inflammasome activation and IL-1beta and IL-18 processing during infection. Trends Immunol. 2011;32(3):110–6. https://doi.org/10.1016/j.it.2011.01.003.
31. Silva GK, Gutierrez FR, Guedes PM, Horta CV, Cunha LD, Mineo TW, et al. Cutting edge: nucleotide-binding oligomerization domain 1-dependent responses account for murine resistance against *Trypanosoma cruzi* infection. J Immunol. 2010;184(3):1148–52. https://doi.org/10.4049/jimmunol.0902254.
32. Silva GK, Costa RS, Silveira TN, Caetano BC, Horta CV, Gutierrez FR, et al. Apoptosis-associated speck-like protein containing a caspase recruitment domain inflammasomes mediate IL-

1beta response and host resistance to *Trypanosoma cruzi* infection. J Immunol. 2013;191(6):3373–83. https://doi.org/10.4049/jimmunol.1203293.

33. Dey N, Sinha M, Gupta S, Gonzalez MN, Fang R, Endsley JJ, et al. Caspase-1/ASC inflammasome-mediated activation of IL-1beta-ROS-NF-kappaB pathway for control of *Trypanosoma cruzi* replication and survival is dispensable in NLRP3−/− macrophages. PLoS One. 2014;9(11):e111539. https://doi.org/10.1371/journal.pone.0111539.
34. Roffe E, Oliveira F, Souza AL, Pinho V, Souza DG, Souza PR, et al. Role of CCL3/MIP-1alpha and CCL5/RANTES during acute *Trypanosoma cruzi* infection in rats. Microbes Infect. 2010;12(8-9):669–76. https://doi.org/10.1016/j.micinf.2010.04.011.
35. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest. 1998;101(4):746–54. https://doi.org/10.1172/JCI1422.
36. Calzada JE, Nieto A, Beraun Y, Martin J. Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy. Tissue Antigens. 2001;58(3):154–8. https://doi.org/10.1034/j.1399-0039.2001.580302.x.
37. Medeiros GA, Silverio JC, Marino AP, Roffe E, Vieira V, Kroll-Palhares K, et al. Treatment of chronically *Trypanosoma cruzi*-infected mice with a CCR1/CCR5 antagonist (met-RANTES) results in amelioration of cardiac tissue damage. Microbes Infect. 2009;11(2):264–73. https://doi.org/10.1016/j.micinf.2008.11.012.
38. Machado FS, Koyama NS, Carregaro V, Ferreira BR, Milanezi CM, Teixeira MM, et al. CCR5 plays a critical role in the development of myocarditis and host protection in mice infected with *Trypanosoma cruzi*. J Infect Dis. 2005;191(4):627–36. https://doi.org/10.1086/427515.
39. de Oliveira AP, Ayo CM, Bestetti RB, Brandao de Mattos CC, Cavasini CE, de Mattos LC. The role of CCR5 in Chagas disease—a systematic review. Infect Genet Evol. 2016;45:132–7. https://doi.org/10.1016/j.meegid.2016.08.012.
40. Foldes G, Liu A, Badiger R, Paul-Clark M, Moreno L, Lendvai Z, et al. Innate immunity in human embryonic stem cells: comparison with adult human endothelial cells. PLoS One. 2010;5(5):e10501. https://doi.org/10.1371/journal.pone.0010501.
41. Ba X, Gupta S, Davidson M, Garg NJ. *Trypanosoma cruzi* induces ROS-PARP-1-RelA pathway for up regulation of cytokine expression in cardiomyocytes. J Biol Chem. 2010;285(15):11596–606. https://doi.org/10.1074/jbc.M109.076984.
42. Tousoulis D, Andreou I, Antoniades C, Tentolouris C, Stefanadis C. Role of inflammation and oxidative stress in endothelial progenitor cell function and mobilization: therapeutic implications for cardiovascular diseases. Atherosclerosis. 2008;201(2):236–47. https://doi.org/10.1016/j.atherosclerosis.2008.05.034.
43. Schultz K, Murthy V, Tatro JB, Beasley D. Endogenous interleukin-1 alpha promotes a proliferative and proinflammatory phenotype in human vascular smooth muscle cells. Am J Physiol Heart Circ Physiol. 2007;292(6):H2927–34. https://doi.org/10.1152/ajpheart.00700.2006.
44. Tanowitz HB, Gumprecht JP, Spurr D, Calderon TM, Ventura MC, Raventos-Suarez C, et al. Cytokine gene expression of endothelial cells infected with *Trypanosoma cruzi*. J Infect Dis. 1992;166(3):598–603. https://doi.org/10.1093/infdis/166.3.598.
45. Saavedra E, Herrera M, Gao W, Uemura H, Pereira MA. The *Trypanosoma cruzi* trans-sialidase, through its COOH-terminal tandem repeat, upregulates interleukin 6 secretion in normal human intestinal microvascular endothelial cells and peripheral blood mononuclear cells. J Exp Med. 1999;190(12):1825–36. https://doi.org/10.1084/jem.190.12.1825.
46. Hassan GS, Mukherjee S, Nagajyothi F, Weiss LM, Petkova SB, de Almeida CJ, et al. *Trypanosoma cruzi* infection induces proliferation of vascular smooth muscle cells. Infect Immun. 2006;74(1):152–9. https://doi.org/10.1128/IAI.74.1.152-159.2006.
47. Waghabi MC, de Souza EM, de Oliveira GM, Keramidas M, Feige JJ, Araujo-Jorge TC, et al. Pharmacological inhibition of transforming growth factor beta signaling decreases infection and prevents heart damage in acute Chagas' disease. Antimicrob Agents Chemother. 2009;53(11):4694–701. https://doi.org/10.1128/AAC.00580-09.
48. Mukherjee S, Machado FS, Huang H, Oz HS, Jelicks LA, Prado CM, et al. Aspirin treatment of mice infected with *Trypanosoma cruzi* and implications for the pathogenesis of Chagas disease. PLoS One. 2011;6(2):e16959. https://doi.org/10.1371/journal.pone.0016959.
49. Kabashima K, Shiraishi N, Sugita K, Mori T, Onoue A, Kobayashi M, et al. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. Am J Pathol. 2007;171(4):1249–57. https://doi.org/10.2353/ajpath.2007.070225.
50. Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell Mol Immunol. 2015;12(1):5–23. http://www.ncbi.nlm.nih.gov/pubmed/25263488.
51. de Carvalho TU, de Souza W. Cytochemical localization of NADH and NADPH oxidases during interaction of *Trypanosoma cruzi* with activated macrophages. Parasitol Res. 1987;73(3):213–7. https://doi.org/10.1007/BF00578506.
52. Cardoni RL, Antunez MI, Morales C, Nantes IR. Release of reactive oxygen species by phagocytic cells in response to live parasites in mice infected with *Trypanosoma cruzi*. Am J Trop Med Hyg. 1997;56(3):329–34. https://doi.org/10.4269/ajtmh.1997.56.329.
53. Dhiman M, Garg NJ. NADPH oxidase inhibition ameliorates *Trypanosoma cruzi*-induced myocarditis during Chagas disease. J Pathol. 2011;225(4):583–96. https://doi.org/10.1002/path.2975.
54. Santiago HC, Gonzalez Lombana CZ, Macedo JP, Utsch L, Tafuri WL, Campagnole-Santos MJ, et al. NADPH phagocyte oxidase knockout mice control *Trypanosoma cruzi* proliferation, but develop circulatory collapse and succumb to infection. PLoS Negl Trop Dis. 2012;6(2):e1492. https://doi.org/10.1371/journal.pntd.0001492.
55. Dhiman M, Garg NJ. P47phox−/− mice are compromised in expansion and activation of $CD8^+$ T cells and susceptible to *Trypanosoma cruzi* infection. PLoS Pathog. 2014;10(12):e1004516. https://doi.org/10.1371/journal.ppat.1004516.
56. Bogdan C. Nitric oxide synthase in innate and adaptive immunity: an update. Trends Immunol. 2015;36(3):161–78. https://doi.org/10.1016/j.it.2015.01.003.
57. Alvarez MN, Peluffo G, Piacenza L, Radi R. Intraphagosomal peroxynitrite as a macrophage-derived cytotoxin against internalized *Trypanosoma cruzi*: consequences for oxidative killing and role of microbial peroxiredoxins in infectivity. J Biol Chem. 2011;286(8):6627–40. https://doi.org/10.1074/jbc.M110.167247.
58. Piacenza L, Peluffo G, Alvarez MN, Kelly JM, Wilkinson SR, Radi R. Peroxiredoxins play a major role in protecting *Trypanosoma cruzi* against macrophage- and endogenously-derived peroxynitrite. Biochem J. 2008;410(2):359–68. https://doi.org/10.1042/BJ20071138.
59. Piacenza L, Zago MP, Peluffo G, Alvarez MN, Basombrio MA, Radi R. Enzymes of the antioxidant network as novel determiners of *Trypanosoma cruzi* virulence. Int J Parasitol. 2009;39(13):1455–64. https://doi.org/10.1016/j.ijpara.2009.05.010.
60.• Zago MP, Hosakote YM, Koo S-J, Dhiman M, Piñeyro MD, Parodi-Talice A, et al. TcI isolates of *Trypanosoma cruzi* exploit the antioxidant network for enhanced intracellular survival in

macrophages and virulence in mice. Infect Immun. 2016;84(**6**): 1842–1856. **This article describes how *T. cruzi* arrests macrophage ability to mount strong ROS and NO response**. https://doi.org/10.1128/IAI.00193-16.

61. Pineyro MD, Parodi-Talice A, Arcari T, Robello C. Peroxiredoxins from *Trypanosoma cruzi*: virulence factors and drug targets for treatment of Chagas disease? Gene. 2008;408(1-2):45–50. https://doi.org/10.1016/j.gene.2007.10.014.
62. Flohe L. The trypanothione system and its implications in the therapy of trypanosomatid diseases. Int J Med Microbiol. 2012;302(4-5):216–20. https://doi.org/10.1016/j.ijmm.2012.07.008.
63. Hall BS, Tam W, Sen R, Pereira ME. Cell-specific activation of nuclear factor-kappaB by the parasite *Trypanosoma cruzi* promotes resistance to intracellular infection. Mol Biol Cell. 2000;11(1):153–60. https://doi.org/10.1091/mbc.11.1.153.
64. Huang H, Petkova SB, Cohen AW, Bouzahzah B, Chan J, Zhou JN, et al. Activation of transcription factors AP-1 and NF-kappa B in murine Chagasic myocarditis. Infect Immun. 2003;71(5):2859–67. https://doi.org/10.1128/IAI.71.5.2859-2867.2003.
65. Dias WB, Fajardo FD, Graca-Souza AV, Freire-de-Lima L, Vieira F, Girard MF, et al. Endothelial cell signalling induced by trans-sialidase from *Trypanosoma cruzi*. Cell Microbiol. 2008;10(1):88–99. https://doi.org/10.1111/j.1462-5822.2007.01017.x.
66. Pinto AM, Sales PC, Camargos ER, Silva AM. Tumour necrosis factor (TNF)-mediated NF-kappaB activation facilitates cellular invasion of non-professional phagocytic epithelial cell lines by *Trypanosoma cruzi*. Cell Microbiol. 2011;13(10):1518–29. https://doi.org/10.1111/j.1462-5822.2011.01636.x.
67.•• Wan X, Wen JJ, Koo SJ, Liang LY, Garg NJ. SIRT1-PGC1alpha-NFkappaB pathway of oxidative and inflammatory stress during *Trypanosoma cruzi* infection: benefits of SIRT1-targeted therapy in improving heart function in Chagas disease. PLoS Pathog. 2016;12(**10**):e1005954. **This report describes the significance of SIRT1 in maintaing metabolic and inflammatory homeostasis in chagasic disease**. https://doi.org/10.1371/journal.ppat.1005954.
68. Gupta S, Bhatia V, Wen J-J, Wu Y, Huang M-H, Garg NJ. *Trypanosoma cruzi* infection disturbs mitochondrial membrane potential and ROS production rate in cardiomyocytes. Free Radic Biol Med. 2009;47(10):1414–21. https://doi.org/10.1016/j.freeradbiomed.2009.08.008.
69. Ba X, Garg NJ. Signaling mechanism of PARP-1 in inflammatory diseases. Am J Pathol. 2010;178:946–55.
70. Keating SM, Deng X, Fernandes F, Cunha-Neto E, Ribeiro AL, Adesina B, et al. Inflammatory and cardiac biomarkers are differentially expressed in clinical stages of Chagas disease. Int J Cardiol. 2015;199:451–9. https://doi.org/10.1016/j.ijcard.2015.07.040.
71. 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(3):163–5. https://doi.org/10.1016/j.autrev.2010.09.019.
72. Garg NJ, Soman KV, Zago MP, Koo SJ, Spratt H, Stafford S, et al. Changes in proteome profile of peripheral blood mononuclear cells in chronic Chagas disease. PLoS Negl Trop Dis. 2016;10(2):e0004490. https://doi.org/10.1371/journal.pntd.0004490.
73. Ferreira LR, Ferreira FM, Nakaya HI, Deng X, Candido DD, de Oliveira LC, et al. Blood gene signatures of Chagas disease cardiomyopathy with or without ventricular dysfunction. J Infect Dis. 2016:jiw540. https://doi.org/10.1093/infdis/jiw540.
74. Souza PE, Rocha MO, Menezes CA, Coelho JS, Chaves AC, Gollob KJ, et al. *Trypanosoma cruzi* infection induces differential modulation of costimulatory molecules and cytokines by monocytes and T cells from patients with indeterminate and cardiac Chagas' disease. Infect Immun. 2007;75(4):1886–94. https://doi.org/10.1128/IAI.01931-06.
75. Souza PE, Rocha MO, Rocha-Vieira E, Menezes CA, Chaves AC, Gollob KJ, et al. Monocytes from patients with indeterminate and cardiac forms of Chagas' disease display distinct phenotypic and functional characteristics associated with morbidity. Infect Immun. 2004;72(9):5283–91. https://doi.org/10.1128/IAI.72.9.5283-5291.2004.
76. Machado FS, Dutra WO, Esper L, Gollob KJ, Teixeira MM, Weiss LM, et al. Current understanding of immunity to *Trypanosoma cruzi* infection and pathogenesis of Chagas disease. Semin Immunopathol. 2012;34(6):753–70. https://doi.org/10.1007/s00281-012-0351-7.
77. Shantsila E, Kamphuisen PW, Lip GY. Circulating microparticles in cardiovascular disease: implications for atherogenesis and atherothrombosis. J Thromb Haemost. 2010;8(11):2358–68. https://doi.org/10.1111/j.1538-7836.2010.04007.x.
78.•• Chowdhury IH, Koo S, Gupta S, Liang LY, Bahar B, Silla L, et al. Gene expression profiling and functional characterization of macrophages in response to circulatory microparticles produced during *Trypanosoma cruzi* infection and Chagas disease. J Innate Immun. 2016;9(2):203–16. **This study provided evidence for the potential role of cellular damage in inflammatory activation of macrophages in chronic Chagas disease**
79. Gupta S, Silva TS, Osizugbo JE, Tucker L, Garg NJ. Serum mediated activation of macrophages reflects Tcvac2 vaccine efficacy against Chagas disease. Infect Immun, 2014;82(4):1382–89
80. Dhiman M, Zago MP, Nunez S, Nunez-Burgio F, Garg NJ. Cardiac oxidized antigens are targets of immune recognition by antibodies and potential molecular determinants in Chagas disease pathogenesis. PLoS One. 2012;7(1):e28449. https://doi.org/10.1371/journal.pone.0028449.
81. Wen JJ, Garg NJ. Mitochondrial generation of reactive oxygen species is enhanced at the Q(o) site of the complex III in the myocardium of *Trypanosoma cruzi*-infected mice: beneficial effects of an antioxidant. J Bioenerg Biomembr. 2008;40(6):587–98. https://doi.org/10.1007/s10863-008-9184-4.
82. Wen J-J, Garg NJ. Mitochondrial complex III defects contribute to inefficient respiration and ATP synthesis in the myocardium of *Trypanosoma cruzi*-infected mice. Antioxid Redox Signal. 2010;12(1):27–37. https://doi.org/10.1089/ars.2008.2418.
83. Wen JJ, Dhiman M, Whorton EB, Garg NJ. Tissue-specific oxidative imbalance and mitochondrial dysfunction during *Trypanosoma cruzi* infection in mice. Microbes Infect. 2008;10(10-11):1201–9. https://doi.org/10.1016/j.micinf.2008.06.013.
84. Perez-Fuentes R, Guegan JF, Barnabe C, Lopez-Colombo A, Salgado-Rosas H, Torres-Rasgado E, et al. Severity of chronic Chagas disease is associated with cytokine/antioxidant imbalance in chronically infected individuals. Int J Parasitol. 2003;33(3):293–9. https://doi.org/10.1016/S0020-7519(02)00283-7.
85. de Oliveira TB, Pedrosa RC, Filho DW. Oxidative stress in chronic cardiopathy associated with Chagas disease. Int J Cardiol. 2007;116(3):357–63. https://doi.org/10.1016/j.ijcard.2006.04.046.
86. Wen J-J, Yachelini PC, Sembaj A, Manzur RE, Garg NJ. Increased oxidative stress is correlated with mitochondrial dysfunction in chagasic patients. Free Radic Biol Med. 2006;41(2):270–6. https://doi.org/10.1016/j.freeradbiomed.2006.04.009.
87. Wan X-X, Gupta S, Zago MP, Davidson MM, Dousset P, Amoroso A, et al. Defects of mtDNA replication impaired the mitochondrial biogenesis during *Trypanosoma cruzi* infection in human cardiomyocytes and Chagasic patients: the role of Nrf1/2 and antioxidant response. J Am Heart Assoc. 2012;1:e003855.

88. Wen J-J, Gupta S, Guan Z, Dhiman M, Condon D, Lui CY, et al. 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(22):2499–508. https://doi.org/10.1016/j.jacc.2010.02.030.
89. Wen JJ, Wan X, Thacker J, Garg NJ. Chemotherapeutic efficacy of phosphodiesterase inhibitors in Chagasic cardiomyopathy. JACC Basic Transl Sci. 2016;1(4):235–50. https://doi.org/10.1016/j.jacbts.2016.04.005.
90. Macao LB, Filho DW, Pedrosa RC, Pereira A, Backes P, Torres MA, et al. Antioxidant therapy attenuates oxidative stress in chronic cardiopathy associated with Chagas' disease. Int J Cardiol. 2007;123(1):43–9. https://doi.org/10.1016/j.ijcard.2006.11.118.
91.•• Wen JJ, Porter C, Garg NJ. Inhibition of NFE2L2-ARE pathway by mitochondrial ROS contributes to development of cardiomyopathy and left ventricular dysfunction in Chagas disease. Antioxid Redox Signal. 2017;**27**(**9**):**550**–66. https://doi.org/10.1089/ars.2016.6831. **This study provides mechanistic evidence for the role of mitochondrial ROS in regulating antioxidant response in the heart**.
92. Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. Free Radic Biol Med. 2011;50(7):777–93. https://doi.org/10.1016/j.freeradbiomed.2011.01.003.
93. Bouzahzah B, Yurchenko V, Nagajyothi F, Hulit J, Sadofsky M, Braunstein VL, et al. Regulation of host cell cyclin D1 by *Trypanosoma cruzi* in myoblasts. Cell Cycle. 2008;7(4):500–3. https://doi.org/10.4161/cc.7.4.5327.
94. Huang YF, Gong KZ, Zhang ZG. Different roles of ERK(1/2) and p38 MAPK(alpha/beta) in cellular signaling during cardiomyocyte anoxia preconditioning. Sheng Li Xue Bao. 2003;55(4):454–8.
95. Adesse D, Lisanti MP, Spray DC, Machado FS, Meirelles Mde N, Tanowitz HB, et al. *Trypanosoma cruzi* infection results in the reduced expression of caveolin-3 in the heart. Cell Cycle. 2010;9(8):1639–46. https://doi.org/10.4161/cc.9.8.11509.
96. Nagajyothi F, Desruisseaux M, Bouzahzah B, Weiss LM, Andrade Ddos S, Factor SM, et al. Cyclin and caveolin expression in an acute model of murine Chagasic myocarditis. Cell Cycle. 2006;5(1):107–12. https://doi.org/10.4161/cc.5.1.2284.
97. Cohen AW, Park DS, Woodman SE, Williams TM, Chandra M, Shirani J, et al. Caveolin-1 null mice develop cardiac hypertrophy with hyperactivation of p42/44 MAP kinase in cardiac fibroblasts. Am J Physiol Cell Physiol. 2003;284(2):C457–74. https://doi.org/10.1152/ajpcell.00380.2002.
98. Woodman SE, Park DS, Cohen AW, Cheung MW, Chandra M, Shirani J, et al. Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. J Biol Chem. 2002;277(41):38988–97. https://doi.org/10.1074/jbc.M205511200.
99. Park DS, Woodman SE, Schubert W, Cohen AW, Frank PG, Chandra M, et al. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and non-muscle caveolae, and develop a severe cardiomyopathic phenotype. Am J Pathol. 2002;160(6):2207–17. https://doi.org/10.1016/S0002-9440(10)61168-6.
100.•• Morillo CA, Marin-Neto JA, Avezum A, Sosa-Estani S, Rassi A Jr, Rosas F, et al. Randomized trial of benznidazole for chronic Chagas cardiomyopathy. N Engl J Med. 2015;373(**14**):1295–1306. **This is an important study demonstrating that anti-parasite drug therapy alone is not sufficient to control chronic Chagas disease.** https://doi.org/10.1056/NEJMoa1507574.