Disease Tolerance and Pathogen Resistance Genes May Underlie Trypanosoma cruzi Persistence and Differential Progression to Chagas Disease Cardiomyopathy

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Chagas disease is caused by infection with the protozoan Trypanosoma cruzi and affects over 8 million people worldwide. In spite of a powerful innate and adaptive immune response in acute infection, the parasite evades eradication, leading to a chronic persistent infection with low parasitism. Chronically infected subjects display differential patterns of disease progression. While 30% develop chronic Chagas disease cardiomyopathy (CCC)—a severe inflammatory dilated cardiomyopathy—decades after infection, 60% of the patients remain disease-free, in the asymptomatic/indeterminate (ASY) form, and 10% develop gastrointestinal disease. Infection of genetically deficient mice provided a map of genes relevant for resistance to T. cruzi infection, leading to the identification of multiple genes linked to survival to infection. These include pathogen resistance genes (PRG) needed for intracellular parasite destruction, and genes involved in disease tolerance (protection against tissue damage and acute phase death—DTG). All identified DTGs were found to directly or indirectly inhibit IFN-γ production or Th1 differentiation. We hypothesize that the absolute need for DTG to control potentially lethal IFN-γ PRG activity leads to T. cruzi persistence and establishment of chronic infection. IFN-γ production is higher in CCC than ASY patients, and is the most highly expressed cytokine in CCC hearts. Key DTGs that downmodulate IFN-γ, like IL-10, and Ebi3/IL27p28, are higher in ASY patients. Polymorphisms in PRG and DTG are associated with differential disease progression. We thus hypothesize that ASY patients are disease tolerant, while an imbalance of DTG and IFN-γ PRG activity leads to the inflammatory heart damage of CCC.

Keywords: disease tolerance gene, pathogen resistance genes, Trypanosoma cruzi, chagas cardiomyopathy, interferon gamma
INTRODUCTION

Chagas disease is caused by infection with *Trypanosoma cruzi*, an obligatory intracellular parasite in the mammalian host. It is endemic in Latin America and affects over 8 million people worldwide, causing thousands of deaths each year. The pathogen naturally infects animals from central Argentina to the southern United States. Acute infection may lead to death in a small proportion of hosts, and survivors live with persistent infection with low parasitism. Chronically infected patients display differential progression. Nearly 30% of infected patients may develop life-threatening chronic heart disease due to an excessive inflammatory response, most others remaining in the asymptomatic/indeterminate (ASY) form with no heart disease, associated with a more immunomodulatory profile. Genes encoding resistance strategies that are shared against intracellular pathogens (1) have evolved for hundreds of millions of years, including the pathogen resistance receptors, TNF-α, and the IFN-γ-dependent immune response, among others (2). Most pathogen resistance genes (PRG) inhibit infection by directly reducing pathogen burden, and are related to immune-driven mechanisms—which, when in excess, can lead to death. Disease tolerance is an alternative strategy to avoid death after infection, whereby the pathogen’s damaging effect on the host is mitigated. Disease tolerance is defined as the situation where an organism can bear a pathogen load without tissue damage and in the absence of a disease state. Disease tolerance genes (DTG)—which do not limit infection, but reduce its fitness costs—operate to minimize tissue damaging effects of the pathogen, leading to stress and damage reduction responses; DTG can also operate by counteracting excessive, tissue-damaging PRG activity (1, 3, 4). One such stress response pathways involved in disease tolerance is the oxidative stress response, whose master regulator is nuclear respiratory factor 2 (Nrf2) (3). By not limiting infection, the host remains a pathogen reservoir enabling transmission, in the absence of health consequences for itself, providing an evolutionary advantage for both host and pathogen. Disease tolerance is frequent in pathogens and hosts who have coevolved; while African monkeys which coevolved with the African virus Simian Immunodeficiency Virus (SIV) develop chronic infection with no disease, the same virus causes deadly disease in Asian macaques (5, 6). Evolutionary selection of DTG can be even more effective than that of PRG (4). A possible example of evolutionary selection of DTG against *T. cruzi* are the South American didelphid marsupials which coevolved with *T. cruzi* for over 40 million years and maintain high and long-lasting *T. cruzi* parasitemias in the absence of disease (7). We here hypothesize that the absolute need for DTG to control potentially lethal PRG activity against *T. cruzi* leads to parasite persistence and establishment of chronic infection. Our second hypothesis is that PRG and DTG also determine the differential progression of chronic Chagas disease toward tissue damage (CCC). According to this hypothesis, ASY patients are disease tolerant, while tissue damage in CCC is a consequence of insufficient DTG and/or excessive PRG activity. Along the review, we will provide evidence supporting both hypotheses.

PATHOGEN RESISTANCE GENES IN *T. cruzi* INFECTION

Most known pathogen resistance mechanisms against *T. cruzi* are immune-driven, directed at the intracellular forms of the parasite, and can be harmful if excessive. *T. cruzi* evades the powerful immune response and establishes a persistent infection with low parasitism. In order to obtain a list of known PRG and DTG, we surveyed the literature on *T. cruzi* infection of genetically deficient knockout mice. PRG were defined as genes essential for control of *T. cruzi* parasitism and needed for survival of infection; operationally, we identified as PRG those genes whose knockout led to increased pathogen load and mortality. DTG were defined as genes whose presence reduced mortality without any effect on *T. cruzi* control. We identified as DTG those genes whose knockout led to reduced parasitism and increased mortality. Table 1 lists the PRG and DTG identified in our literature review. Most PRG belong to the TLR-MYD88-IL12-IFNG pathway, IL17 pathway, cell migration, inflammasome and other pathways involved in restriction of intracellular pathogen growth. Mice genetically deficient on TLR4, TLR7, and TLR9, MYD88, and UNC93B1 display increased blood parasitism and mortality (8–13). Likewise, mice genetically deficient of IL12A, IL12B, and STAT4, essential for the differentiation of IFN-γ-producing Th1 cells, also display intense tissue and blood parasitism with increased mortality (15, 33). Along with TLR genes, IFNG is one of the main PRG involved in *T. cruzi* parasite control (43). Mice genetically deficient on IFNG or STAT1 display drastically augmented *T. cruzi* parasitism and 100% mortality 13 days after infection (20, 21, 24). It was shown that *T. cruzi* amastigotes themselves dephosphorylate STAT1 serine residues, inhibiting IFN-γ signaling; evasion of IFN-γ signaling is further proof of the importance of the IFN-γ in the control of intracellular parasitism (44). IFN-γ-dependent PRG, like TNFA and NOS2, play a major role in resistance to *T. cruzi* (45, 46). The key role of TNF-α in *T. cruzi* control has been shown in TNFA-receptor 1 knockout mice (TNFRSF1A−/−), which display an increased number of blood and tissue parasites and shortened survival time (26). Platelet-activating factor (PAF) KO mice are more susceptible to *T. cruzi* infection than wildtype mice, and its protective effects depend on TNF-α-dependent NO production. In the context of protection against *T. cruzi*, IFN-γ, and TNF-α synergistically induce NF-kb activation to control *T. cruzi* parasitism and mortality in mice, by upregulating the expression of the PRG inducible nitric oxide synthase (NOS2), leading to the production of large amounts of NO and microbicidal reactive nitrogen species (RNS) (45, 46). NOS2-KO mice are susceptible to *T. cruzi* infection, with increased parasite burden and mortality due to lack of NO production (15, 47). Interestingly, constitutive NOS1-KO mice also showed increased parasitism and mortality (27). Genetic deficiency of macrophage PI3KCG increases susceptibility to *T. cruzi* infection; PI3KCG expression correlates with IFNG expression in CCC myocardium (38). IFN-γ increases ROS generation through induction of NADP oxidases (NOX2) and mitochondrial ROS via NF-kb...
activation (47, 48). Mice knockout for NOX2 displayed increased *T. cruzi* tissue parasitism and mortality due to the lack of type 1 cytotoxic T cells (29). IFN-γ-induced ROS enhances peroxynitrite anion (ONOO−) production, a strong oxidant arising from the reaction of NO with superoxide radical (O2−) (49). ONOO− induces damage to multiple molecules and is one of the ultimate effectors of parasite killing. Peroxynitrite promotes morphological disruption of internalized parasites, and trypanothione depletion, severely impairing parasite redox homeostasis (50, 51). In addition, IFN-γ can also exert its protective effects *in vivo* in a NO-independent manner, through complementary events of protection against *T. cruzi*, such as production of other macrophage-derived effector molecules, MHC class II induction, CD4+ T cell polarization, IgG isotype switch to IgG2a and TLR induction (52–54). IRGM1, an IFN-γ-dependent GTPase, plays a key role in phagosome maturation and in the killing of intracellular pathogens contained in vacuoles (55).

In addition to inflammatory cells, several other cell types, including cardiomyocytes, fibroblasts and astrocytes, bear IFN-γ receptors and respond to the cytokine (56, 57). IFN-γ activates *T. cruzi*-infected macrophages and cardiomyocytes to produce TNF-α, NO, microbical ROS, and RNS. *In vitro* treatment with IFN-γ/TNF-α and IL1-β of mouse cardiomyocytes infected with *T. cruzi* resulted in NO production and elevated trypansomal activity (58, 59). The primary role of IFN-γ mediating protection against intracellular *T. cruzi* infection is depicted in Figure 1.

IL17A is also a key PRG, and Th17 T cells may confer stronger protection against *T. cruzi*-related mortality than Th1 cells (60). IL-17A signaling is mainly dependent on TNF-α receptor associated factor 6 (TRAF6), but it can strongly promote TNF-α-induced NF-kB signaling by stabilizing pro-inflammatory mRNAs (61). Indeed, IL17A KO mice displayed an increase in blood and tissue parasitism with increased mortality. This was related to an impairment of leukocyte migration and activation of immune cells in the sites of parasite infection, as well as by reduced production of NO (18, 19). IL-17A also increases the persistence time of *T. cruzi* in the parasitophorous vacuole, enhancing exposure time of *T. cruzi* to the antimicrobial environment of endolysosomes, which can be further enhanced by IFN-γ-induced mechanisms (18). Genetic depletion of key inflammasome-related genes CASP1 and PYCARD/Asc induce enhanced mortality and increased parasitism (28). Mice knockout for genes involved in migration pathways, like chemokines/receptors CCL2, CCR5, and adhesion molecule ICAM1 also develop increased parasitism and decreased survival, in line with the impaired recruitment of leukocytes to sites of parasite replication (30–33). CD28 KO mice display T cell activation defects, and NCF1 (p47phox/NOX2) display a reduced type 1 CD8+ T cell response, leading to impaired control of parasitism and increased susceptibility to infection (29, 34). Although IL27RA KO mice develop increased parasitism and mortality and was a priori classified as a PRG, these mice develop grossly increased IFN-γ and inflammatory cytokine production, and death was related to the IFN-γ dependent tissue inflammation, a phenotype similar to that of IL10 KO mice (62). This is in line with the known effect of IL27RA signaling in the control of IFN-γ and inflammatory cytokines. Phospholipase A2 γ knockout mice show increased susceptibility to *T. cruzi* infection, and decreased arachidonic acid and prostaglandin E2 production; the mechanism of protection is still obscure (37). Galectin-1 KO mice displayed increased parasitism and mortality upon infection with the Tulahuen strain, classifying it as a PRG (36).

However, subsequent studies with infection of Galectin-1 with the RA strain showed reduced parasitism and mortality, which is not a PRG profile (63). This suggests the phenotypes may change according to the *T. cruzi* strain being tested and site of infection.

| Symbol | Name |
|--------|------|
| ifng  | Interferon-γ |
| stat1 | Signal transducer and activator of transcription 1 |
| stat4 | Signal transducer and activator of transcription 4 |
| trf1a | TNF receptor superfamily member 1A |
| nos1  | Nitric oxide synthase 1 |
| nos2  | Nitric oxide synthase 2, inducible |
| casp1 | Caspase 1 |
| pycard | Asc/PyD and CARD domain containing |
| ncf1  | P47phox/neutrophil cytosolic factor 1 |
| ccl2  | C–C motif chemokine ligand 2 |
| cor5  | C–C motif chemokine receptor 5 |
| icam1 | Interleukin 1 interleukin 6 |
| cd28  | CD28 antigen |
| vgrm1 | Immunity-related GTPase family M member 1 |
| ptaf  | Platelet-activating factor receptor |
| igals1 | Galectin-1/lectin, galactose binding, soluble 1 |
| pnpla8 | Phospholipase A2 γ (IPLA2γ)/patatin-like phospholipase domain containing 8 |
| pik3cg | Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma |

| Symbol | Name |
|--------|------|
| ifh   | Intercellular adhesion molecule 1 |
| i10   | Interleukin 6 |
| i23   | Interleukin 10 |
| i17ra | Interleukin 17 receptor A |
| ebv3  | Epstein-Barr virus induced gene 3 |

| Symbol | Name |
|--------|------|
| tbl4  | Toll-like receptor 4 |
| tbl7  | Toll-like receptor 7 |
| tbl9  | Toll-like receptor 9 |
| unc93b1 | Unc-93 homolog B1, TLR signaling regulator |
| myd88 | Myeloid differentiation primary response 88 |
| i6    | Interleukin 6 |
| il2b  | Interleukin 12A |
| il12a | Interleukin 12B |
| il12b | Interleukin 17A |
| il17a | Interleukin 17A |

Figure 1
DISEASE TOLERANCE GENES IN T. cruzi INFECTION

Table 1 shows the T. cruzi DTG. Remarkably, all T. cruzi DTG (IL10, Ebi-IL27p28, IL17RA, IL23, IL6) shared as a common feature the ability to reduce IFN-γ production or Th1 differentiation. In addition to decreased parasitism and increased mortality, inflammation was upregulated in all DTG-deficient mice. Infection of IL-10 deficient mice is accompanied by increased release of IFN-γ, TNF-α, IL-12, and RNS (40). Mechanistically, IL-10 is a potent inhibitor of monocyte-macrophage activation and NK cell activity and can inhibit the synthesis of TNF-α and IL-12 and IFN-γ (64, 65). Mice genetically deficient of IL-17RA or IL-23 showed increased mortality due to a shift to a Th1 profile after infection and augmented IFN-γ and TNF-α levels in the heart (39, 42, 66). IL17RA signaling downregulated T-bet expression, and reduced Th1 T cell differentiation, and further downregulated IFN-γ production in acute infection by recruiting IL-10-producing suppressive neutrophils (42). Paradoxically, IL-17A is a PRG (18). It is possible that a engagement of the IL-17 receptor by a different IL-17 family member can be responsible for the DTG effect of IL17RA. IL-23 negatively regulates IL-12-induced IFN-γ production in CD8+ T cells by reducing STAT4 phosphorylation, independently of IL-17A, IL-17F, or IL-22 (18, 25, 67, 68). In addition, IL-23 is a key stimulatory cytokine for Th17 and innate "type 17" cells that can respond immediately to pathogenic insults; IL-23 may thus also suppress IFN-γ by promoting IL17RA signaling (69). Infection of Ebi3/IL-27p28 deficient mice is accompanied by increased IFN-γ production, with augmented Th1 immune response (70). Mechanistically, Ebi3 signaling modulates overproduction of IFN-γ, by inducing a population of IL-10 producing Tr1 T cells (39). On the other hand, IL-6 can be both a PRG and a DTG, depending on the model of T. cruzi infection. IL-6's DTG activity may be secondary to inhibition of Th1 differentiation through enhancing IL-4 production in CD4+ T cells (71), while its PRG activity may be explained by its ability to upregulate endothelial adhesion molecules, facilitating lymphocyte migration into non-lymphoid tissues (14, 71). We will discuss evidence that IFNG, a major PRG that operates as a key player in pathogen protection and is the culprit of tissue damage in Chagas disease, is the main target of modulation by DTGs with relevance in both the acute infection and differential progression of chronic disease.

IMMUNE DYNAMICS IN ACUTE T. cruzi INFECTION

T. cruzi subverts a highly conserved cellular pathway for the repair of plasma membrane lesions and explores endogenous cellular machinery for invasion, escape from the parasitophorous vacuole, which allows intracytoplasmic survival, and replication (72). The intracellular life cycle of T. cruzi is a major target of the antiparasite response (73). Extracellular T. cruzi components, such as trypomastigote-derived glycosylphosphatidylinositol (tGPI) and glycoinositolphospholipid (eGIPL) engage membrane Toll-like receptors (TLR) 2 and 4 (74). Trypanosoma cruzi is internalized by several different mechanisms, but end up in the phagolysosomal compartment, where T. cruzi DNA and RNA engage TLR7 and TLR9. TLR engagement promotes the Myd88-mediated activation of NF-kB. Lysosome acidification promotes escape of T. cruzi to the cytoplasm, where it differentiates into the replicative amastigote forms. Amastigote replication in the
cytoplasm leads to activation of inflammasomes that can induce inflammatory cytokines and NF-κB activation. This induces pro-inflammatory cytokines including IL-12, a PRG which elicits differentiation of IFN-γ-producing Th1 cells soon after infection, promoting Th1 cell differentiation. IFN-γ induces expression of multiple other PRGs, such as TNFA and NOS2 (45, 46). Recent studies have reported that IL17A is a key PRG, plays a major protective role during the acute phase of infection (60). A strong antibody response is also triggered, but apparently has a lower effect on parasitism than innate immunity and acquired IFN-γ-dependent CD4+ and CD8+ T cell responses (53).

Although powerful, the immune response that occurs during acute infection leads to partial parasite control. *T. cruzi* evades complete eradication, leading to the establishment of a chronic persistent infection with low parasitism. *T. cruzi*-infected individuals maintain increased production of inflammatory/Th1 cytokines like IFN-γ and TNF-α as compared to healthy individuals, as a result of persistent stimulus of innate and specific immunity (75).

### IMMUNE DYNAMICS AND DIFFERENTIAL DISEASE PROGRESSION TO CCC

Chronically infected individuals display differential disease progression. While 60% of infected individuals remain disease-free for life, 30% develop life-threatening CCC—a severe inflammatory dilated cardiomyopathy, years after infection. Ten percent develop gastrointestinal disease. CCC has a worse prognosis than cardiomyopathies of non-inflammatory etiologies, such as ischemic or idiopathic cardiomyopathy (76). CCC patients show an increased number of IFN-γ-producing Th1 T cells and plasma TNF-α levels as compared with ASY. Conversely, numbers of IL-10-producing CD4+CD25+ regulatory T cells (Tregs) CD4+CD25+FoxP3+Tregs and Th17 cells, as well as Eb3/IL-27p28 levels are lower as compared with ASY (28, 31, 39, 44, 60, 77) (Figure 2). The exacerbated Th1 response observed in the peripheral blood of CCC patients is reflected on the Th1-rich inflammatory infiltrate predominantly secreting IFN-γ and TNF-α. A lower, but significant, production of IL-4, IL-6, IL-7, IL-15, IL-18 was found in their heart tissue (57, 78–83). Indeed, IFN-γ is the most upregulated cytokine in CCC heart tissue; concordantly, we observed significant expression of Tbet, the hallmark Th1 transcription factor, in the CCC myocardium (84). We found a positive correlation between Tbet expression and left ventricular dilation, corroborating the pathogenic role of Tbet positive/IFN-γ producing T cells toward CCC. Conversely, mRNA expression of GATA3, RORγT, and FoxP3, hallmark transcription factors of Th1-antagonizing Th2, Th17, and Treg populations was low or undetectable. In agreement, mRNA expression of their signature cytokines IL4, IL13, IL17, IL10, and Treg molecular markers FOXP3 and CTLA4 was also low or undetectable (84). IFN-γ-producing CCR5+CXCR3+ Th1 T cells are more abundant in CCC than ASY (85), and the same cells were identified in CCC heart tissue, along with their chemokine ligands (CCL3, CCL4, CCL5, CXCL9, and CXCL10, respectively). CCL5 and CXCL9 were the most highly expressed chemokine mRNAs, and the intensity of the myocardial inflammation was positively correlated with CXCL9 mRNA expression (86, 87). Together, this suggests that locally produced Th1 T cell-attracting chemokines play a role in the selective accumulation of Th1 T cells in CCC hearts. Moreover, it indicates that the Th1 infiltrate in CCC myocardium is essentially unopposed by regulatory cells or cytokines, suffering little regulation. This lack of regulation could explain the destructiveness of the inflammatory infiltrate, most likely due to excessive collateral damage by IFN-γ-producing T cells as described in acutely *T. cruzi*-infected mice. IFN-γ is thus considered the culprit of CCC. We believe the unopposed IFN-γ action is linked to the fact that DTGs that reduce IFN-γ production and/or Th1 T cell differentiation IL10, Eb3/IL27p28, and genes involved in IL-17 signaling are downregulated in CCC patients.

The immunomodulatory profile of ASY patients, with increased levels of DTG and lower levels of the PRG IFN-γ, indicates that ASY patients are in a state of disease tolerance. It is interesting to notice that the majority of chronic Chagas disease patients ~60% are disease-tolerant ASY patients. This is in line with evolutionary studies indicating that selection of DTG is dominant over PRG, leading to higher frequencies of individuals expressing DTG than PRG.

### DELETEROUS EFFECTS OF IFN-γ

While IFN-γ can control parasites, excessive levels can cause tissue damage and death in the acute and chronic phases. IFN-γ effectively regulates the expression of over 1,000 genes through activation of Janus tyrosine kinase (JAK) and phosphorylation of transducer and activator of transcription 1 (STAT-1) pathway, along with other mechanisms (88, 89). Among key IFN-γ-inducible inflammatory genes are TNF-α, and several other inflammatory cytokines and chemokines, interferon-inducible
factor 1 (IRF1) and other PRG, including inducible nitric oxide synthase (NOS2) (90). Multiple findings suggest that IFN-γ plays a central pathogenic role in the myocarditis and heart failure in CCC patients. Systemic T. cruzi persistence drives continued production of IFN-γ by T cells, important for parasite control through NOS production, as well as activating ROS through induction of NADP oxidases and mitochondrial ROS through NF-κB (47, 48). However, T. cruzi is highly resistant to ROS; IFN-γ and the accompanying NOS and ROS can also induce severe disturbances of heart function. IFN-γ can modulate gene expression in immune cells and cardiomyocytes. A significant proportion of genes modulated in the CCC myocardium and hearts from acutely infected mice are inducible by IFN-γ (86, 91).

Significantly, transgenic mice overexpressing IFN-γ develop a TNF-α-dependent myocarditis and cardiomyopathy (92, 93). In cardiomyocytes, IFN-γ treatment reduced contractility, induced NO/peroxynitrite-dependent cardiomyocyte apoptosis, reduced cardiomyocyte area, and also induced atrial natriuretic factor and production of chemokines CCL3, CCL5, and CXCL1 (94–96). IFN-γ regulates cardiac fibrosis by increasing fibroblast proliferation, production of hyaluronan and metalloproteinases 2 and 9 (95, 97, 98).

**IFN-γ AND MITOCHONDRIA: DANGEROUS LIAISONS**

Evidence of mitochondrial dysfunction has been found in hearts of animal models of Chagas disease, as well as the myocardium of CCC patients. This is especially relevant for CCC pathogenesis, since mitochondrial dysfunction is a paramount feature of heart failure of diverse etiologies (99). Nisha Garg and her group pioneered and studied in detail mitochondrial dysfunction in hearts of murine models of acute and chronic Chagas disease and in vitro infection models (100). Regarding mitochondrial damage in human CCC, our group described altered expression of mitochondrial genes and 16S mitochondrial rRNA in CCC heart lesions (57). We also found a selective reduction of protein expression of ATP synthase and creatine kinase activity—key mitochondrial energy metabolism enzymes—in CCC heart lesions (101). In agreement, mitochondrial DNA content was found to be reduced in CCC heart tissue (102), further indicating that mitochondrial function is compromised in CCC. Evidence indicates that many damaging effects of IFN-γ are secondary to promoting peroxynitrite-dependent and independent mitochondrial dysfunction and oxidative stress. IFN-γ effects on mitochondria include inhibition of the oxidative metabolism (103) an increased rate of ATP depletion (104) and inhibition of creatine kinase expression (105, 106). Moreover, IFN-γ+TLR ligand (LPS) treatment of cardiomyocytes does not T. cruzi infection per se—downregulates expression of multiple mitochondrial genes. These genes were involved in the electron transport chain, mitochondrial fission, mitophagy, mitochondrial, and nuclear gene transcription (51). NF-kB activation is one of the main mechanisms of mitochondrial damage induced by IFN-γ. Although IFN-γ does not directly activate NF-kB, it enhances TNF-α-induced NF-kB nuclear translocation. IFN-γ/TNF-α-driven NF-kB activation is known to cause dissipation of the proton gradient and impairment of the mitochondrial membrane potential (MMP) and ATP synthesis, leading to apoptosis (106, 107). Persistent activation of NF-kB by IFN-γ/TNF-α has been described to potentiate ROS release, which can in turn increase tissue damage and mitochondrial energy imbalance (106). Indeed, inhibition of NF-kB has been shown to improve MMP with substantial decrease of NOS2/NO induction and ROS release. Taken together, these reports strongly suggest that IFN-γ may play a significant role in mitochondrial dysfunction and heart failure. Excessive mitochondrial ROS production by cardiomyocytes is considered as a central cause of heart failure (108, 109).

A significant crosstalk occurs between NF-kB and mitochondrion-protecting proteins. NF-kB signaling down-regulates sirtuin-1 (SIRT1) activity through the expression of IFN-γ, ROS, and NO (110). SIRT1, an antioxidant and anti-inflammatory protein, regulates the oxidative respiration and cellular survival and is highly expressed in the heart, acting as an inhibitor of NF-kB inflammatory signals (111). While NF-kB stimulates glycolytic energy flux in acute inflammation, SIRT1 inhibits NF-kB and enhances mitochondrial oxidative metabolism through 5′ AMP-activated protein kinase (AMPK) resulting in the resolution of inflammation (110). Treatment of T. cruzi-infected mice with SIRT1 and/or AMPK agonists SRT1720, resveratrol and metformin reduced myocardial NF-kB transcriptional activity, inflammation and oxidative stress, resulting in beneficial results for restoration of cardiac function.
Preserving Nrf2 activity was shown to arrest the mitochondrial and cardiac oxidative stress, cardiac fibrosis, and heart failure in murine *T. cruzi* infection (113). Nrf2 is the master regulator of the antioxidant response, a transcription factor controlling expression of hundreds of genes (114), and promotes mitochondrial biogenesis (115). HMOX1 (Heme oxygenase 1), a key effector of the Nrf2 antioxidative response, is upregulated in the hearts of acutely infected mice (91), but not in the myocardium of CCC patients. Furthermore, its paralog HMOX2 is downregulated in CCC myocardium (ECN and CC, submitted for publication), indicating that Nrf2-dependent antioxidative defenses are even lower in CCC myocardium than in acute *T. cruzi* myocarditis (91). It has been recently observed that mitochondrial damage due to any stimulus leads to inflammation. Figure 3 shows the interplay between IFN-γ, DTG, NF-kB, and its antagonists, mitochondrial protective factors SIRT1, Nrf2, and AMPK vs. the outcome of mitochondrial function and inflammation.

ROS activate NF-kB, and dysfunctional or damaged mitochondria release mitochondrial DAMPs (damage-associated molecular patterns) including mitochondrial DNA (mtDNA) leading to the activation of the TLR9/Myd88/NF-kB, cGas/Type I IFN, and NRLP3 inflammasome pathways (116). In summary, the release of mitochondrial DAMPs in response to initial inflammatory stimuli and infection can establish a sterile inflammation process, a self-perpetuating positive feedback loop of mitochondrial damage and inflammation. This process has been found to occur in heart failure of diverse etiologies, and has been called sterile cardiac inflammation (117). Such sterile inflammation could be a contributing factor for the maintenance of inflammation in the absence of *T. cruzi* and may potentiate inflammatory damage in CCC (Figure 4). Together, these results indicate that mitochondrial dysfunction and mitochondrial ROS production are major pathogenic factors and therapeutic targets in CCC.

### HOST GENETIC FACTORS ASSOCIATED TO DIFFERENTIAL DISEASE PROGRESSION

The finding that 30% of Chagas disease patients develop CCC suggested the participation of genetics in differential disease progression. This was reinforced by the finding of familial aggregation of cases of CCC in endemic area settings (118). CCC patients display a more intense inflammatory response...
than the ASY patients, who seem to have a more regulated immune response. Given the importance of inflammatory mechanisms for CCC pathogenesis, genetic susceptibility to CCC may result from functionally relevant genetic polymorphisms that lead to variations in the intensity of the innate or acquired immune response and in inflammatory cytokines and chemokines involved in the pathogenesis of the disease. Common genetic association studies compare frequencies of a genetic polymorphism (single nucleotide polymorphism, SNP) in a candidate gene (picked by the investigator) in two populations (typically disease and control). If a gene variant is more frequent in the disease group, it is said to be associated with the disease and assumed to confer risk toward developing disease. These studies show genetic contributions that are typically small, explaining <10% of the phenotype of complex, polygenic diseases like CCC. A SNP found to be associated with a disease may be directly connected to a phenotype (e.g., a polymorphism in a transcription factor-binding sequence in the promoter region, affecting gene expression) or merely a marker of a linked biologically relevant polymorphism. In the case of Chagas disease, we performed a thorough literature search at the PUBMED database and retrieved 145 association studies addressing polymorphisms in 76 genes, which disclosed 62 SNPs from 44 genes to be associated with CCC. From those, 9 SNPs/genes were associated with CCC severity—SNPs were more frequent among severe CCC patients, with significant left ventricular dysfunction (ejection fraction <40%), as compared to the remaining CCC patients.

In our literature search, we found that 7 genes with polymorphisms associated with CCC or severe CCC were also defined as PRG or DTG in mouse knockout infection studies (Table 2). The 5 PRG belonged to the TLR, Th1, or chemokine pathways/processes. Since TNF-α signaling is key in both the acute infection and CCC, we added TNFA and TNF-receptor alpha (TNFRSF1A) to this table, even though TNFA was only assessed in association studies (120–123, 132, 133), and TNFRSF1A only in knockout infection experiments (26). The two DTG Ebi3/IL27p28 and IL-10 both modulate IFN-γ via downregulation of IFN-γ production and Th1 differentiation. Such genes are central in the pathogenesis of CCC, as IFN-γ and TNF-α-producing cells migrate to CCC heart tissue in response to chemokines CCL2 and CCR5. While the−308 variant of TNFA has been associated with increased production, this has not been validated every time. Significantly, polymorphisms in several other genes belonging to the same pathways (TLR-IL12-IL18-IFNG-TNFA-NF-kB, chemokines/receptors, inflammasome including both IL1B, and IL18) have been associated with CCC.

The heterozygous TLR4-D299G/T399I genotype occurred more frequently in ASY infected subjects than CCC patients (OR = 5.4, 95% CI = 1.03–52.6, P = 0.02) (119). In humans, D299G/T399I reduces TLR4 responsiveness to lipopolysaccharide and associates with increased susceptibility and/or severity of numerous infectious and inflammatory diseases. The TIRAP variant S180L was found to be more prevalent in the ASY group than in the CCC group (OR = 0.31, 95%CI = 0.16–0.60, P = 0.0084) (134). Mutation leads to a decrease in signal transduction upon ligation of TLR2 or

### Table 2 | PRG and DTG gene polymorphisms associated with CCC, severe CCC, or death.

| Gene Type | Gene Name | Gene Type (KO studies) | Associated polymorphisms (patients) | Phenotype | Association study references |
|-----------|-----------|------------------------|-------------------------------------|-----------|-----------------------------|
| TLR PATHWAY | TLR4 | Toll Like Receptor 4 | PRG | D299G/T399I | CCC | (119) |
| TNF/TNFR | TNFA | Tumor necrosis factor alpha | PRG | rs1799984, rs1800629, TNFa−238, TNFa−308 | CCC | (120–122) |
| TNFA | Tumor necrosis factor alpha | PRG | ~308, TNFa2 | CCC - death | (123) |
| Th1/IFNγ | IL12B | Interleukin 12A | PRG | IL12B+1188 | CCC | (124) |
| IFNG | Interferon gamma | PRG | rs2430561 | CCC | (125, 126) |
| CHEMOKINES | CCL2 | C-C Motif Chemokine Ligand 2 | PRG | CCL2-2518, rs2530797, rs4586, rs3917891 | CCC | (127) |
| CCR5 | C-C Motif Chemokine Receptor 5 | PRG | CCR5+59029, rs1799987, rs2856758, rs2734648, rs3176763, rs11575815, rs1799988 | CCC | (87,127–129) |
| CCR5 | C-C Motif Chemokine Receptor 5 | PRG | rs1800024 | CCC - severity | (130) |
| IFNγ-INHIBITING GENES | IL10 | Interleukin 10 | DTG | IL10−1082 | CCC | (131) |
| EBI3 | Epstein-Barr Virus Induced 3/IL-27p28 | DTG | rs4740, rs4905 | CCC severity | (39) |

CCC severity, associated with CCC with ventricular dysfunction (Left ventricular ejection fraction <40%).
TLR4 to their respective ligands. Weitzel et al. have shown a similar trend of association for TLR4 (119). A similar study on a large Brazilian population found that TIRAP rs8177376A/A is associated with an increased susceptibility to CCC (OR = 1.36; 95% CI = 1.19–1.80, P = 0.037) (127). This same TIRAP variant confers protection against tuberculosis, pneumococcal disease, and bacteremia (135). Polymorphisms in the TNFA-LTA region have been extensively studied. TNFA–LTA polymorphisms (~308, ~244, and ~238) were studied on a Peruvian cohort with negative results (132), while it was found to be associated in a Mexican population (OR = 3.03, 95% CI = 1.29–7.12, P = 0.008) (120). Pissetti et al. have shown that the LTA (+252) polymorphism was significantly associated with Chagas disease as compared with healthy seronegative individuals in a Brazilian population (133). Pissetti et al. have shown that this allele is associated with higher TNF-α production in Brazilian Chagas disease patients as compared to healthy seronegative individuals (OR = 1.846, 95% CI = 1.057–3.223, P = 0.03) (121). Similar results were detected on a Colombian cohort (122). Severe CCC patients with significant ventricular dysfunction carrying the susceptibility genotypes TNFA–308 or TNFA A2 microsatellite display a significantly shorter survival time than those carrying other alleles (OR = 0.51, 95% CI = 0.27–0.97, P = 0.04) (123). CCL2 is an important chemokine attracting monocytes/macrophages to inflamed tissue. The plasma concentrations CCL2 are associated with myocardial dysfunction in patients with severe CCC, dilated cardiomyopathy and acute myocardial infarction (55, 136, 137). Ramasawmy et al. have shown on their Brazilian population that the variant at position CCL2–2518A allele conferred susceptibility to CCC in a Brazilian population (OR = 4.1; 95% CI = 1.7–11; P = 0.001) (138), a result that was subsequently confirmed (rs2530797: OR = 1.46; 95% CI = 1.11–1.92, P = 0.007) (127). Multiple studies have identified associations between different polymorphisms in the CCR5 gene (87, 127–129, 139–142). A polymorphism in IFNG rs2430561 was found to be associated with CCC (126). The IL12B +1188C allele was found to be associated with CCC in a similar population (OR = 3.39, 95% CI = 1.3–9.15, P = 0.015) (124).

Finally, genes tagged as DTG were also associated in genetic studies. The functional IL10 gene polymorphism –1082G/A is associated with the development of CCC in a Brazilian population (OR = 0.84, 95% CI = 1.44–4.95, p < 0.01) (131). Similar data were obtained in Colombia (143). Ebi3/IL-27p28 regulates IFN-γ-mediated myocarditis by promoting an anti-inflammatory environment through IL-10. Medina et al. has shown that Ebi3 polymorphism (SNP rs4740) is less frequent in patients with severe cardiopathy (6.6%) compared to patients with the indeterminate form (OR 3.157, 95% CI = 1.15–8.64, p = 0.025) (39).

### Does T. cruzi Genetic Variability Play a Role in Determining the Outcome of Human Chagas Disease?

In addition to host genetic susceptibility factors, genetic variation in T. cruzi may also play a role in the outcome of Chagas disease. In animal models of infection, different T. cruzi strains show very distinct patterns of acute phase death, induction/activation of cytokines, PRGs and DTGs, tissue tropism, and development of chronic infection (144, 145).

Major subgroups of T. cruzi genetic variability have been classified into 6 discrete type units (DTU): TcI to TcVI, each with a distinct geographical distribution. It has been reported that TcI (Colombian) and TcII (Y) strains differentially activate human monocytes (146). However, there was no difference in the DTUs found in the CCC and the ASY groups in a same geographical region; heart disease is caused by the DTUs that are prevalent in a given region (147–150). Although DTUs were not clearly associated with Chagas disease outcomes, T. cruzi genetic complexity surpasses the simple DTU classification. It is likely that yet undisclosed genetic variants of T. cruzi may influence the development of CCC, as shown in mice (145), which will certainly interact with the host genome to lead to different outcomes. Infection of BALB/c or C57BL/6 mice leads to differential activation of PRGs (151).

### CONCLUDING REMARKS

The finding that all identified T. cruzi DTGs converge on the inhibition of IFN-γ production and/or Th1 cell differentiation indicates the importance of this homeostatic control to avoid IFN-γ-induced death in the acute phase. Downmodulation of the IFN-γ response by DTGs favors T. cruzi evasion and the establishment of chronic infection. Among chronically infected patients, disease progression is associated with distinct immune profiles. In chronically infected ASY patients, IFN-γ production levels are adequately modulated by DTG IL10, Ebi3/IL27p28, Th17/IL17RA signaling, along with Treg, promoting a state of disease tolerance. Conversely, in CCC, an imbalance between DTG and IFNG causes increased IFN-γ production associated with inflammatory cardiac damage. Excessive myocardial IFN-γ signaling can lead to mitochondrial dysfunction. In turn this leads to energy imbalance, impairment of contractile function. Mitochondrial dysfunction induces further inflammation, in a positive feedback loop that can help perpetuate myocardial inflammation in CCC.

### AUTHOR CONTRIBUTIONS

EC-N and CC participated in conceptual design, manuscript drafting, and revision. JN and RA participated in manuscript drafting, and revision. AF, RP, and MN participated in manuscript drafting. JK participated in manuscript revision.

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REFERENCES

1. He X, Berland R, Mekasha S, Christensen TG, Alroy J, Kramnik I, et al. The st1 resistance locus regulates evasion of type I interferon signaling by Chlamydia pneumoniae as a disease tolerance mechanism. PLoS Pathog. (2013) 9:e1003569. doi: 10.1371/journal.ppat.1003569

2. Savan R, Ravichandran S, Collins JR, Sakai M, Young HA. Structural and functional conservation of interferon gamma among vertebrates. Cytokine Growth Factor Rev. (2009) 20:115–24. doi: 10.1016/j.cytogfr.2009.02.006

3. Soares MP, Goelzello R, Weiss T. Tissue damage control in disease tolerance. Trends Immunol. (2014) 35:483–94. doi: 10.1016/j.it.2014.08.001

4. Roy BA, Kirchner JW. Evolutionary dynamics of pathogen resistance and tolerance. Evolution (2000) 54:51–63. doi: 10.1554/0014-3820(2000)054[0051:EDOPRA]2.0.CO;2

5. Harris LD, Tabb B, Sodora DL, Piaidini M, Klatt NR, Douek DC, et al. Downregulation of robust acute type I interferon responses distinguishes nonpathogenic simian immunodeficiency virus (SIV) infection of natural hosts from pathogenic SIV infection of rhesus macaques. J Virol. (2010) 84:7886–91. doi: 10.1128/JVI.02612-09

6. Harris LD, Klatt NR, Vinton C, Briant JA, Tabb B, Ladell K, et al. Mechanisms underlying γ T-cell subset perturbations in SIV-infected Asian rhesus macaques. Blood (2010) 116:4148–57. doi: 10.1182/blood-2010-05-283549

7. Iannone AM, Xavier SC, Roque AL. The multiple and complexable scenarios of the Trypanosoma cruzi transmission cycle in the sylvatic environment. Acta Trop. (2015) 151:1–15. doi: 10.1016/j.actatropica.2015.07.018

8. Oliveira AC, de Alencar BC, Tzelepis F, Klezewsky W, da Silva RN, Neves FS, et al. Impaired innate immunity in Thr4(-/-) mice but preserved CD8+ T cell responses against Trypanosoma cruzi in Thr4+, Thr2-, Thr- and Thr8-deficient mice. PLoS Pathog. (2010) 6:e1000870. doi: 10.1371/journal.ppat.1000870

9. Caetano BC, Carmona 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) 187:1903–11. doi: 10.4049/jimmunol.11003911

10. Bafica A, Santiago HC, Goldszmid R, Roptet 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) 177:3515–9

11. Campos MA, Closel M, Valente EP, Cardoso JE, Jirásek A, Alvarez-Leite JJ, et al. Impaired production of proinflammatory cytokines and host resistance to acute infection with Trypanosoma cruzi in mice lacking functional myeloid differentiation factor 88. J Immunol. (2004) 172:1711–8. doi: 10.4049/jimmunol.172.3.1711

12. Koga R, Hamano S, Kuwata H, Arashita K, Ogawa M, Hisaeda H, et al. TLR-dependent induction of IFN-beta mediates host defense against Trypanosoma cruzi. J Immunol. (2006) 177:7059–66. doi: 10.4049/jimmunol.177.7.7059

13. Gonçalves VM, Matteucci KC, Buzo CL, Miotto BH, Ferrante D, Torreilhas AC, et al. NLPR3 controls Trypanosoma cruzi infection through a caspase-1-dependent IL-1R-independent NO production. PLoS Negl Trop Dis. (2013) 7:e2469. doi: 10.1371/journal.pntd.0002469

14. Gao W, Pereira MA. Interleukin-6 is required for parasite specific response and host resistance to Trypanosoma cruzi. Int J Parasitol. (2002) 32:167–70. doi: 10.1016/S0140-525X(01)00322-8

15. Michailowsky V, Silva NM, Rocha CD, Vieira LQ, Lannes-Vieira J, Gazzinelli RT. Pivotal role of interleukin-12 and interferon-gamma axis in controlling tissue parasitism and inflammation in the heart and central nervous system during Trypanosoma cruzi infection. Am J Pathol. (2001) 159:1723–33. doi: 10.1016/S0002-9440(10)63019-2

16. Müller U, Köhler G, Mossmann H, Schaub GA, Alber G, Di Santo JP, et al. IL-12-independent IFN-gamma production by T cells in experimental Chagas’ disease is mediated by IL-18. J Immunol. (2001) 167:3346–53. doi: 10.4049/jimmunol.167.6.3346

17. Bombeiro AL, Gonçalves LA, Penha-Gonçalves C, Marinho CR, D’Imprèrio Lima MR, Chadi G, et al. IL-12p40 deficiency leads to uncontrolled Trypanosoma cruzi dissemination in the spinal cord resulting in neuronal death and motor dysfunction. PLoS ONE (2012) 7:e49022. doi: 10.1371/journal.pone.0049022

18. Erdmann H, Rossagel C, Bohme J, Iwakura Y, Jacobs T, Schaible UE, et al. IL-17A promotes macrophage effector mechanisms against Trypanosoma cruzi by trapping parasitoids in the endosomes of compartment. Immunobiology (2013) 218:910–23. doi: 10.1016/j.imbio.2012.10.005

19. Miyazaki Y, Hamano S, Wang S, Shimamoe Y, Iwakura Y, Yoshida H. IL-17 is necessary for host protection against acute-phase Trypanosoma cruzi infection. J Immunol. (2010) 185:1150–7. doi: 10.4049/jimmunol.0900047

20. Martins GA, Vieira LQ, Cunha FQ, Silva JS. Gamma interferon modulates CD95 (Fas) and CD95 ligand (Fas-L) expression and nitric oxide-induced apoptosis during the acute phase of Trypanosoma cruzi infection: a possible role in immune response control. Infect Immun. (1999) 67:3864–71

21. Cummings KL, Tarleton RL. Inducible nitric oxide synthase is not essential for control of Trypanosoma cruzi infection in mice. Infect Immun. (2004) 72:4081–9. doi: 10.1128/iai.72.7.4081-4089.2004

22. Arantes RM, Marche HH, Bahia MT, Cunha FQ, Rossi MA, Silva JS. Interferon-gamma-induced nitric oxide causes intrinsic intestinal derangement in Trypanosoma cruzi-infected mice. Am J Pathol. (2004) 164:1361–8. doi: 10.1002/ajpath.1063222-1

23. Caniato BC, Closel M, Rey JA, Rottet C, Arentes RM, Cheever A, et al. Mice deficient in LRG-47 display enhanced susceptibility to Trypanosoma cruzi infection associated with defective hemopoiesis and intracellular control of parasite growth. J Immunol. (2005) 175:8165–72. doi: 10.4049/jimmunol.175.12.8165

24. Kulkaarni MM, Varikuti S, Terrazas C, Kimble JL, Satoskar AR, McGwire BS. Signal transducer and activator of transcription 1 (STAT-1) plays a critical role in control of Trypanosoma cruzi infection. Immunology (2015) 145:225–31. doi: 10.1111/jimm.12438

25. Tarleton RL, Grusby MJ, Zhang L. Increased susceptibility of Stat4-deficient and enhanced resistance in Stat6-deficient mice to infection with Trypanosoma cruzi. J Immunol. (2000) 165:1520–7. doi: 10.4049/jimmunol.165.3.1520

26. Pérez AR, Roggero E, Nicora A, Palazzi J, Besedovsky HO, Del Rey A, et al. Thymus atrophy during Trypanosoma cruzi infection. J Leukoc Biol. (2009) 86:1239–46. doi: 10.1189/jlb.0309187
31. 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) 191:627–36. doi: 10.1086/427515

32. Hardison IL, Wrightsman RA, Carpenter PM, Kuziel WA, Lane TÉ, Manning JE. The CC chemokine receptor 5 is important in control of parasite replication and acute cardiac inflammation following infection with *Trypanosoma cruzi*. *Infect Immun.* (2006) 74:1335–43. doi: 10.1128/IAI.74.1.133-143.2006

33. Michailowsky V, Celes MR, Marino AP, Silva AA, Vieira LQ, Rossi MA, et al. Intercellular adhesion molecule 1 deficiency limits impaired recruitment of T lymphocytes and enhanced host susceptibility to infection with *Trypanosoma cruzi*. *J Immunol.* (2004) 173:463–70. doi: 10.4049/jimmunol.173.1.463

34. Martins GA, Campanneli AP, Silva RB, Tadokoro CE, Russo M, Cunha FQ, et al. CD28 is required for T cell activation and IFN-gamma production by CD4+ and CD8+ T cells in response to *Trypanosoma cruzi* infection. *Microbes Infect.* (2004) 6:1133–44. doi: 10.1016/j.micinf.2004.07.002

35. Talvani A, Santana G, Barcelos LS, Ishii S, Shimizu T, Romania AF, et al. Experimental *Trypanosoma cruzi* infection in platelet-activating factor receptor-deficient mice. *Microbes Infect.* (2003) 5:789–96. doi: 10.1016/S1286-4579(03)00146-1

36. Benatar AF, García GA, Bua J, Ceriliani JP, Postan M, Tasso LM, et al. Galectin-1 prevents infection and damage induced by *Trypanosoma cruzi* on cardiac cells. *PLoS Negl Trop Dis.* (2015) 9:e0004148. doi: 10.1371/journal.pntd.0004148

37. Sharma J, Eickhoff CS, Hoft DE, Ford DA, Gross RW, McHowat J. The absence of myocardial calcium-independent phospholipase A2gamma results in impaired prostaglandin E2 production and decreased survival in mice with acute *Trypanosoma cruzi* infection. *Infect Immun.* (2013) 81:2278–87. doi: 10.1128/iai.00497-12

38. Silva MC, Davoli-Ferreira M, Medina TS, Sesti-Costa R, Silva GK, Lopes CD, et al. Canonical PI3Kγ signaling in myocardial cells restricts *Trypanosoma cruzi* infection and dampens chagasic cardiomyopathy. *Nat Commun.* (2019) 9:1513. doi: 10.1038/s41467-018-03986-3

39. Medina TS, Oliveira GG, Silva MC, David BA, Silva GK, Fonseca DM, et al. Ebi3 prevents *Trypanosoma cruzi*-induced myocarditis by dampening IFN-y-driven inflammation. *Front Immunol.* (2017) 8:1213. doi: 10.3389/fimmu.2017.01213

40. Holscher C, Mohrs M, Dai WJ, Köhler G, Ryffel B, Schaub GA, et al. Defective nitric oxide effector functions lead to extreme susceptibility of *Trypanosoma cruzi*-infected mice deficient in gamma interferon receptor or inducible nitric oxide synthase. *Infect Immun.* (1998) 66:1208–15.

41. Wuy Antony S, Juhasz A, Lu J, Ge Y, Jiang G, et al. Up-regulation and sustained activation of Stat1 are essential for interferon-gamma (IFN-gamma)-induced dual oxidase 2 (Duox2) and dual oxidase A2 (Duox2A) expression in human pancreatic cancer cell lines. *J Biol Chem.* (2011) 286:12245–56. doi: 10.1074/jbc.M111.191301

42. Rakshit S, Chandrasekar BS, Saha B, Victor E, Majumdar S, Nandi D. Interferon-gamma induced cell death: regulation and contributions of nitric oxide, c-Jun N-terminal kinase, reactive oxygen species and peroxynitrite. *Biochim Biophys Acta.* (2014) 1843:2645–61. doi: 10.1016/j.bbamc.2014.06.014

43. Hunter CA, Ellis-Neyes LA, Slifer T, Kanaly S, Grünig G, Fort M, et al. Interleukin-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *Infect Immun.* (2006) 286:6627–40. doi: 10.1074/jbc.M111.167247

44. Koo SJ, Chowdhury HJ, Szczesny B, Wan X, Garg NJ. Macrophages promote oxidative metabolism to drive nitric oxide generation in response to *Trypanosoma cruzi*. *Infect Immun.* (2016) 84:3527–41. doi: 10.1128/IAI.00809-16

45. Schroeder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol.* (2004) 75:163–89. doi: 10.1189/jlb.0603252

46. Marinho CR, Nuñez-Apaza LN, Martinez-Santos R, Bastos KR, Bompérol AL, Bucci DZ, et al. IFN-gamma, but not nitric oxide or specific IgG, is essential for the in vivo control of low-virulence Sylvio X10/4 *Trypanosoma cruzi* parasites. *Scand J Immunol.* (2007) 66:297–308. doi: 10.1111/j.1365-3083.2007.01958.x

47. Schroeder K, Lichtinger M, Irvine KM, Brion K, Trieu A, Ross IL, et al. Protective role of interferon-gamma-signaling in parasite-infected cells. *Circulation* (2014) 130:e0004148. doi: 10.1161/circulationaha.113.003510

48. Cunha-Neto E, Dzau VJ, Allen PD, Stamatiou D, Benvenutti L, Higuchi ML, et al. Ebi3 prevents Trypanosoma cruzi-driven inflammation. *Front Immunol.* (2017) 8:1577–90. doi: 10.3389/fimmu.2017.01577

49. Talvani A, Rocha MO, Barcelos LS, Gomes YM, Ribeiro AL, Teixeira MM. Elevated concentrations of CCL2 and tumor necrosis factor-alpha in chagas cardiomyopathy. *Clin Infect Dis.* (2004) 38:943–50. doi: 10.1086/381892

50. Silva RR, Mariente RM, Silva AA, dos Santos AL, Roiff E, Santiago H, et al. IFN-gamma regulates the expression of cytokines by *Trypanosoma cruzi* infection. *PLoS ONE* (2015) 10:e0118600. doi: 10.1371/journal.pone.0118600

51. Cunha-Neto E, Dzau VJ, Allen PD, Desta M, Benvenuti L, Higuchi ML, et al. Cardiac gene expression profiling provides evidence for cytokinopathy as a molecular mechanism in Chagas’ disease cardiomyopathy. *Am J Pathol.* (2005) 167:305–13. doi: 10.1016/S0002-9440(10)62976-8

52. Machado FS, Martins GA, Alberti JC, Mestriner FL, Cunha FQ, Silva JS. *Trypanosoma cruzi*-infected cardiomyocytes promote chemokines and cytokines that trigger potent nitric oxide-dependent trypanocidal activity. *Circulation* (2000) 102:3003–8. doi: 10.1161/01.cir.102.24.3003

53. Vespa GN, Cunha FQ, Silva JS. Nitric oxide is involved in control of Trypanosoma cruzi-infected parasitemia and directly kills the parasite in vitro. *Infect Immun.* (1994) 62:5177–82.

54. Cai CW, Blase JR, Zhang X, Eickhoff CS, Hoft DF. Th17 cells are more protective than Th1 cells against the intracellular parasite *Trypanosoma cruzi*. *PLoS Pathog.* (2016) 12:e1005902. doi: 10.1371/journal.ppat.1005902

55. Gu C, Wu L, Li X. IL-17 family: cytokines, receptors and signaling. *J Biol Chem.* (2013) 288:677–85. doi: 10.1074/jbc.2013.07.022

56. Hamano S, Himeno K, Miyazaki Y, Ishii K, Yamanaka A, Takeda A, et al. WSX-1 is required for resistance to *Trypanosoma cruzi* infection. *J Immunol.* (2013) 189:657–67.

57. Vila-del Sol V, Peñón C, Fresno M. IFN-gamma-induced TNF-alpha expression is regulated by interferon regulatory factors 1 and 8 in mouse macrophages. *J Immunol.* (2008) 181:4461–70.

58. Holscher C, Köhler G, Müller U, Mommßn H, Schaubs GA, Brombacher F. Defective nitric oxide effector functions lead to extreme susceptibility of *Trypanosoma cruzi*-infected mice deficient in gamma interferon receptor or inducible nitric oxide synthase. *Infect Immun.* (1998) 66:1208–15.

59. Abrahamsohn IA, Coffman RL. IFN-γ promotes infection of astrocytes by *Trypanosoma cruzi*. *Infect Immun.* (2004) 38:943–50. doi: 10.1086/381892
Chevillard et al. PRG/DTG Balance in Chagas Disease

66. da Matta Guedes PM, Gutierrez FR, Maia FL, Milanezi CM, Silva GK, Pavaneli WR, et al. IL-17 produced during Trypanosoma cruzi infection plays a central role in regulating parasite-induced myocarditis. PLoS Negl Trop Dis. (2010) 4:e604. doi: 10.1371/journal.pntd.0000604

67. Sieve AN, Meeks KD, Lee S, Berg RE. A new immunoregulatory function for IL-23: inhibition of IL-12-dependent IFN-γ production. Eur J Immunol. (2010) 40:2236–47. doi: 10.1002/eji.200939575

68. Aggarwal S, Ghlardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4+ T cell activation state characterized by the production of interleukin-17. J Biol Chem. (2003) 278:910–4. doi: 10.1074/jbc.M207577200

69. Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol. (2014) 14:585–600. doi: 10.1038/nri3707

70. Böhme J, Roeffner C, Jacobs T, Behrends J, Hölscher C, Erdmann H. Epstein-Barr virus–induced gene 3 suppresses T helper type 1, type 1, and type 2 immune responses after Trypanosoma cruzi infection and inhibits parasite replication by interfering with alternative macrophage activation. Immunology (2016) 147:338–48. doi: 10.1111/imm.12565

71. Diel S, Rincón M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol. (2002) 39:531–6. doi: 10.1016/S0161-5890(02)00210-9

72. Fernandes MC, Andrews NW. Host cell invasion by Trypanosoma cruzi: a unique strategy that promotes persistence. FEMS Microbiol Rev. (2012) 36:734–47. doi: 10.1111/j.1574-6976.2012.02033.x

73. Cardoso MS, Reis-Cunha R, Bartholomeu DC. Evasion of the immune response by Trypanosoma cruzi during acute infection. Front Immunol. (2015) 5:6659. doi: 10.3389/fimmu.2015.00659

74. Rodrigues MM, Oliveira AC, Bellio M. The immune response to Trypanosoma cruzi: role of Toll-like receptors and perspectives for vaccine development. J Parasitol Res. (2012) 2012:507874. doi: 10.1155/2012/507874

75. De Alba-Alvarado M, Salazar-Schettino PM, Jiménez-Álvarez L, Cabrera-Bravo M, García-Sancho C, Zenteno E, et al. Th-17 cytokines are associated with severity of Trypanosoma cruzi chronic infection in pediatric patients from endemic areas of Mexico. Acta Trop. (2018) 178:134–41. doi: 10.1016/j.actatropica.2017.11.009

76. Bestetti RB, Muccillo G. Clinical course of Chagas' heart disease: a comparison with dilated cardiomyopathy. Int J Cardiol. (1997) 60:187–93.

77. Sousa GR, Gomes JA, Damasio MP, Nunes MC, Costa HS, Medeiros NL, et al. The role of interleukin-17-mediated immune response in Chagas disease: high level is correlated with better left ventricular function. PLoS ONE (2017) 12:e0172833. doi: 10.1371/journal.pone.0172833

78. Iguchi Mde L, Gutierrez PS, Aiello VD, Palomino S, Bocchi E, Kalil J, Reis DD, Jones EM, Tostes S Jr, Lopes ER, Gazzinelli G, Colley DG, et al. Myocardial chemokine expression and intensity of myocarditis in chagas cardiomyopathy are controlled by polymorphisms in CXCL9 and CXCL10. PLoS Negl Trop Dis. (2012) 6:e1816. doi: 10.1371/journal.pntd.0001867

79. Higuchi Mde L, Gutierrez PS, Aiello VD, Palomino S, Bocchi E, Kalil J, Reis DD, Jones EM, Tostes S Jr, Lopes ER, Gazzinelli G, Colley DG, et al. Cardiac gene expression profiling provides evidence for cytokinopathy as a molecular mechanism in Chagas’ disease cardiomyopathy. Am J Pathol. (2005) 167:305–13. doi: 10.1016/j.ajpath.2004.10.029

80. Nogueira LG, Santos RHB, Ianni BM, Fiorelli AL, Mairena EC, Benvenuti LA, et al. Myocardial chemokine expression and intensity of myocarditis in Chagas cardiomyopathy are controlled by polymorphisms in CXCL9 and CXCL10. PLoS Negl Trop Dis. (2012) 6:e1816. doi: 10.1371/journal.pntd.0001867

81. Ehrt S, Schnappiger D, Bekiranov S, Drenkov J, Shi S, Gingeras TR, et al. Reprogramming of the macrophage transcriptome in response to interferon-gamma and Mycobacterium tuberculosis: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. J Exp Med. (2001) 194:1123–40. doi: 10.1084/jem.194.8.1123

82. Levick SP, Goldspink PH. Could interferon-gamma be a therapeutic target for treating heart failure? Heart Fail Rev. (2014) 19:227–36. doi: 10.1007/s10741-013-9393-8

83. Krause CD, He W, Kotenko S, Pestka S. Modulation of the activation of Stat1 by the interferon-gamma receptor complex. Cell Res. (2006) 16:113–23. doi: 10.1038/sj.cr.7100115

84. Ferreira LRP, Ferreira FM, Laugier L, Cabantous S, Navarro IC, da Silva Cândido D, et al. Integration of miRNA and gene expression profiles suggest a role for miRNAs in the pathobiological processes of acute Trypanosoma cruzi infection. Sci Rep. (2017) 7:17199. doi: 10.1038/s41598-017-18080-9

85. Reifenberg K, Lehr HA, Torzewski M, Steige G, Wiese E, Küpper I, et al. Interferon-gamma induces chronic active myocarditis and cardiomyopathy in transgenic mice. Am J Pathol. (2007) 171:463–72. doi: 10.2353/ajpath.2007.060906

86. Torzewski M, Wenzel P, Kleinert H, Becker C, El-Masri J, Wiese E, et al. Chronic inflammatory cardiomyopathy of interferon γ-overexpressing transgenic mice is mediated by tumor necrosis factor-α. Am J Pathol. (2012) 180:73–81. doi: 10.1016/j.ajpath.2011.09.006

87. Patten M, Krämer E, Bünemann J, Wenck C, Thoenes M, Wieland T, et al. Endotoxin and cytokines alter contractile protein expression in cardiac myocytes in vivo. Pflugers Arch. (2001) 442:920–7. doi: 10.1007/s0042401010621

88. Wang Z, Jiang B, Brecher P. Selective inhibition of STAT3 phosphorylation by sodium salicylate in cardiac fibroblasts. Biochem Pharmacol. (2002) 63:1197–207. doi: 10.1016/S0006-2952(02)00853-5

89. Chae HJ, Ha KC, Kim DS, Cheung GS, Kwak YG, Kim HM, et al. Catalase protects cardiomyocytes via its inhibition of nitric oxide synthesis. Nitric Oxide (2006) 14:189–99. doi: 10.1016/j.niox.2005.11.008

90. Dai B, Cui M, Zhu M, Su WL, Qiu MC, Zhang H. STAT1/3 and ERK1/2 synergistically regulate cardiac fibrosis induced by high glucose. Cell Physiol Biochem. (2013) 32:960–71. doi: 10.1159/000345499

91. Wu AL, Laffrerie RM, Park C, Apinhasmit W, Chen ZJ, Birkedal-Hansen H, et al. Modulation of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) by interferon-gamma in a human salivary gland cell line. J Cell Physiol. (1997) 171:117–24. doi: 10.1002/(SICI)1097-4652(199705)171:2<117::AID-JCP1>3.0.CO;2-R

92. Brown DA, Perry JB, Allen ME, Sabbath HN, Stauffer BL, Stauffer HN. Interleukin-17 as a molecular mechanism in Chagas disease. PLoS Pathog. (2016) 12:e1005954. doi: 10.1371/journal.ppat.1005954
101. Teixeira PC, Santos RH, Fiorelli AI, Bilate AM, Benvenuti LA, Stolf NA, et al. Selective decrease of components of the creatine kinase system and ATP synthase complex in chronic Chagas disease cardiomyopathy. PLoS Negl Trop Dis. (2011) 5:e205. doi: 10.1371/journal.pntd.0002105

102. Wan X, Gupta S, Zago MP, Davidson MM, Dousset P, Amoroso A, et al. Defects of mtDNA replication impaired mitochondrial biogenesis during Trypanosoma cruzi infection in human cardiomyocytes and chagasic patients: the role of Nrfl2 and antioxidant response. J Am Heart Assoc. (2012) 1:e003855. doi: 10.1161/JAHA.112.003855

103. Luss H, Watkins SC, Freewick PD, Imro AK, Nussler AK, Billiar TR, et al. Characterization of inducible nitric oxide synthase expression in endotoxin rat cardiac myocytes in vivo and following cytokine exposure in vitro. J Mol Cell Cardiol. (1995) 27:25–29.

104. Wang D, McMillin JB, Bick R, Buja LM. Response of the neonatal rat cardiomyocyte in culture to energy depletion: effects of cytokines, nitric oxide, and heat shock proteins. Lab Invest. (1996) 75:809–18.

105. Kalovidováres AI, Plotkin Z, Graesser D. Interferon-gamma inhibits proliferation, differentiation, and creatine kinase activity of cultured human muscle cells. II. A possible role in myositis. J Rheumatol. (1995) 20:1718–23.

106. Lee HJ, Oh YK, Rhee M, Lim JY, Hwang JY, Park YS, et al. Zorova LD, Popkov V A, Plotnikov EY, Silachev DN, Pevzner IB, Jankauskas A, et al. Mitochondrial membrane potential. J Mol Biol. (2005) 369:967–84. doi: 10.1016/j.jmb.2007.03.072

107. Caruso R, Marafini I, Franzè E, Stolfi C, Zorzi F, Monteleone I, et al. Defective Nrf2 regulates ROS production by mitochondria in cardiomyocytes in culture to energy depletion: effects of cytokines, nitric oxide, and heat shock proteins. Lab Invest. (1996) 75:809–18.

108. Wan X, Gupta S, Zago MP, Davidson MM, Dousset P, Amoroso A, et al. Defects of mtDNA replication impaired mitochondrial biogenesis during Trypanosoma cruzi infection in human cardiomyocytes and chagasic patients: the role of Nrfl2 and antioxidant response. J Am Heart Assoc. (2012) 1:e003855. doi: 10.1161/JAHA.112.003855

109. Paiva CN, Medei E, Bozza MT. ROS and NADPH oxidase. Biochim Biophys Acta. (2015) 1850:794–801. doi: 10.1016/j.bjbiol.2014.11.021

110. Pissetti CW, de Oliveira RF, Correia D, de Oliveira RF, Llaguno MM, Balarin MA, et al. Defects of mtDNA replication impaired mitochondrial biogenesis during Trypanosoma cruzi infection in human cardiomyocytes and chagasic patients: the role of Nrfl2 and antioxidant response. J Am Heart Assoc. (2012) 1:e003855. doi: 10.1161/JAHA.112.003855

111. Rodríguez-Pérez JM, Cruz-Robles D, Hernández-Pacheco G, Pérez-Hernández N, Murguia LE, Granados J, et al. Tumor necrosis factor-alpha promoter polymorphism in Mexican patients with Chagas' disease. Immunol Lett. (2005) 98:97–102. doi: 10.1016/j.imlet.2004.10.017
139. Calzada JE, Nieto A, Beraún Y, Martín J. Chemokine receptor CCR5 polymorphisms and Chagas’ disease cardiomyopathy. *Tissue Antigens*. (2001) 58:154–8. doi: 10.1034/j.1399-0039.2001.580302.x

140. Fernández-Mestre MT, Montagnani S, Layrisse Z. Is the CCR5-59029-G/G genotype a protective factor for cardiomyopathy in Chagas disease? *Hum Immunol.* (2004) 65:725–8. doi: 10.1016/j.humimm.2004.05.002

141. de Oliveira AP, Ayo CM, Bestetti RB, Mattos LC. The role of CCR5 in Chagas disease - a systematic review. *Infect Genet Evol.* (2016) 45:132–7. doi: 10.1016/j.meegid.2016.08.012

142. Stein RW, Whelan J. Insulin gene enhancer activity is inhibited by adenovirus 5 E1a gene products. *Mol Cell Biol.* (1989) 9:4531–4.

143. Flórez O, Martín J, González CI. Interleukin 4, interleukin 4 receptor-α and interleukin 10 gene polymorphisms in Chagas disease. *Parasite Immunol.* (2011) 33:506–11. doi: 10.1111/j.1365-3024.2011.01314.x

144. Meza SK, Kaneshima EN, Silva Sde O, Gabriel M, de Araújo SM, Gomes ML, et al. Comparative pathogenicity in Swiss mice of *Trypanosoma cruzi* IV from northern Brazil and *Trypanosoma cruzi* II from southern Brazil. *Exp Parasitol.* (2014) 146:34–42. doi: 10.1016/j.exppara.2014.08.014

145. Magalhães LM, Viana A, Chiari E, Galvão LM, Gollob KJ, Dutra WO. Differential Activation of human monocytes and lymphocytes by distinct strains of *Trypanosoma cruzi*. *PLoS Negl Trop Dis.* (2015) 9:e0003816. doi: 10.1371/journal.pntd.0003816

146. Teixeira MM, da Silva FM, Marcili A, Umezawa ES, Shikanai-Yasuda MA, Cunha-Neto E, et al. Short communication: *Trypanosoma cruzi* lineage I in endomyocardial biopsy from a north-eastern Brazilian patient at end-stage chronic Chagasic cardiomyopathy. *Trop Med Int Health*. (2006) 11:294–8. doi: 10.1111/j.1365-3156.2006.01575.x

147. Cura CI, Lucero RH, Bisio M, Oshiro E, Formichelli LB, Burgos JM, et al. *Trypanosoma cruzi* discrete typing units in Chagas disease patients from endemic and non-endemic regions of Argentina. *Parasitology* (2012) 139:516–21. doi: 10.1017/S0033582911002186

148. Risso MG, Sartor PA, Burgos JM, Briceño L, Rodríguez EM, Guhl E, et al. Immunological identification of *Trypanosoma cruzi* lineages in human infection along the endemic area. *Am J Trop Med Hyg.* (2011) 84:78-84. doi: 10.4269/ajtmh.2011.10-0177

149. Muñoz-San Martín C, Zulantay I, Saavedra M, Fuentalba C, Muñoz G, APT W. Discrete typing units of *Trypanosoma cruzi* detected by real-time PCR in Chilean patients with chronic Chagasic cardiomyopathy. *Acta Trop.* (2018) 185:280–4. doi: 10.1016/j.actatropica.2018.05.004

150. Carrera-Silva EA, Eugenio Antonio CS, Cano RC, Carolina CR, Guíñazú N, Natala G, et al. TLR2, TLR4 and TLR9 are differentially modulated in liver lethally injured from BALB/c and C57BL/6 mice during *Trypanosoma cruzi* acute infection. *Mol Immunol.* (2008) 45:3580–8. doi: 10.1016/j.molimm.2008.05.004

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