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THE ROLE OF NITRIC OXIDE IN THE PATHOGENESIS OF CHAGAS DISEASE

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1. ABSTRACT

In this chapter we summarize the protective and toxic effects of nitric oxide (NO) that are frequently seen in parallel during the infection with *Trypanosoma cruzi.* The killing of trypomastigotes is dependent on the production of NO which is catalyzed by the inducible NO synthase (iNOS). The cytokines IFN-gamma and TNF-alpha and several chemoatractants molecules, which act on G protein-coupled serpentine receptors, are produced during the acute infection. They play major roles in the induction of iNOS, and in the NO production-dependent killing of *T. cruzi* by murine macrophages. On the other hand, TGF-beta and IL-10, which are also produced during the infection, are negative regulators of NO production. In addition to mediating resistance against the infection, NO can also suppress the immune response to *T. cruzi* via the induction of apoptosis of T cells. Furthermore, the expression of cardiac iNOS has been associated with myocardial dysfunction. In fact, we discuss here the evidences indicating that iNOS/NO pathway is involved in the pathogenesis of neuronal and myocardial dysfunction seen in patients and in experimental models.

2. INTRODUCTION

Nitric oxide (NO), a product of the oxidation of L-arginine to L-citrulline by a family of NADPH-dependent enzymes (nitric oxide synthases, or NOS) plays a pivotal role in numerous and diverse pathophysiological processes. NO is potentially able to react with the redox forms of oxygen, thiols, amines and transition metals (reviewed in ref. 1) and can also lead to the S-nitrosylation or nitration of proteins. These properties enable NO to be involved in many biological functions, from neurotransmittion to microbicidal activity. Moreover, depending on its concentration, the biological redox milieu and the involvement/induction of intracellular mechanisms, NO can interfere with cell proliferation and death by either inducing or suppressing apoptosis (2, 3).

NO-related species include NO and nitrosonium ion equivalents (NO^+) with one less electron than NO, as well as a nitroxyl anion (NO^-) with one additional electron compared to $NO^{\cdot}$ It has being suggested that these redox-related forms or their functional equivalents are important pharmacologically and physiologically, participating in distinctive chemical reactions (4). The toxicity of NO is more likely to result from the diffusion-limited reaction of NO with superoxide ($O^{\cdot-}_2$) to produce the toxic oxidant peroxynitrite ($ONOO^-$), which is a binary toxin assembled spontaneously whenever NO and superoxide are produced together (4-5). In fact, NO reacts rapidly with a selected range of molecules that have orbital with unpaired electrons, which are typically other free radicals, and with transition metals like heme iron. These properties allow NO to react with a broad range of molecules, from guanylate cyclase, which is activated by NO, to oxyhemoglobin, which leads to NO inactivation (1,4).

There are three different NO-generating enzymes: a constitutively expressed neuronal NOS (nNOS or NOS1), an endothelial NOS (eNOS or NOS3) and inducible NOS (iNOS or NOS2). These enzymes require three co-substrates (L-arginine, NAPH and O_2) and five co-factors or prosthetic groups (FAD, FMN, calmodulin, tetrahydrobibiopterin and heme). The constitutively expressed NOS depend on intracellular Ca^{2+} levels to be active and lead to the production of low amounts of NO. In contrast, the inducible form of NOS is Ca^{2+} independent and when induced by diverse stimuli, such as microbial and/or cytokines, is able to generate far higher and enduring NO levels (6-7). Inducible NOS is expressed in many cell types including macrophages, muscle cells,

hepatocytes, fibroblasts, astrocytes and endothelial cells (reviewed in ref. 8). However, there is a marked variability in the expression of iNOS and the production of NO in different tissues and different species (8-13). For example, stimuli known to readily induce iNOS expression in murine tissue macrophages do not induce iNOS expression in human mononuclear phagocytes purified from healthy human blood (10-12). However, iNOS expression can be induced in human macrophages by alternative stimulatory mechanisms, such as culturing in presence of anti-IgE receptor (CD23) (13). Moreover, iNOS expression can be found in human tissue macrophages (11) and, to a lesser extent, in blood monocytes after infection with certain pathogens (7,10).

3. REGULATION OF NITRIC OXIDE PRODUCTION

Production of NO is tightly regulated at multiple levels (e.g. transcriptional, post-transcripitional, and post-translational) (6-7). The cytokines IFN-gamma and TNF-alpha (14) and chemokines such as JE/MCP-1 (15), RANTES, MIP-1alpha, MIP-1beta, MIP-2 and CRG-2 up-regulate iNOS expression and NO production (16-18), whereas other cytokines such as IL-10, IL-4, IL-13, and TGF-beta can block NO production with consequent inhibition of the antimicrobial activity (19-20).

IFN-gamma induces NO production alone or in synergy with bacterial and protozoan products, such as lipopolysaccharide (LPS) (21), glycosylphosphatidylinositol (GPI)- anchors (22) and staphylococcal enterotoxin B (23), and with cytokines such as TNF-alpha, which provides a second signal to induce microbicidal activity in activated macrophages (18, 24, 25). The crucial role of IFN-gamma as a NO-inducing factor is illustrated by observations from studies using mice with targeted disruption of the IFN-γ gene (26). These mice are deficient in the expression of iNOS and in the production of toxic nitrogen oxides (26, 27) and developed fatal *M. tuberculosis* infections with markedly increased bacillary loads (27, 28). In another murine model, transgenic mice with disruption of the IFN regulatory factor 1 gene have also been shown to be more susceptible to *Mycobacterium bovis* infection (29). In addition, IFN-gamma is crucial for the resistance against *Brucella abortus* infection in mice (30), and has a protective role in limiting viral replication after respiratory syncytial virus (RSV) infection in BALB/c mice (31). Moreover, the absence of IFN-gamma gene resulted in increased susceptibility to infection with *T. cruzi* (32-33).

The IFN-gamma-induced NO-mediated macrophage microbicidal activity can be inhibited by IL-10 (19, 34), which suppresses the arginine-dependent pathway that leads to NO production. Thus, IL-10 joins IL-4 and TGF-beta as one of the few purified and cloned factors able to inhibit macrophage activation (19-20). Reed et al (34) demonstrated that in a *T. cruzi*-susceptible mouse strain, administration of neutralizing anti-IL-10 antibodies confers resistance to the infection and that this is related to increased production of IL-12 and IFN-gamma and possibly NO. Similarly, IL-10 interferes with the ability of IFN-gamma to stimulate TNF-alpha production, resulting in decrease NO production and diminished killing of larval of *Schistosoma mansoni* (35). Furthermore, in conjunction with IL-4, IL-10 was involved in the failure of P strain mice to respond to vaccination against schistosomiasis (36). In that regard, IL-10 reactivity differed from that of TGF-β and IL-4, which inhibited the cytotoxic function of macrophages without modulating TNF-alpha production (37).

NO production by activated macrophages can also be down regulated by IL-13, a cytokine that shares activities with IL-4 (38) and is produced by Th2 cells. The suppression of NO by IL-13 leads to a decrease parasiticidal activity by activated macrophages (39). Moreover, IL-13 deficient BALB/c mice are highly resistant to *L. major*, whereas over expression of IL-13 gene in resistant C57Bl/6 mice rendered them susceptible to *L. major* even in the absence of IL-4 (40-41).

The inhibition of NO production by TGF-beta is mediated by the TGF-beta1 isoform, which suppress the production of both superoxide (42) and NO (43) by macrophages. TGF-beta1 is a potent suppressor of the expression of iNOS in numerous cell types, including cardiac myocytes (44), fibroblasts (45), and macrophages (43). TGF-beta inhibits the NO-dependent cytocidal activity of activated macrophages for several parasites including *T. cruzi* (46), *L. major* (47) and *S. mansoni* (48). The mechanisms implied in the TGF-beta mediated suppression of iNOS expression include at least three distinct pathways: decreased stability of iNOS mRNA, decrease translation of iNOS mRNA, and increased degradation of iNOS protein (49). Similarly to the described to IL-10 effects, administration of TGF-beta to mice infected with *T. cruzi* leads to increased parasitemia and mortality, which is associated with decreased production of IFN-gamma (50) and possibly of NO.

NO production can also be induced by chemokines, a novel class of inflammatory mediators, which play a major role in mediating the migration and accumulation of specific leukocyte subsets in acute and chronic inflammatory processes in several diseases (51). Chemokines are produced by different cell types after activation and have potent chemotactic activity both in vitro and in vivo. In addition to having profound effects on the locomotion of leukocytes, chemokines appear to affect several other biological phenomena, including T-lymphocyte proliferation (52), Th1-Th2 differentiation (53), NK cell migration and activation (54-55), and cytokine production by cells such as macrophages (56). These effects contribute in modulating host resistance to microbial agents, such as virus (57), fungi (58-59) and helminths (53).

More recently, we and others have shown that chemokines such as JE/MCP-1, RANTES, MIP-1alpha and MIP-1beta can also induce NO production and NO-dependent killing of *T. cruzi* by murine macrophages and cardiac myocytes (16-18). Moreover, NO production on the murine cardiac myocytes can also be induced by MIP-2 and

Table 1. Antimicrobicidal actions of NO

Protozoa	Bacteria	Viruses
Trypanosoma cruzi [65, 95-96]	*Staphilococcus aureus* [140]	*Herpes simplex virus type 1* [73-75]
Trypanosoma brucei [138]	*Chlamydia pneumoniae* [141]	*Murine cytomegalovirus* [148]
Toxoplasma gondii [84]	*Listeria monocytogenes* [142]	*Coxsakie virus B3* [81]
Leishmania major [87]	*Mycobacterium tuberculosis* [82, 83]	*Ectromelia virus* [76-78]
Leishmania donovani [110]	*Klebsiella pneumonia* [143]	
Plasmodium berghei [66]	*Salmonella typhimurium* [144]	**Helminth**
Plasmodium chabaudi [139]	*Chamydia trachomatis* [145]	*Schistosoma mansoni* [70]
Plasmodium falciparum [68, 69]	*Helicobacter pylori* [146]	
	Shigella flexneri [147]	

CRG-2 (18). Besides inducing NO production, JE/MCP-1 acts synergistically with IFN-gamma to control *T. cruzi* replication. We concluded that chemokine-induced NO production is mediated by the iNOS activation, since addition of L-NMMA, the specific inhibitor of iNOS, almost completely abrogate the parasiticidal activity. Moreover, addition of EGTA to the culture medium did not abolish NO production (18). Similarly, MIP-1alpha and MCP-1 induce anti-leishmania activity in murine macrophages via generation of NO by iNOS activation (60).

In addition to chemokines, other chemoattractant molecules which act on G protein-coupled serpentine receptors, such as platelet-activating factor (PAF) and leukotriene B_4, also participate in the cascade of events leading to NO production and parasite killing (61, 62). Akin to bacterial LPS and Mycoplasma LPG, *T. cruzi* trypomastigotes express GPI-anchored mucin-like glycoproteins (tGPI-mucins) on their surface which are capable of activating IFN-gamma-primed murine macrophages to induce NO and the production of pro-inflammatory cytokines, such as TNF-alpha and IL-12 (22).

4. ANTIMICROBIAL ACTIONS OF NITRIC OXIDE

Production of NO is induced in multiple cells types of the immune system, including mast cells, dendritic cells, NK cells and phagocytic cells (neutrophils, eosinophils, macrophages, microglia cells, Kupffer cells) as well as other cells involved in immune reactions, such as vascular smooth cells, keratinocytes, hepatocytes, fibroblasts, cardiomyocytes, chondrocytes, mesangial cells, epithelial and endothelial cells (19, 63). Numerous studies have documented the potent antimicrobial activity of NO against intracellular and extracellular pathogens, including protozoan, virus, fungi and bacteria (Table 1) (22, 64, 65). Many of these studies showed that these infectious agents are directly or indirectly controlled by RNIs *in vivo.* In fact, NO produced in vivo during infection with many different pathogens seems to represent an intrinsic mechanism of antimicrobial defense against pathogens even in cells others than macrophages (66-70). For example, the IFN-gamma-induced inhibition of intracellular replication of malaria parasites in hepatocytes was inhibited by arginase and L-NMMA (66-69). Murine endothelial cells can also be activated by cytokines to kill schistosome larvae through NO production (70).

The role of NO in mediating resistance against infection with *S. mansoni* and *P. falciparum* (71, 72), was extended by recent observations showing that protective immunity against malaria and schistosomiasis conferred by vaccination with irradiated *P. berghei or Schistosoma* eggs antigen, respectively is at least in part mediated by production of NO (71, 72). The NO microbiocidal activity can also be extended to viruses, including: herpes simplex virus type 1, vaccinia virus, ectromelia and flavivirus (73-75, 76-78). The ability of IFN-gamma-activated macrophages to restrict virus replication is mediated to a large extent through the induction of iNOS (74). NO acts in vitro by preventing viral DNA replication and late protein synthesis (79), which is a consequence of inactivation of enzymes containing iron-sulfur centers (80). iNOS is also a critical antiviral effector against *Coxsackievirus B3* infection (81). This virus replicates to higher titers in iNOS null mice, which develop more severe myocarditis and clear virus slowly as compared to the wild type mice (81). NO is also required for macrophage-mediated effective killing of several other microorganisms, including *Mycobaterium tuberculosis* (28, 82, 83), *Toxoplasma gondii* (84, 85) and *Leishmania* (86, 87).

4.1. Trypanocidal actions of nitric oxide

The ability of IFN-gamma-activated macrophages to control *T. cruzi* growth *in vitro* (88, 89) and *in vivo* (90) was initially attributed to the generation of hydrogen peroxide. However, the implication of oxygen-independent mechanisms in mediating macrophage trypanocidal activity was claimed by studies showing that the parasite it self was unable to trigger or increase the respiratory burst of activated macrophages (91). Moreover, the exhaustion of respiratory burst by treatment with phorbol myristate, or treatment with scavengers of respiratory burst metabolites failed to inhibit macrophage ability to kill *T. cruzi* in vitro (91). Further reports demonstrating that a cell line defective in the respiratory burst was fully able to kill *T. cruzi* upon IFN-gamma and LPS activation (65) and that the production of hydrogen peroxide *in vivo* did not correlated with trypanocidal activity (92), supported the existence of the oxygen-independent macrophage trypanocidal activity.

Further studies revealed that the IFN-gamma and TNF-alpha–induced trypanocidal activity in murine (65, 93) or human (94) macrophages *in vitro* were mediated by L-arginine-dependent NO production and could be blocked

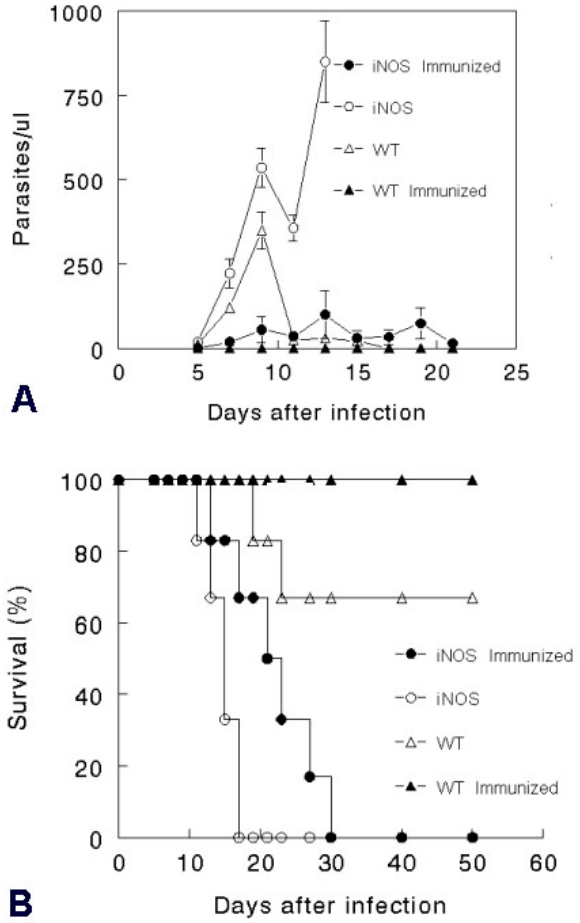

Figure 1. Protection against T.cruzi infection conferred by immunization with non-infective parasite forms is abrogated in the absence of iNOS. C57Bl/6 wild type (WT) (triangles) and C57Bl/6iNOS-/- (circles) mice were inoculated twice with 1 x 107 alive epimastigote forms (filled symbols) or only PBS (empty symbols), with a 15 days interval between the inoculations. Seven days after the last epimastigote forms inoculation all mice were infected with 1000 bloodstream trypomastigote forms (Y strain) and the parasitemia (A) and mortality (B) determined.

by the administration of the competitive inhibitor of L-arginine, NG-monomethyl-L-arginine (L-NMMA), in the cultures. In addition, NO-mediated trypanocidal activity was observed in cells other than macrophages, including cardiac myocytes (16).

Subsequently, the dependence of NO biosynthesis on the mechanisms that control intracellular multiplication of *T. cruzi in vivo* was clearly established by studies evaluating the effects of NO synthesis inhibitors, which limit the expression of iNOS, and in iNOS null mice. Thus, treatment of mice with L-arginine analogues, such as L-NMMA (95), aminoguanidine (AG) (our unpublished results), L-iminoethyl-L-ornithine (L-NIL) (96) or of Wistar rats with N-nitro-l-arginine (97), leads to increased parasitemia and mortality after *T. cruzi* infection. Similarly, pre-treatment with monoclonal antibodies against the NO-inducing cytokines IFN-gamma or TNF-alpha prevented iNOS expression, NO production and resulted in greater parasitemia and mortality (98). In agreement, infected iNOS null mice are highly susceptible to infection with different strains of *T. cruzi,* including the Colombian (99) Tulahuen (95,119), and Y strains (96). Moreover, protection against the infection conferred by the immunization with non-infecting epimastigote forms was dependent on NO production, in such a manner that although the immunization of iNOS null mice significantly decreased parasitemia levels and resulted in delayed mortality, it was unable to confer lasting protection as observed to occur in wild type mice immunized in the same way (Figure 1). In agreement with this crucial role of NO in controlling parasite growth, the NO donor drug S-nitroso-acetyl-penicillamine (SNAP) has been shown to kill *T. cruzi* trypomastigotes *in vitro* in the absence of any host cells, indicating that NO directly mediates killing of this parasite (96).

Although the molecular mechanisms through which NO mediates its cytotoxic effects against *T. cruzi* are not completely understood, it appears that NO is capable of direct interference with the parasite metabolism. NO efficiently inhibits the activity of cruzipain (100), a major cysteine proteinase expressed in all life-cycle stages of the parasite and which is abundant in the replicating forms (101). Cruzipain plays an important role in the parasite nutrition and cell invasion, and in the mechanisms used by the parasite escape of the immune response (102). As such, NO-mediated inactivation of cruzipain may represent an important mechanism of impairment of parasite growth.

The rapid generation of peroxynitrite anion ($ONOO^-$) from NO and superoxide (O_2^-) anion, which is produced in macrophages and other leukocytes, or even inside the pathogens - such as demonstrated by a recent study in which NO generated from host cells was showed to react with bacterial-derived O_2^- inside the microbe and form antibacterial $ONOO^-$ (103)- might also play a role in the trypanocidal activity of NO. In fact, it has been shown that $ONOO^-$ kills *T. cruzi* in a dose–dependent manner (104) by a mechanism probably involving impairment of calcium uptake by the parasites (105) and inactivation of the thiol-containing enzymes required for the parasite energetic metabolism (106).

In addition to these direct effects, the trypanocidal activity of NO might also depend on indirect actions. Similarly to the observed in another intracellular pathogens, *T. cruzi* also depends on the host cell input of arginine from which the parasite synthesizes polyamines, required for its growth. In addition, L-arginine is able to inhibit apoptosis induced in *T. cruzi* amastigotes as recently reported (107). Thus, consumption of arginine by iNOS activation and NO generation could indirectly impair parasite survival and growth. In deed, the inhibition of NO production by TGF-β seems to favor *T. cruzi* growth, through a mechanism that depends on the parasite synthesis of polyamines from the L-arginine remaining from iNOS inactivation (108).

Interestingly, it has been shown that iNOS activity is required for full IL-12 mediated-activation via STAT-4 phosphorylation in NK cells from *Leishmania* infected-mice (109). Since the production of IFN-γ in *T. cruzi* –infected mice is highly dependent on IL-12, it is tempting to speculate that indirect modulation of IFN-γ production through modulation of IL-12 activity could consist in another indirect mechanism of the anti-trypanocidal NO activity.

Whereas the need for NO as a trypanocidal agent is consistently demonstrated, it must be taken in to account that similar to the observed in mice infected with other parasites, including *L. donovani* (110) some experimental evidence have rose the possibility that protective antimicrobial effects of NO in mice are restricted to the acute phase of the *T. cruzi* infection. This issue will certainly require further studies, but a recent report showed that administration of iNOS inhibitor to *T. cruzi* (Tulahuen strain)-infected Balb/c mice in the late acute or chronic phase of the infection, did not result in parasitemia reappearance or increased mortality rates (95). Despite the observation that the same treatment would result in 100% mortality if performed in the early acute phase of the infection in this mice, these data indicate that NO is not implied in the mechanisms that keep parasite under control in the late phases of the infection. This is in accordance with previously published data showing that although the production of NO is greatly increased in the early acute phase of the infection it is decreased soon after parasitemia control and it is maintained at the basal levels after that (96). In spite of this, since NO plays a crucial role in controlling parasite growth and spread in the acute phase it can be envisaged that it plays an indirect role in promoting the establishment of a benign chronic infection.

5. ROLE OF NITRIC OXIDE IN CHAGASIC HEART DISEASE

A significant proportion of *T. cruzi*-infected patients will progressively develop myocarditis and congestive heart failure which characterize the chronic cardiac form of Chagas disease. The pathogenic mechanisms associated with the development of chagasic cardiomyopathy have been subject of extensive investigation but still require further elucidation. Initial studies with transplanted neonatal hearts in to the external ear of isogenic mice (111), associated to the inability to detected the presence of *T. cruzi* parasites in the myocardium and the supposed lack of correlation between parasite presence and the occurrence of myocardial inflammatory infiltrate has supported the hypothesis that chronic Chagas' cardiomyopathy could be due to autoimmunity (112). However, recent studies showed that parasite presence could be directly associated to the heart rejection in the transplanted heart experiments and that parasite antigens or DNA is found in the myocardium of chronically infected individuals (113-114), favoring the hypothesis that the parasite presence directly participate in the pathogenesis of the chagasic cardiomyopathy (114-115).

Despite the incomplete understanding of the mechanisms that raise the inflammatory response in the heart after the infection, it is known that CD4+ and CD8+ T lymphocytes are the major components of the inflammatory cell infiltrate that characterizes the human chronic chagasic myocarditis. Further characterization of the inflammatory infiltrate in human and T. cruzi infected animals also showed the presence of cytokines such as IFN-gamma, TNF-alpha, and IL-1 (115-116). In mice infected with T. cruzi the presence of these inflammatory cytokines in the myocardium seems to correlate with iNOS activation and NO production (116). Furthermore, results from our and other laboratories have shown that iNOS activity is abundant in the heart of T. cruzi-infected mice (19, 117-119) and chagasic patients (V. Rodrigues, unpublished results).

In vitro studies showed that isolated fetal murine cardiomyocytes cultured in presence of trypomastiogotes express mRNA for the cytokines TNF-alpha and IL-1beta and for iNOS, strongly suggesting that these cells could be the potential source of cytokines and iNOS in vivo. Moreover, following T. cruzi infection of cultured myocytes we have observed iNOS protein induction and NO2- production, which could be blocked by selective iNOS inhibitors (L-NIO and aminoguanidine), demonstrating that the parasite induced NO production in cardiomyocytes via upregulation of the expression of the inducible isoform of NOS (16).

The mechanism by which iNOS expression is induced by T. cruzi in cardiac myocytes remains unresolved. One possibility is that parasite-secreted products, such as GPI mucins (120) or LPS-like molecules (121) may induce the enzyme directly. Alternatively, iNOS expression may result from autocrine stimulation by cytokines and chemokines released by cardiomyocytes following T. cruzi infection. In this regard, T. cruzi has been shown to induce production of beta-chemokines by macrophages (59) and expression of JE/MCP-1, RANTES, KC, MIP-2, Mig and Crg-2 mRNA in cardiomyocytes. The parasites can also induce TNF-alpha and IL-12 synthesis by macrophages, which results in IFN-gamma production by NK cells (121-122). The presence of IFN-gamma and chemokines in the heart tissue of infected mice, in association with IL-1beta and TNF-alpha could lead to induction of iNOS.

Analogous to the observed in T. cruzi-infected macrophages, the production of NO by cardiac myocytes likely controls parasite replication in the heart. Indeed, incubation of cardiomyocytes with cytokines or chemokines resulted not only in NO synthesis but also in significant trypanocidal activity. Addition of selective iNOS inhibitors significantly inhibited NO production and parasite killing, convincingly demonstrating that cardiac myocyte-derived NO possesses significant trypanocidal activity (16). The finding that the iNOS null infected mice present an increased number of amastigote nests in the heart (our unpublished observations) could additionally support the existence of a NO-mediated cardiac myocyte trypanocidal activity.

Nevertheless, the supposed beneficial effects of NO produced in the myocardium during *T. cruzi* infection could be beyond the participation in controlling parasite growth: The finding that *T. cruzi*-infected and L-NMMA-treated mice have an increased severity of lesions in

skeletal muscle and liver (123) together with the observation that iNOS null mice have increased myocardial inflammation and a different pattern of chemokine production when infected with the Y strain of *T. cr*uzi (our unpublished results) suggest that NO might be broadly implicated in modulating inflammatory responses through modulation of Th1 cytokine production in the acute phase of the infection. In this regard, one could presume that Th1 cells (or IFN-γ producers) predominantly in inflammatory lesions in infected iNOS null mice as compared to the infected wild-type. This is a tempting possibility, but still remains to be investigated. Moreover, the enhanced inflammatory activity in the absence of iNOS could be related to the interference that NO can exert on cell migration. As recently reported, NO is able to inhibit leukocyte adhesion and migration through the endothelial cell layer by down regulating expression of selectins, vascular cell adhesion molecule (VCAM) and intracellular adhesion molecule 1 (ICAM-1) (124-125). Furthermore, P-selectin expression was found to be impaired in the presence of NO (126). Since, P- E-selectins mediate recruitment of Th1 but not Th2 cells into inflamed tissue (127) it is conceivable that NO could preferentially down regulate the accumulation of Th1 cells at the sites of chronic inflammation by interfering with the adhesion process.

Nitric oxide decreases endothelial cell activation and expression of cell-surface adhesion molecules that mediate neutrophil and monocyte adhesion as well as platelet aggregation. It also diminishes microvascular permeability. These properties could also enable NO to participate in modulating the inflammatory process in the heart after the infection. In addition, NO has been reported to increase left ventricular relaxation, an action which in combination with its direct coronary vasodilator capacity provides a mechanism by which it could help prevent progressive deterioration in myocardial performance. In line with this, treatment of patients with heart failure with vasodilating drugs that ultimately act through release of NO is unquestionably beneficial. NO has also been shown to have anti-arrhythmic properties. As arrhythmias are a major cause of death in heart failure, increased NO production in the heart might be protective. Nevertheless, iNOS activity and NO production has been reported to occur in the myocardium in circumstances other than *T. cruzi* infection, including heart failure, ischemia, allograft cardiac rejection (128), viral induced myocarditis (129) and in some of these circumstances NO seems to contribute to the disease progression. Consistent with a pathogenic role of NO in such conditions, iNOS null mice had better outcomes after cardiac allograft transplantation (130) and were resistant to LPS-induced septic shock (131). Moreover, administration of iNOS inhibitor resulted in increased survival after myocardium infarction in rabbits (130). Strikingly, a recent report showed that in mice experimentally infected with the Tulahuen strain of *T. cruzi*, iNOS-derived NO is implied in the development and progression of ventricular dilatation and systolic dysfunction in acute myocarditis (119).

The mechanisms modulating the causative or exacerbating role of NO in cardiac pathologies are not fully understood. Nevertheless, NO has been shown to exert a negative inotropic effect in contraction in both: isolated cardiac myocyte and perfused working hearts (132). This ability in promoting contractile depression could be related to the fact that NO is able to decrease the intracellular levels of cAMP in response to beta-adrenergic stimulation in cardiac myocytes, at least in part through a cGMP-mediated mechanism (133).

Nitric oxide is also a regulator of apoptosis. Induction of cardiac myocytes apoptosis could be another mechanism by which NO interferes with myocardial homeostasis. In fact, time-course studies have implied iNOS-derived NO as a mediator of cardiac myocyte apoptosis (134) and, high levels of NO production by iNOS is able to kill cardiac myocytes by triggering apoptosis, possibly by a p53-mediated mechanism (135), and exposure of rat ventricular myocytes to combinations of IL-1beta, TNF-alpha and IFN-gamma or co-culture with activated macrophages resulted in myocyte injury or death, which could be prevented by addition of NOS inhibitors or TGF-beta (136). NO has been implicated as a mediator of apoptosis in immune cells in the periphery during the acute phase of T. cruzi infection in mice (98), and apoptosis occurs also in the myocardium of acutely infected mice (our unpublished results). However, studies performed in situ in preserved heart tissue from chagasic patients were unable to detect a significant increase in DNA fragmentation or p53 expression in myocardial cells or mononuclear cells (137).

6. SUMMARY

Experimental evidence obtained so far certainly implicate NO as a important mediator of parasite killing in mice experimentally infected with T. cruzi. Some more recent experimental observations also indicate that NO is potentially implied in regulating many other processes required to the efficient immune response against this parasite. While there is now clear evidence that NO production is induced in the myocardium after T. cruzi infection, the consequences of this are far beyond to be completely understood. There are many questions to be answered regarding the exact contribution of NO to the pathogenesis of this infection. Since NO contributes to many biological process, elucidation of the mechanisms in which it can participate will certainly favor the development of future therapies against other pathological circumstances than the one caused by T. cruzi infection.

8. ACKNOWLEDGEMENTS

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