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Prostaglandin and nitric oxide regulate TNF-α production during *Trypanosoma cruzi* infection

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Abstract

The mechanisms that control TNF-α production by macrophages during *Trypanosoma cruzi* infection are still unknown. Destruction of intracellular forms by cytokine activated macrophages is considered to be a major mechanism of parasite elimination. Although in vitro TNF-α contributes to enhanced parasite destruction by macrophages, previous work in vivo has shown that as the parasite burden increases, serum TNF-α levels decline. In this report we show that TNF-α production by peritoneal adherent cells is elevated at the initial phase of *T. cruzi* infection. As infection progresses TNF-α production decreases. The observed reduction is partly due to inhibition, largely exerted by endogenous PG and secondarily by NO. Inhibition of their synthesis partially restored the ability to produce high levels of TNF-α to macrophages upon stimulation by LPS. Neither endogenous IL-10 nor TGF-β seem to be involved in the negative regulation of TNF-α production.

Keywords: *Trypanosoma cruzi*; Macrophage; TNF-α; Prostaglandins; Nitric oxide

1. Introduction

Trypanosoma cruzi, the etiologic agent of Chagas' disease in man, is a dygenetic protozoan capable of invading and multiplying in virtually any type of nucleated cell including macrophages [1]. Cytokine activated macrophages become capable of destroying the intracellular parasites and are an important effector mechanism of parasite elimination both by H_2O_2-dependent and independent mechanisms [2–4]. Interferon-γ, by itself or in combination with TNF-α or IL-4, is one of the most efficient activators of macrophages to a trypanocidal function [5–11]. However the addition of TNF-α either alone [12] or in combination with LPS [13] or with IFN-γ [8] also effectively activates macrophage cultures to a trypanocidal state. Both adherent and non-adherent spleen cells from *T. cruzi* infected mice produce TNF-α [7]. In vivo, it has been shown that spontaneous production of this cytokine is low but can be stimulated by LPS injection [14]. Moreover, other studies have shown increased production of TNF-α during the early phases of infection, as assessed by serum levels of this cytokine, followed by their sharp decline as infection progressed [7] and no correlation could be established in vivo between the decrease in parasite blood counts and enhanced production of

Abbreviations: COX, cyclooxygenase; IFN-γ, interferon γ; iNOS, inducible NO-synthase; LPS, *E. coli* lipopolysaccharide; NO, nitric oxide; PG, prostaglandins; PMA, phorbol-12-myristate-13-acetate; PM, peritoneal macrophages; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor-α.

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TNF-α. However, in vivo administration of high doses of rTNF-α delayed mortality of *T. cruzi* infected mice [15]. The importance of endogenous TNF-α in the resistance to *T. cruzi* infection has been recently confirmed: TNF-α role as a major factor in resistance provided by the innate immune system was described in mice deprived of immune cells (RAG knock-out mice) [16]; in addition, it also participates in the resistance to *T. cruzi* observed in immunocompetent animals [16,17]. Moreover, resistance to the infection mediated by in vivo treatment with IL-12 depends on both IFN-γ and TNF-α synthesis [18].

So far, there have been no studies to address how TNF-α production is regulated during the course of *T. cruzi* infection. We focused our investigation on the role of: (a) NO on TNF-α production, as this reactive nitrogen intermediate can affect several functions of the macrophage [19] and large amounts of this molecule are generated during *T. cruzi* infection [20–24]; (b) PG as they are potent inhibitors of TNF-α synthesis [25]; (c) IL-10 and TGF-β as both inhibit TNF-α synthesis by macrophages [28,29] and have been shown to act as potent deactivators of macrophage trypanocidal function [4,28].

2. Materials and methods

2.1. Mice and parasites

C57BL/6 mice (6–8 weeks old) were obtained from the breeding colonies of the Faculdade de Medicina da Universidade de São Paulo and housed in a clean conventional colony at the Instituto Butantan. The mice were injected s.c. with 4000 *T. cruzi* F strain blood trypomastigotes obtained from infected Swiss mice [30]. The course of infection was monitored by counting (optical microscope, 40× objective) the number of trypomastigotes in 5-μl samples of citrated blood drawn from the tail veins, as described by Brener [31].

2.2. Peritoneal macrophage cultures

Peritoneal cells were collected from six to eight normal or from five to six *T. cruzi* infected mice by i.p. injection of 10 ml sterile 0.01 M PBS containing 40 μg/ml gentamycin. The fluid was withdrawn and the cells washed by centrifugation (200 × *g*, 4°C, 10 min) and re-suspended in RPMI-1640 complete medium containing 5% FCS, 2 mM L-glutamine and 40 μg/ml gentamycin (Sigma, St. Louis, MO). The cells were cultured in 96-well flat-bottom plates at 4×10^5 cells/well. The plates were incubated for 1 h at 37°C in 5% CO_2 atmosphere. Non adherent cells were removed by vigorous washing with warm medium. The adherent cells were mostly medium or large, cytoplasm-rich cells. Intracellular parasites could be detected in approximately 20% of the adherent cells coming from infected mice. The cultures of adherent cells will be referred to as peritoneal macrophage (PM) cultures.

2.3. Experimental design

Peritoneal macrophage cultures from normal or infected mice were stimulated with *E. coli* 026:B6 lipopolysaccharide LPS (0.5 μg/ml) (Sigma) for 6 h or just maintained in culture medium for the same time (spontaneous production of TNF-α) and the supernatants collected and frozen at −20°C. Treatments of PM cultures with anti-cytokine mAbs or with inhibitors of PG and NO synthesis were done by incubating the cultures for 18 h with the appropriate inhibitor or mAb or only in complete medium as controls; the cultures were then washed with warm medium and further incubated with the same inhibitor or mAb together with 0.5 μg/ml LPS for an additional 6 h, after which the supernatants were collected and frozen. The following treatments were used: indomethacin (Indo) (Sigma); N^G-monomethyl-L-arginine (NMLA) (Calbiochem, San Diego, CA); mAb anti-TGF-β 1, 2, 3, at 25 μg/ml (Genzyme, Cambridge, MA); mAb anti-mouse IL-10 (rat IgG1, designated JES5-2A5) or an isotype matched rat anti-β galactosidase control mAb (GL-113), both used at 20 μg/ml; these last two mAbs were purified from the respective hybridoma supernatants in our laboratory and the cells were a gift from Dr Robert Coffman, DNAX Research Institute, Palo Alto, CA.

2.4. Measurement of TNF-α activity, nitrite and prostaglandin production in macrophage culture supernatants

TNF-α was measured by cytotoxicity of the TNF-α-sensitive L929 cell line [32], modified in order to quantify the viable cells by the colorimetric MTT test at the end of the assay [33]. TNF-α levels in supernatants from unstimulated (spontaneous) and LPS-stimulated cultures obtained as described were calculated from a standard curve generated with serially diluted recombinant murine TNF-α, a generous gift from Dr G.R. Adolf (Ernest-Boehringer Institute, Germany). The cytotoxic activity in macrophage culture supernatants was characterized as TNF-α, as it was completely neutralized by the co-incubation with an anti-murine TNF-α specific antiserum (Genzyme).

The amount of nitrite accumulated in peritoneal macrophage culture supernatants was determined by a spectrophotometric method using the Griess reagent. The absorbances were read at 540 nm, and the concentration of nitrite was obtained by comparison with a standard curve of serially diluted sodium nitrite as previously described [34].

Table 1
TNF-α, prostaglandins, and nitrite production by peritoneal macrophages from *T. cruzi*-infected mice

Days after infection	Parasites/ml[a] ($\times 10^6$)	TNF (pg/ml)		Prostaglandin (ng/ml)		Nitrite (μM)	
		Spontaneous	LPS-stimulated[b]	Spontaneous	LPS-stimulated	Spontaneous	LPS-stimulated
0	0	68 ± 8[c]	138 ± 15	0.82	2.33	3.9	10.9
14	0.9 ± 0.6	340 ± 63	516 ± 120	3.75	6.19	12.4	17.6
21	12 ± 0.2	24 ± 0.5	84 ± 1.3	4.15	19.4	22.2	21.0
28	20 ± 0.2	15 ± 2	88 ± 22	57.8	84.2	26.4	32.8

[a] Parasites per ml of blood; mean ± SEM ($n = 5$).
[b] Peritoneal macrophage cultures (4×10^5/well) from uninfected or from infected mice were stimulated with LPS (0.5 μg/ml) and the supernatants collected after 6 h.
[c] TNF determinations expressed as means ± SD; PG and nitrite concentrations presented as means; SD did not exceed 20% of the means.

The levels of prostaglandins in the same supernatants were measured by a colorimetric competition assay using the EIA kit for PGE_2 (514010), purchased from Cayman, Ann Arbor, MI.

2.5. Statistical analysis

The results were expressed as arithmetic means and respective standard errors of the means (SEM) or standard errors (SD). Tukey-Kramer multiple comparisons test was used to analyze the data.

3. Results

3.1. TNF-α production by PM from T. cruzi is markedly reduced as the host's parasite load increases and correlates with increased prostaglandin synthesis

Infection of C57/BL6 mice with the highly virulent *T. cruzi* F strain is characterized by progressively higher parasitism that eventually leads to host death by the fifth week of infection. Production of TNF-α (both unstimulated and LPS-stimulated) by peritoneal macrophages (PM) was elevated during the second week of infection and from then on declined coincident with the steep rise of the number of blood parasites (Table 1). By 3 weeks of infection, TNF-α production by unstimulated or by LPS-stimulated peritoneal macrophages had fallen to levels below those observed in macrophage cultures from uninfected mice. Macrophages obtained from 21 and 28 days-infected mice produced very low levels of TNF-α but still responded to LPS stimulation with three- to fivefold increases in its production. The marked decrease in TNF-α production by PM observed at this stage of the infection was not due to a decrease of the numbers of adherent cells nor to their accelerated death during the culture period as checked by cell density counts and viability testing (data not shown). The decrease of TNF-α levels in PM cultures from infected mice could also not be ascribed to a neutralizing or inhibitory effect by the parasites present in these cultures on the activity of secreted TNF-α, as direct incubation of rTNF-α with live parasites did not alter the concentration of this cytokine measured by the bioassay (data not shown).

The markedly reduced TNF-α synthesis observed in the fourth week of infection was accompanied by increasingly higher levels of prostaglandins (PG) in LPS-stimulated cultures (Table 1). By day 28 of infection, very high PG levels were produced by LPS-stimulated and by unstimulated macrophage cultures coincident with the lowest TNF-α levels detected in this last situation.

The morphological appearance of PM as large, spread out, ruffled cells on microscopic examination suggested that these cells were highly activated in spite of their basal levels of TNF-α secretion. The elevated levels of nitrite produced in the same cultures were indicative of increased NO synthesis by activation of the inducible NO synthase pathway of macrophages (Table 1). Nitrite secretion by macrophages from infected mice was not significantly increased by LPS.

3.2. NO and PG production by PM cells from infected mice are inhibitable by NMLA

Synthesis of nitrite by PM could be inhibited by the addition of the competitive inhibitor of NO production, NMLA, to the cultures; cultures were incubated overnight in the presence of NMLA, washed, and re-incubated with the inhibitor for the duration of the 6-h period of supernatant collection. NMLA was used at the concentrations of 0.1 mM, 0.5 mM and 1 mM that inhibited respectively 55, 62 and 80% of the nitrite production by PM from day 28-infected mice. At a concentration of 1 mM of NMLA, nitrite levels were brought down to the levels obtained in normal PM cultures treated with the inhibitor (4 ± 2 mM).

The effect of NO on prostaglandin synthesis was investigated by treating LPS-stimulated PM cultures from infected mice with the inhibitor NMLA in the dose of 1 mM, that effectively blocked NO synthesis. As shown in Fig. 1, treatment with NMLA significantly decreased PG production by PM from days 21- and 28-infected mice while minimally affecting its production by macrophages from normal uninfected mice or from 14 days-infected animals. This result is consistent with the higher NO production levels observed during the fourth week of infection (Table 1). However, PG synthesis by 14 days-infected mice was not affected by NO inhibition, yet the levels of LPS-stimulated NO production by 14 days PM are not significantly different from those found by 21 days of infection (Fig. 1 versus Table 1). Nevertheless, by day 14 of infection, TNF-α (that is a potent stimulator of PG synthesis) levels are high and, as described below, were further significantly increased by NMLA treatment (Table 1 versus Fig. 2A,B).

3.3. Inhibition of NO synthesis augments TNF-α production by PM from normal and from infected mice

NO can affect macrophage biosynthetic pathways in a number of ways [16]. Therefore, we investigated its effect on TNF-α synthesis by PM. Inhibition of NO production by NMLA increased, in a dose-dependent fashion, LPS-stimulated TNF-α production by PM from normal and from infected mice. In a range of doses from 0.1 to 1.0 mM, the latter was the most effective that could be used without inducing cytotoxicity (data not shown). Higher concentrations of NMLA (0.5 mM and 1 mM) were needed to augment TNF-α production in 21 or 28 days-infected LPS-stimulated PM cultures than in normal cell cultures, where 0.1 mM doubled TNF-α levels (data not shown). This is in agreement with the much higher production of NO by PM from infected mice (Table 1).

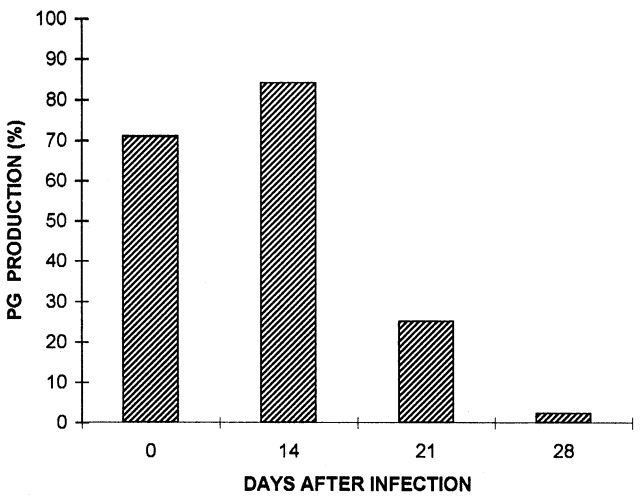

Fig. 1. Inhibition of prostaglandin (PG) synthesis by blocking nitric oxide synthesis in LPS-stimulated macrophage cultures from *T. cruzi* infected mice. Cultures were pre-treated with 1 mM NMLA, washed and stimulated with LPS in the presence of NMLA. Supernatants were harvested after 6 h for PG determination. Results from a representative experiment are expressed as a percentage of PG levels in NMLA-treated cultures relative to those obtained in untreated cultures.

The effect of inhibiting NO synthesis on TNF-α production by PM is shown in Fig. 2A,B. Treatment with NMLA (1 mM) was mostly effective at augmenting TNF-α production by unstimulated and by LPS-activated PM from normal mice and by LPS-activated PM obtained from 14 days-infected mice. Although less impressive, NMLA still significantly ($P < 0.01$) increased unstimulated and LPS-stimulated TNF-α production in 21 days-infected PM cultures and in LPS-stimulated cultures (but not in unstimulated cultures) from 28 days-infected mice. However, restoration of TNF-α production by NMLA was only partial as the levels of this cytokine did not reach half the levels observed in cultures from uninfected mice and from 14 days-infected mice submitted to the same treatment. That PM from infected mice produced high levels of NO and that TNF-α production could be partially restored by inhibiting NO production in these cell cultures indicated that, as suggested by their morphology, these PM were highly activated but TNF-α synthesis was being negatively controlled by other molecules in addition to NO.

3.4. Inhibition of cyclooxygenase by indomethacin markedly increased LPS-stimulated TNF-α production by PM from normal and from infected mice

Prostaglandins of the E series, in particular PGE_2, are known regulators of TNF-α production [25]. In order to investigate the effect of endogenous PG production on TNF-α synthesis by PM, adherent cell cultures from normal and from infected mice were treated for 18 h with indomethacin, washed and further incubated with indomethacin and LPS as a stimulant of TNF-α production. As shown in Fig. 2A, significant increases of TNF-α production were observed after indomethacin treatment (20 μg/ml) of unstimulated PM cultures from normal mice and of LPS-stimulated cultures from normal mice and 14, 21 or 28 days-infected mice. TNF-α levels in 21 or 28 days-infected mice were still 50% of those obtained for identically treated 14 days-infection cultures and 30% of those obtained for normal mice. No increase in TNF-α levels was seen upon indomethacin treatment of non-stimulated cultures from 14, 21, or 28 days-infected mice (Fig. 2B). Treatment of PM cultures from infected mice with 1–20 μg/ml indomethacin enhanced TNF-α production whereas in cultures from uninfected mice this occurred in the presence of 10 or 20 μg/ml of this COX inhibitor (data not shown).

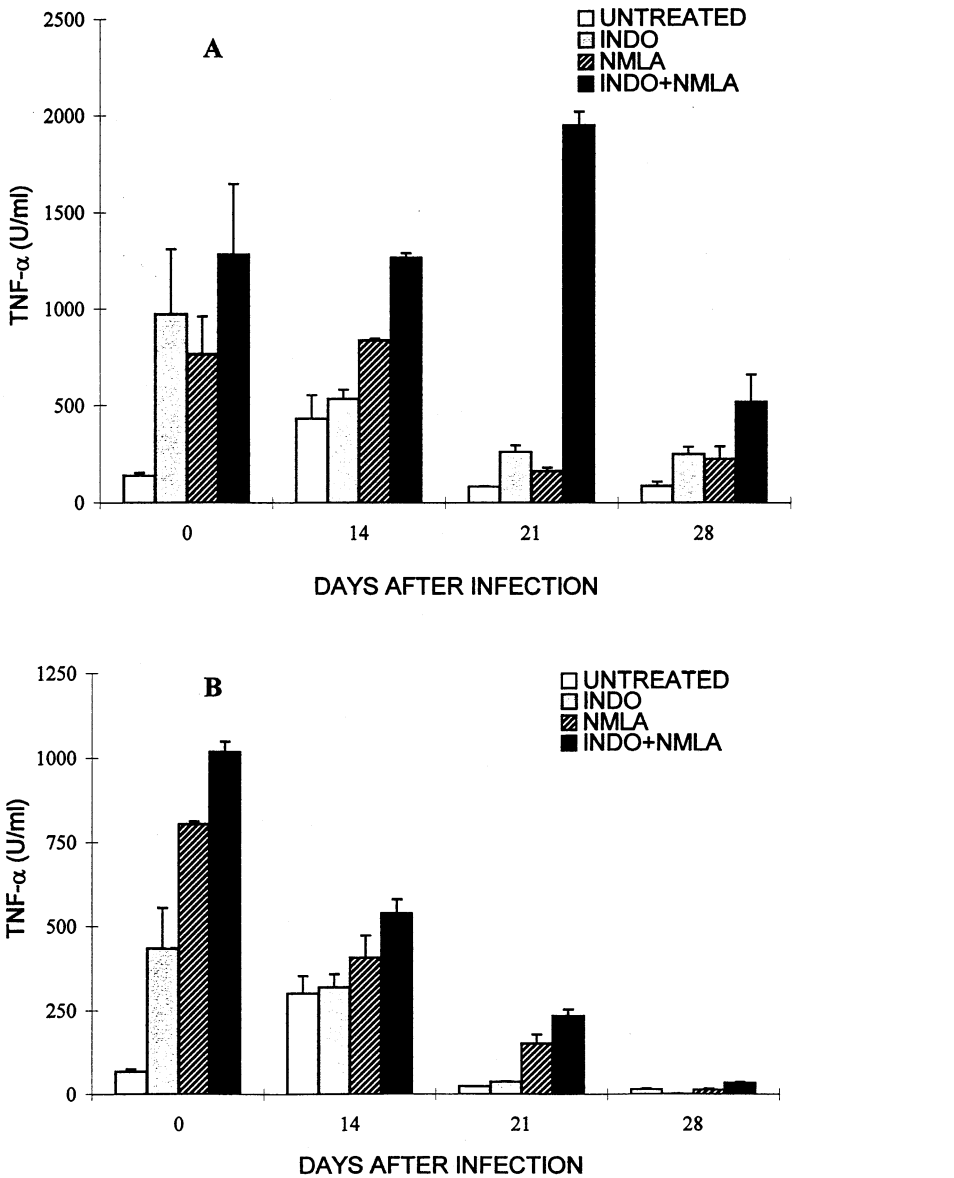

Fig. 2. Enhancement of TNF-α production by treatment of peritoneal macrophages from *T. cruzi* infected mice with indomethacin and/or NMLA. (A) LPS-stimulated cultures; (B) Non-stimulated cultures. Notice different range of y-axis in (A) vs. (B). NMLA treatment: $P < 0.01$ at all time-points shown in (A) and for zero and 21 days in (B); days 14 and 28 in (B): $P > 0.05$. Indomethacin treatment: $P < 0.01$ at all time points in (A) and in normal (zero) unstimulated cultures in (B); days 14, 21 and 28 in (B): $P > 0.05$. NMLA + Indomethacin treatment $P < 0.01$ at all time points in (A) and (B).

3.5. Inhibiting both COX and iNOS had a further additive or potentiating effect on TNF-α production by normal or infected mice PM cultures

As treatment with indomethacin and NMLA individually resulted in a significant increase of TNF-α production, we next studied the effect of their simultaneous addition to the cultures. The combined treatment of LPS-stimulated cultures with both inhibitors, resulted in TNF-α production levels significantly ($P < 0.01$) higher than the highest levels measured obtained in cultures treated with either NMLA or indomethacin (Fig. 2A). This additive effect was also seen for TNF-α produced in non-LPS-stimulated PM cultures from normal and infected mice (Fig. 2B).

3.6. Treatment of PM cultures with anti-TGF-β or with anti-IL-10 neutralizing antibodies did not increase either spontaneous or LPS-stimulated TNF-α production

TGF-β and IL-10 are efficient antagonists of macrophage activation, as has been shown for macrophages infected with *T. cruzi* [28,29], and negatively regulate TNF-α and reactive nitrogen intermediates' production [26,27]. We investigated the role of

these cytokines in controlling TNF-α secretion by PM from infected and normal mice. The cultures were incubated for 18 h with the mAb 2A5 or with anti-TGF-β, washed, and reincubated with either neutralizing Ab and LPS as the stimulus for TNF-α secretion. Anti-IL-10 treatment only slightly augmented TNF-α levels in cultures of both infected and normal PM and the effect was not significant (data not shown). Anti-TGF-β treatment had no effect at all on TNF-α production by PM from either normal or infected mice (data not shown).

Taken together, the results indicate that suppression of LPS-stimulated TNF-α production by peritoneal macrophages from infected mice is largely mediated by endogenous prostaglandins and secondarily by NO. Inhibition of their synthesis restored the ability of these cells to produce high levels of TNF-α upon LPS stimulation but did not significantly enhance production of this cytokine to parasite stimuli intrinsic to the culture.

4. Discussion

We show in this paper that, during infection with *T. cruzi* F strain, TNF-α production by peritoneal macrophages is very high at the second week of infection and markedly decreases as the host's parasite load increases during the course of infection. The previous report that serum TNF-α levels reach high levels early in the course of infection and suffer a sharp decline at the time of maximum parasitemia [7] suggests that the reduction of TNF-α production by macrophages which we observed is neither restricted to the peritoneal cell compartment nor to the *T. cruzi* strain used in our experiments.

Infection of mice with the F strain of *T. cruzi* used in this study determines an intense mononuclear inflammatory reaction in the peritoneal cavity. Inflammatory macrophages can be easily collected from this site by simple aspiration obviating the need to use inflammation-inducing agents that might by themselves provide signals for macrophage activation. Thus, this model can provide useful information on the state of activation of inflammatory macrophages recruited to and directly involved in an inflammatory response to the presence of the parasite.

By 21 and 28 days of infection, TNF-α production in non-LPS-stimulated macrophage cultures was lower than observed for normal macrophages and LPS stimulation elicited TNF-α levels comparable to those obtained in LPS-stimulated normal macrophages. These results were intriguing as they paralleled progressively higher parasite loads and increasing nitrite accumulation and prostaglandin secretion. Nitric oxide as assessed by nitrite accumulation, indicated that the iNOS pathway was being activated and thus it was unlikely that the macrophages were in a state of general deactivation. Nevertheless, we tested the effects of neutralizing endogenous IL-10 and TGF-β, both potent inhibitors of the synthesis of TNF-α and of reactive oxygen and nitrogen intermediates [4,26,27]. Treatment of macrophage cultures with mAbs to either cytokine in doses that are maximally effective at neutralizing their activities [23] did not increase spontaneous or LPS-induced TNF-α production. Taken together, these results suggested that TNF-α synthesis could be under negative regulation by nitric oxide and/or by prostaglandins.

Indeed, treating PM cultures from 21 or 28 days-infected mice with NMLA, a competitive inhibitor of NO production, significantly enhanced LPS-induced TNF-α production. In addition, treatment with indomethacin, a blocker of prostaglandin synthesis that acts by directly inhibiting COX activity also increased TNF-α production in response to LPS.

The augmentation of TNF-α synthesis by NMLA could be attributed to several mechanisms, none mutually exclusive, that have to be further investigated. Nitric oxide can affect macrophage biosynthetic pathways or may account for apoptosis in highly stimulated macrophages [35]. However, our results show that by inhibiting NO synthesis in LPS-stimulated cultures on days 21 and 28 of infection, PG production is also significantly decreased by 60% and 90% respectively. Prostaglandins are known inhibitors of LPS-induced TNF-α production [25] but do not inhibit NO production [38]. Thus the results indicate that when elevated NO production co-exists with increased prostaglandin secretion, as is the case of macrophages from intensely parasitized mice, the enhancing effect of NMLA on TNF-α synthesis results from both inhibition of NO and a marked inhibition of PG synthesis. In this regard, it has been shown that inhibition of NO production decreases PG production by cultured macrophages [36] or by inflammatory exudate cells [37] suggesting that NO activates the induced form of cyclooxygenase (COX). In this situation, inhibition of NO would be indirectly expected to partially release TNF-α production by PGE_2-suppressed macrophages as we observed in our model. Nonetheless unstimulated or LPS-stimulated normal macrophages produce only low levels of PG suggesting that mechanisms other than inhibition of PG synthesis account for the enhancing effect of NMLA on TNF-α production in these cultures.

That the production of TNF-α to LPS stimulation in infected macrophage cultures was indeed being negatively regulated by PG was shown by the increased cytokine levels after treatment with indomethacin. It has been described that suppression of TNF-α synthesis by PGE_2 involves the augmentation of IL-10 production, via the increase of cAMP levels and that significant but not complete reversal of the PGE_2 effect was achieved by anti-IL-10 treatment [38]. We did not

observe the involvement of IL-10 in the PGE_2-mediated pathway of suppression in *T. cruzi* infected peritoneal macrophages as IL-10 neutralization had no effect on TNF-α production; neutralization of TGF-β by mAb was also without effect on TNF-α levels. However, by day 28 of infection, TNF-α production by cultures treated with both NMLA and indomethacin were low when compared to that of normal peritoneal cells suggesting that other distinct inhibitory molecules might control TNF-α production at this stage of infection.

Among the tested treatments, indomethacin was only effective at increasing TNF-α production in macrophage cultures from infected mice stimulated with LPS. This finding suggests that relieved from PG-mediated suppression, production of TNF-α to parasite stimuli intrinsic to the culture would require a second signal, possibly mediated by the stimuli provided by macrophage-activating cytokines.

The role of TNF-α as a required co-stimulator of macrophage to trypanocidal activity is still a matter of debate. One study [9] has shown that rTNF-α alone did not induce trypanocidal activity and that the combination of TNF-α with IFN-γ did not have a significant effect on reducing parasite numbers in macrophage cultures below that obtained with IFN-γ alone. However, other studies have shown that in vitro, TNF-α in association with LPS [13] or with IFN-γ [8] promotes activation to *T. cruzi* killing.

When TNF-α was administered in vivo, reduction of mortality was only achieved with high doses of the cytokine [15]. Recently, however, experiments of TNF-α neutralization by mAb in *T. cruzi* infected mice devoid of T and B cells by disruption of RAG genes (RAG/KO) have shown the importance of this cytokine in early resistance conferred by innate immunity mechanisms [16]. As infection progressed, the high levels of IFN-γ produced [11,39] would provide a powerful stimulus to further enhance TNF-α production by macrophages. Together, these cytokines stimulate NO production [16] and resistance to the infection [16–18]. Thus, TNF-α release in a situation of predominant Th1 cytokine activation [23,39,40] would have to be tightly controlled (by PG and/or by NO) in order to avoid deleterious effects to the host and to the ongoing immune response. In addition to inhibiting TNF-α production, prostaglandins might exert other important anti-inflammatory effects at sites of intense parasite destruction possibly by stimulating IL-10 synthesis by macrophages [38]. It has been shown recently that TNF-α is a potent suppressor of antigen-specific and mitogen-driven T lymphocyte proliferation in *T. cruzi* infection [23].

Desensitization of macrophages to LPS is an efficient way to prevent excessive TNF-α production. This has been accomplished in vitro by pretreatment of macrophages with low doses of LPS; desensitization can be blocked by inhibition of COX and pretreatment with PGE_2 can mimic desensitization [41]. This mechanism could also play a role in inhibition of TNF-α production by macrophages from *T. cruzi* infected mice: macrophages as removed from the infected host would be desensitized and the subsequent in vitro incubation in the presence of indomethacin would restore LPS responsiveness to the cells.

In summary our results show that, during the phase of high parasitemia, prostaglandins and nitric oxide control spontaneous and LPS-induced TNF-α production by macrophages from *T. cruzi* infected mice. TNF-α production upon LPS stimulation was shown to be negatively regulated by prostaglandins and by nitric oxide but not by IL-10 or TGF-β. These findings suggest that mechanisms that control excessive production of TNF-α in other models of acute inflammation also operate to control TNF-α release during the acute phase of *T. cruzi* infection.

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