Leukotriene B₄ Induces Nitric Oxide Synthesis in *Trypanosoma cruzi*-Infected Murine Macrophages and Mediates Resistance to Infection

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The production of nitric oxide (NO) by gamma interferon (IFN- γ)-activated macrophages is a major effector mechanism during experimental *Trypanosoma cruzi* infection. In addition to IFN- γ , chemoattractant molecules, such as platelet-activating factor (PAF) and CC chemokines, may also activate macrophages to induce NO and mediate the killing of *T. cruzi* in an NO-dependent manner. Here we investigated the ability of leukotriene B₄ (LTB₄) to induce the production of NO by macrophages infected with *T. cruzi* in vitro and whether NO mediated LTB₄-induced parasite killing. The activation of *T. cruzi*-infected but not naive murine peritoneal macrophages with LTB₄ induced the time- and concentration-dependent production of NO. In addition, low concentrations of LTB₄ acted in synergy with IFN- γ to induce NO production. The NO produced mediated LTB₄-induced microbicidal activity in macrophages, as demonstrated by the inhibitory effects of an inducible NO synthase inhibitor. LTB₄-induced NO production and parasite killing were LTB₄ receptor dependent and were partially blocked by a PAF receptor antagonist. LTB₄ also induced significant tumor necrosis factor alpha (TNF- α) production, and blockade of TNF- α suppressed LTB₄-induced NO release and parasite killing. A blockade of LTB₄ or PAF receptors partially inhibited IFN- γ -induced NO and TNF- α production but not parasite killing. Finally, daily treatment of infected mice with CP-105,696 was accompanied by a significantly higher level of blood parasitemia, but not lethality, than that seen in vehicle-treated animals. In conclusion, our results suggest a role for LTB₄ during experimental *T. cruzi* infection. Chemoattractant molecules such as LTB₄ not only may play a major role in leukocyte migration into sites of inflammation in vivo but also, in the event of an infection, may play a relevant role in the activation of recruited leukocytes to kill the invading microorganism in an NO-dependent manner.

Chagas' disease is a protozoan infection caused by *Trypanosoma cruzi* and is an important public health problem in much of Latin America. The murine model of Chagas' disease has been used to understand the pathophysiological mechanisms underlying the disease and host protection. In this model, host resistance developed against parasites is dependent on the production of inflammatory cytokines, especially interleukin-12, which triggers the production of gamma interferon (IFN- γ) by NK or T cells, and tumor necrosis factor alpha (TNF- α) (1, 5–7). These cytokines can in turn activate macrophages to produce nitric oxide (NO), the main effector molecule that controls intracellular parasite replication (5, 10, 15, 31). In macrophages, NO is generated from the guanidino nitrogen atom of L-arginine by an inducible NADPH-dependent enzyme called inducible NO synthase (iNOS) (19, 22). More recently, it was shown that chemoattractant molecules which act on G protein-coupled serpentine receptors, such as che-

mokines and platelet-activating factor (PAF), may also participate in the cascade of events leading to NO production and parasite killing (2, 3). On the other hand, interleukin-10 and transforming growth factor β appear to modulate negatively the production of NO and *T. cruzi* killing induced by proinflammatory cytokines (28, 29).

Leukotrienes are metabolites of arachidonic acid and have been shown to induce leukocyte activation and/or recruitment in several models of inflammation (9, 14). Leukotriene B₄ (LTB₄) acts via G protein-coupled receptors and is enzymatically produced through the 5-lipoxygenase pathway (8, 33). A previous study demonstrated the ability of LTB₄ to induce the uptake and killing of *T. cruzi* by murine macrophages (32). However, the mechanisms underlying LTB₄-induced killing and whether this was relevant in vivo were not evaluated. Here we investigated the ability of LTB₄ to induce the production of NO and whether NO mediated LTB₄-induced parasite killing by peritoneal macrophages infected with *T. cruzi* in vitro. As the actions of LTB₄ may be partially dependent on the release of secondary mediators, such as PAF (13, 16, 18, 24), we also evaluated whether LTB₄-induced NO production and parasite killing were PAF dependent. Finally, we investigated whether endogenous production of LTB₄ played a relevant role in the infection of mice with *T. cruzi* in vivo.

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MATERIALS AND METHODS

Experimental animals. Female BALB/c mice, 6 to 8 weeks old, were maintained under standard conditions in the animal houses at our institutions. All procedures had prior approval from the local animal ethics committee.

Parasites and experimental infection. The Y strain of *T. cruzi* was used for all experiments. Trypomastigote forms were cultured and purified from the monkey kidney fibroblast cell line LLC-MK2 for experiments in vitro. BALB/c mice were infected intraperitoneally with 10^4 blood trypomastigote forms, and the number of parasites in 5 μ l of blood collected from a tail vein was measured daily as previously described (17).

In vivo treatment with CP-105,696. Three hours before the infection with blood forms of *T. cruzi*, mice were pretreated via the oral route with 250 μ l of the LTB₄ receptor (BLT) antagonist CP-105,696 [(+)-1-(3S,4R)-[3-(4-phenyl-benzyl)-4-hydroxy-chroman-7-yl]-cyclopentane carboxylic acid] (20 mg/kg of body weight; diluted in phosphate-buffered saline–0.5% methylcellulose) (25, 26). Control animals received 250 μ l of drug vehicle via the same route. After infection, treatment was administered daily until animals died. The dose of CP-105,696 was based on previously published studies demonstrating its efficacy (11, 12). Parasite levels and mortality rates were evaluated throughout the acute phase of infection, and the in vivo experiments were repeated twice.

Macrophage cultures. Inflammatory macrophages were harvested from the peritoneal cavities of mice 3 days after the injection of 1.5 ml of 3% (wt/vol) sodium thioglycolate (Sigma). Cells (10^6 /ml) were plated on chamber slides in 24-well tissue culture plates (Nunc) and incubated for 2 to 4 h at 37°C. Nonadherent cells were removed by exhaustive washing with Hanks' medium. Adherent cells were then infected at a parasite-to-cell ratio of 1:1 for 120 min.

Microbicidal activity. After incubation with *T. cruzi*, extracellular parasites were removed by six washes with RPMI 1640. Infected macrophages were incubated at 37°C in 5% CO₂ in the presence or absence of various concentrations of PAF (0.01 and 0.1 μ M), LTB₄ (0.01 to 1.0 μ M), CP-105,696 (1 μ M), UK-74,505 (1 μ M) (30), 100 U of recombinant murine IFN- γ (Life Technologies, Bethesda, Md.)/ml, a neutralizing anti-TNF- α polyclonal antibody (100 μ g/ml; R&D Systems, Minneapolis, Minn.), or L-N-monomethyl-arginine (L-NMMA; 200 mM; Sigma). Supernatants were harvested on different days. Nitrite measurements, an index of the NO produced, were obtained by using the Griess reaction (31). Parasite growth in macrophages was evaluated by counting the number of trypomastigotes released from day 3 until day 7 of culturing as previously described (27). Both CP-105,696 and UK-74,505 were dissolved in dimethyl sulfoxide prior to further dilution in culture medium. Concentrations of the drugs of greater than 1 μ M could not be used due to the large amounts of dimethyl sulfoxide necessary in the culture medium (data not shown).

TNF- α measurements. Supernatants from macrophage cultures stimulated with various stimuli (PAF, LTB₄, or IFN- γ in the presence of CP-105,696, UK-74,505, or vehicle) were collected and stored at –20°C until further analysis. The concentration of TNF- α in culture supernatants was evaluated by an enzyme-linked immunosorbent assay with commercially available antibodies according to the protocol of the supplier (Pharmingen).

Statistical analysis. All results are presented as the mean and standard error of the mean (SEM). Normalized data were analyzed by a one-way analysis of variance, and differences between groups were assessed by using the Student-Newman-Keuls posttest. A *P* value of <0.05 was considered significant.

RESULTS

Concentration- and time-dependent NO production induced by the activation of infected peritoneal macrophages with LTB₄. The activation of *T. cruzi*-infected murine peritoneal macrophages with LTB₄ induced the concentration-dependent production of NO (Fig. 1). At 4 days after infection of macrophages, the lipid mediator PAF was more effective and more potent than LTB₄ at inducing NO production (Fig. 1). Concentrations of LTB₄ of greater than 1 μ M did not induce further NO production (data not shown). LTB₄-induced NO production was slower in onset, and maximal levels were detected only after 7 days of culturing (Fig. 2). PAF-induced NO production was faster in onset and seemed to increase until day 7 of culturing (Fig. 2).

A series of experiments were then designed to investigate whether low concentrations of LTB₄ enhanced the ability of

FIG. 1. Concentration-dependent effects of LTB₄ and PAF on the production of NO by *T. cruzi*-infected macrophages. BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes (Y strain) at a parasite-to-host cell ratio of 1:1 for 48 h in the presence of LTB₄ or PAF at 37°C in a humidified chamber containing 5% CO₂. The supernatants were harvested, and nitrite concentrations were measured. Results are the mean and SEM for triplicate samples from one of three independent experiments with LTB₄ and PAF. Med, infected cells cultured in medium alone.

IFN- γ to induce NO production. As shown in Fig. 3, LTB₄ effectively enhanced the ability of IFN- γ to induce NO in infected peritoneal macrophages (Fig. 3). For the experiments described below, we chose to use LTB₄ and PAF at concentrations of 1.0 and 0.1 μ M, respectively, and NO production and parasite killing were assessed after 7 days of culturing. Results obtained after 4 days of infection were qualitatively similar to those obtained after 7 days and are thus not shown.

FIG. 2. Kinetics of the effects of LTB₄ and PAF on the production of NO by *T. cruzi*-infected macrophages. BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes at a parasite-to-cell ratio of 1:1 alone (inverted triangles) or in the presence of LTB₄ (1 μ M; circles) or PAF (0.1 μ M; squares). The supernatants were harvested daily, and nitrite concentrations were measured. Results are the mean and SEM for triplicate samples from one of two independent experiments.

FIG. 3. Synergistic effects of the addition of LTB₄ and IFN- γ on the production of NO by *T. cruzi*-infected macrophages. BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes at a parasite-to-cell ratio of 1:1. Macrophages were then stimulated with IFN- γ alone (open bars) or IFN- γ in the presence of LTB₄ (0.01 μ M; hatched bars). The supernatants were harvested on day 4, and nitrite concentrations were measured. Results are the mean and SEM for triplicate samples from one of two independent experiments. An asterisk indicates a *P* value of <0.05 for a comparison with infected cells cultured in the presence of IFN- γ alone.

Effects of the blockade of LTB₄ and PAF receptors on NO production by *T. cruzi*-infected peritoneal macrophages. Next, we evaluated whether the ability of LTB₄ to induce NO production by infected macrophages was LTB₄ receptor mediated and dependent on the production of intermediate molecules, such as PAF. Pretreatment of macrophages with the LTB₄ receptor antagonist CP-105,696 significantly inhibited by about 50% the production of NO induced by LTB₄-stimulated macrophages (Fig. 4A). Similarly, pretreatment of macrophages with the PAF receptor antagonist UK-74,505 blocked LTB₄-induced NO production by 52% (Fig. 4A). At this concentra-

tion, UK-74,505 inhibited PAF-induced NO production by 54% (Fig. 4B). In contrast to its effect on LTB₄-induced NO production, CP-105,696 failed to alter the release of NO after stimulation with PAF (Fig. 4B). Interestingly, pretreatment of IFN- γ -activated macrophages with either CP-105,696 or UK-74,505 reduced the release of NO by 68 and 77%, respectively (Fig. 4C).

LTB₄-induced parasite killing is NO and TNF- α dependent.

Next, we evaluated whether the ability of LTB₄ to induce NO production was relevant for the reported capacity of LTB₄ to enhance the killing of *T. cruzi* by murine macrophages (32). As in the latter study, the addition of LTB₄ to peritoneal macrophages prevented the replication of *T. cruzi* (Fig. 5B). As shown in Fig. 5A, pretreatment with the iNOS inhibitor L-NMMA reduced the release of NO by infected macrophages activated with LTB₄. This inhibition of NO production was accompanied by effective reversal of the ability of LTB₄ to induce macrophages to control parasite replication (Fig. 5B).

TNF- α has been shown to be a major modulator of NO production by *T. cruzi*-infected macrophages (20, 21, 27). LTB₄ activation of infected macrophages (Table 1) but not noninfected macrophages (noninfected macrophages, 0.6 ± 0.5 μ M nitrite; macrophages plus LTB₄ 1 μ M, 0.6 ± 0.3 μ M, *n* = 3) induced significant production of TNF- α . The TNF- α produced was inhibited by pretreatment with either CP-105,696 or UK-74,505 (Table 1). Similarly, pretreatment of macrophages with either CP-105,696 or UK-74,505 partially reduced IFN- γ -induced TNF- α production by infected macrophages (Table 1). More importantly, pretreatment with a neutralizing anti-TNF- α antibody prevented the ability of LTB₄ to induce NO by infected macrophages (Fig. 5A) and reversed LTB₄-induced parasite killing (Fig. 5B). Together, these results demonstrate that the activation of infected macrophages by LTB₄ induces TNF- α production and that the action of this cytokine is important for the ability of LTB₄-activated, *T. cruzi*-infected macrophages to produce NO and kill the parasites.

IFN- γ -induced parasite killing is not dependent on PAF or

FIG. 4. Effects of pretreatment with an LTB₄ receptor antagonist, CP-105,696, or a PAF receptor antagonist, UK-74,505, on the production of NO by *T. cruzi*-infected macrophages activated with LTB₄ (A), PAF (B), or IFN- γ (C). BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes at a parasite-to-cell ratio of 1:1. Macrophages were activated with LTB₄ (1 μ M), PAF (0.1 μ M), or IFN- γ (100 U/ml) in the presence or absence of CP-105,696 (CP; 1 μ M) or UK-74,505 (UK; 1 μ M). After 7 days of culturing, the supernatants were harvested, and nitrite concentrations were measured. Results are the mean and SEM for triplicate determinations and are representative of three independent experiments.

FIG. 5. Effects of pretreatment with an iNOS inhibitor, L-NMMA, or an anti-TNF- α antibody on the production of NO (A) and parasite killing (B) by *T. cruzi*-infected macrophages activated with LTB₄. BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes at a parasite-to-cell ratio of 1:1. Macrophages were activated with LTB₄ (1 μ M) in the absence or presence of L-NMMA (L-N; 200 mM) or anti-TNF- α antibody (a-TNF; 100 μ g/ml). After 7 days of culturing, the supernatants were harvested, and nitrite concentrations were determined (A). In parallel, trypomastigote forms present in the supernatants were determined (B). Results are the mean and SEM for triplicate determinations and are representative of two independent experiments.

LTB₄ receptor activation. As shown above in Fig. 4C and Table 1, pretreatment with CP-105,696 or UK-74,505 partially inhibited the production of NO and TNF- α by infected macrophages activated with IFN- γ . However, and in contrast to these results, neither CP-105,696 nor UK-74,505 modulated IFN- γ -mediated killing (Table 2). At the concentrations used, both CP-105,696 and UK-74,505 reversed LTB₄-induced parasite killing (Table 2).

Effects of pretreatment with the LTB₄ receptor antagonist CP-105,696 during experimental *T. cruzi* infection in vivo. As shown in Fig. 6, daily treatment with CP-105,696 significantly increased blood parasitemia in mice infected with 10,000 trypomastigotes of the Y strain. At the peak of infection, CP-105,696-treated animals had 55% more blood parasites than

TABLE 1. LTB₄ is an effective inducer of TNF- α production by infected murine macrophages^a

Stimulus	TNF- α in the presence of:		
	Vehicle	CP-105,696	UK-74,505
None (macrophages alone)	33 $\pm$ 3	ND	ND
LTB ₄	2,272 $\pm$ 53 ^b	1,263 $\pm$ 68 ^c	1,482 $\pm$ 27 ^c
PAF	4,601 $\pm$ 124 ^b	4,000 $\pm$ 65	2,614 $\pm$ 85 ^c
IFN- γ	2,780 $\pm$ 24 ^b	1,356 $\pm$ 116 ^c	1,170 $\pm$ 66 ^c

^a BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes at a parasite-to-cell ratio of 1:1. Macrophages were activated with medium, LTB₄ (1 μ M), PAF (0.1 μ M), or IFN- γ (100 U/ml) in the absence (vehicle) or presence of CP-105,696 (1 μ M) or UK-74,505 (1 μ M). After 7 days of culturing supernatants were harvested, and TNF- α levels were measured with a specific enzyme-linked immunosorbent assay. Results are the means and SEMs for triplicate determinations and are representative of two independent experiments. ND, not determined.

^b The *P* value was <0.05 for a comparison with infected cells cultured in medium alone.

^c The *P* value was <0.05 for a comparison with cells cultured with vehicle.

vehicle-treated mice (Fig. 6A). The drug had no significant effect on lethality for mice in this experiment (Fig. 6B).

DISCUSSION

The production of NO by IFN- γ -activated macrophages is thought to be the major effector mechanism during experimental *T. cruzi* infection (21, 23, 27, 31). In addition to IFN- γ , it was recently shown that chemoattractant molecules, such as PAF and CC chemokines, activated macrophages to induce NO and mediated the killing of *T. cruzi* in an NO-dependent manner (2, 3). Interestingly, chemokines also acted in synergy with low concentrations of IFN- γ to mediate microbicidal activity (2). The mechanisms by which chemoattractant agents facilitate NO production and microbicidal activity by macrophages are not known. However, preliminary data from our group suggest that chemokines facilitate the phagocytosis of parasites by macrophages and induce phagocytosis-dependent NO production and subsequent parasite killing (G. C. Santana, A. A. Scianne, J. S. Silva, R. T. Gazzinelli, and M. M. Teixeira,

TABLE 2. Effect of CP-105,696 and UK-74,505 on IFN- γ -induced parasite killing by infected macrophages^a

Stimulus	Trypomastigotes (10 ⁶)
None (macrophages alone)	44.0 $\pm$ 20.0
LTB ₄	5.6 $\pm$ 2.1 ^b
LTB ₄ + CP-105,696	23.6 $\pm$ 0.8 ^c
LTB ₄ + UK-74,505	26.0 $\pm$ 15.3 ^c
IFN- γ	0 $\pm$ 0 ^b
IFN- γ + CP-105,696	1.6 $\pm$ 1.2 ^b
IFN- γ + UK-74,505	0.3 $\pm$ 0.3 ^b

^a BALB/c mouse-derived peritoneal macrophages were cultured with *T. cruzi* trypomastigotes at a parasite-to-cell ratio of 1:1. Macrophages were activated with medium, LTB₄ (1 μ M), or IFN- γ (100 U/ml) in the absence or presence of CP-105,696 (1 μ M) or UK-74,505 (1 μ M). After 7 days of culturing, supernatants were harvested, and trypomastigote forms present in the supernatants were determined. Results are the means and SEMs for triplicate determinations and are representative of two independent experiments.

^b The *P* value was <0.05 for a comparison with infected cells cultured in medium alone.

^c The *P* value was <0.05 for a comparison with cells cultured in the presence of LTB₄.

FIG. 6. Effects of in vivo treatment with an LTB₄ receptor antagonist, CP-105,696, on blood parasitemia (A) and lethality (B) in an experimental model of *T. cruzi* infection. BALB/c mice were treated orally with CP-105,696 (20 mg/kg) or with drug vehicle (0.5% methylcellulose in phosphate-buffered saline). The first dose of the antagonist was given 3 h before infection and then daily during the course of the experiment. The number of parasites in 5 μ l of blood (A) and the number of surviving animals (B) were determined daily. Results are the mean and SEM for 12 animals in each group and are representative of two independent experiments.

Abstr. Proc. XXV Meet. Braz. Soc. Immunol., abstr. Cy-29, p. 124, 2000). Similarly, LTB₄ enhanced the ability of macrophages to phagocytose *T. cruzi* in a PAF-dependent manner (G. C. Santana and M. M. Teixeira, unpublished results). In the present study, we evaluated the ability of the chemoattractant molecule LTB₄ to induce NO production and microbicidal activity by *T. cruzi*-infected peritoneal macrophages.

The activation of macrophages with LTB₄ induced a concentration- and time-dependent increase in NO production by *T. cruzi*-infected macrophages. Maximal NO production occurred at 1 μ M LTB₄ and after 7 days of infection of macrophages. Staining for iNOS immunoreactivity was detected in LTB₄-treated *T. cruzi*-infected macrophages but not in untreated cells (data not shown). Interestingly, LTB₄ induced a significant amount of TNF- α in infected but not naive macrophages, and blockade of the TNF- α produced was associated

with a marked inhibition of NO production. Moreover, and as in other studies (32), activation with LTB₄ was associated with significant control of parasite replication in macrophages. Blockade of NO production with an iNOS inhibitor reduced the microbicidal activity of LTB₄. Overall, these results provide strong experimental evidence to suggest that the activation of *T. cruzi*-infected macrophages with LTB₄ induces the production of TNF- α , which drives the release of NO. The NO produced is then responsible for the control of parasite replication. These results are qualitatively similar to previous findings which demonstrated the ability of chemokines or PAF to induce the production of NO and microbicidal activity by *T. cruzi*-infected peritoneal macrophages (2, 3). Of note, our studies were carried out with macrophages derived from the peritoneal cavities of thyoglycolate-treated mice. Future studies should address whether macrophage populations, stimulated or not, derived from other sources respond in a similar manner.

Pretreatment of macrophages with CP-105,696, an LTB₄ receptor antagonist, significantly inhibited the effects of LTB₄ on TNF- α and NO production and parasite killing, suggesting that the effects of LTB₄ are BLT_{1/2} receptor mediated. Interestingly, a PAF receptor antagonist, UK-74,505, also blocked part of the effects of LTB₄ in our system. The latter finding is in agreement with the results of studies with other experimental systems demonstrating that part of the effects of LTB₄ may be mediated by the autocrine production of PAF (e.g., see references 6, 13, and 24). It is unclear how the endogenous production of PAF may drive the ensuing production of TNF- α and NO and subsequent parasite killing. These issues are being actively investigated in our laboratories. Nevertheless, it was previously demonstrated that, at least in neutrophils, the activation of PAF receptors may be an important regulatory mechanism for the phagocytosis of large particles and phagocytosis-dependent chemokine production (4).

Pretreatment of macrophages with PAF or LTB₄ receptor antagonists partially blocked the production of NO and TNF- α after the activation of infected macrophages with IFN- γ . In contrast, IFN- γ -induced parasite killing was not modified by pretreatment with either of the antagonists. These results suggest that the endogenous production of lipid mediators and action on their respective receptors are responsible for part of the ability of IFN- γ to induce NO and TNF- α . Nevertheless, the partial inhibition is not sufficient to reduce IFN- γ -induced parasite killing. The latter results suggest that with the onset of immune- and, thus, IFN- γ -mediated control of parasite replication, the role of lipid mediators (i.e., PAF and LTB₄) may become less important. However, our results also showed that low concentrations of LTB₄ and IFN- γ act in synergy to induce significant production of NO by *T. cruzi*-infected macrophages. In a similar way, chemokines were also shown to act in synergy with IFN- γ to induce NO production by macrophages. One possibility that stems from the latter results is that with the onset of IFN- γ production, chemoattractant molecules cooperate with the cytokine to produce NO and kill parasites. These cooperative actions may become less important when high concentrations of IFN- γ are present.

Our in vitro studies clearly demonstrate a role for LTB₄ in the control of the replication of *T. cruzi*. However, it was important to determine whether the blockade of LTB₄ recep-

tors in vivo would unravel a role for LTB₄ during experimental *T. cruzi* infection. Here, we demonstrated that there was a marked increase in blood parasitemia in mice infected with the Y strain of *T. cruzi*. However, and in contrast to the effects of the PAF receptor antagonist (3), the LTB₄ receptor antagonist had no effect on animal lethality during the acute infection. Although it is possible that complete LTB₄ receptor blockade may not have been achieved in vivo, CP-105,696 has been shown to effectively modulate LTB₄-dependent inflammation and pathology in several models at the dose used (11, 12). Thus, our results suggest that other pathways may be sufficient to eventually control parasite replication and animal survival. Nevertheless, it is worth pointing out that our experimental infection model is already accompanied by significant animal lethality and that a protective role for LTB₄ might not be disclosed under our conditions. Future studies with other strains of *T. cruzi*, measuring concentrations of relevant proinflammatory cytokines (e.g., TNF- α) and nitrate, and with LTB₄ receptor (BLT₁)-knockout animals may help in the further understanding of the role of LTB₄ in experimental and human *T. cruzi* infections.

In conclusion, we showed that the activation of LTB₄ receptors is accompanied by TNF- α and NO production by *T. cruzi*-infected murine peritoneal macrophages. The effects of LTB₄ are partially dependent on the endogenous production of PAF. Moreover, blockade of LTB₄ receptors in vivo disclosed a role for this lipid mediator during experimental *T. cruzi* infection. The above findings support previous work (2, 3) demonstrating an important role for chemoattractant molecules which activate G protein-coupled serpentine receptors during in vitro and in vivo *T. cruzi* infections. Thus, these molecules not only may play a major role in leukocyte migration into sites of inflammation in vivo but also, in the event of an infection, may play a relevant role in the activation of the recruited leukocytes to kill the invading microorganisms in an NO-dependent manner.

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REFERENCES

- Aliberti, J. C. S., M. A. Cardoso, G. A. Martins, R. T. Gazzinelli, L. Q. Vieira, and J. S. Silva. 1996. Interleukin-12 mediates resistance to *Trypanosoma cruzi* in mice and is produced by murine macrophages in response to live trypomastigotes. *Infect. Immun.* **64**:1961–1967.
- Aliberti, J. C. S., F. S. Machado, R. T. Gazzinelli, M. M. Teixeira, and J. S. Silva. 1999. Platelet-activating factor induces nitric oxide synthesis in *Trypanosoma cruzi*-infected macrophages and mediates resistance to parasite infection in mice. *Infect. Immun.* **67**:2810–2814.
- Aliberti, J. C. S., F. S. Machado, J. T. Souto, A. P. Campanelli, M. M. Teixeira, R. T. Gazzinelli, and J. S. Silva. 1999. β -Chemokines enhance parasite uptake and promote nitric oxide-dependent microbistatic activity in murine inflammatory macrophages infected with *Trypanosoma cruzi*. *Infect. Immun.* **67**:4819–4826.
- Au, B. T., M. M. Teixeira, P. D. Collins, and T. J. Williams. 2001. Blockade of PAF receptors controls IL-8 production by regulating the activation of neutrophil CD11/CD18. *Eur. J. Pharmacol.* **425**:65–71.
- Brener, Z., and R. T. Gazzinelli. 1997. Immunological control of *Trypanosoma cruzi* infection and pathogenesis of Chagas disease. *Int. Arch. Allergy Immunol.* **114**:103–110.
- Cardillo, F., J. C. Voltarelli, S. G. Reed, and J. S. Silva. 1996. Regulation of *Trypanosoma cruzi* infection in mice by gamma interferon and interleukin-10: the role of NK cells. *Infect. Immun.* **64**:128–134.
- Chandrasekar, B., P. C. Melby, D. A. Troyer, J. T. Colston, and G. L. Freeman. 1998. Temporal expression of pro-inflammatory cytokines and inducible nitric oxide synthase in experimental acute Chagasic cardiomyopathy. *Am. J. Pathol.* **152**:925–934.
- Dasari, V. R., J. Jin, and S. P. Kunapuli. 2000. Distribution of leukotriene B₄ receptors in human hematopoietic cells. *Immunopharmacology* **48**:157–163.
- Fermor, B., B. Haribabu, J. B. Weinberg, D. S. Pisetsky, and F. Guilak. 2001. Mechanical stress and nitric oxide influence leukotriene production in cartilage. *Biochem. Biophys. Res. Commun.* **285**:806–810.
- Gazzinelli, R. T., I. P. Oswald, S. Hieny, S. L. James, and A. Sher. 1992. The microbicidal activity of interferon- γ treated macrophages against *Trypanosoma cruzi* involves an L-arginine-dependent, nitrogen oxide-mediated mechanism inhabitable by interleukin-10 and transforming growth factor- β . *Eur. J. Immunol.* **22**:2501–2506.
- Gladue, R. P., L. A. Carrol, A. J. Milici, D. N. Scampoli, H. A. Stukenbrok, E. R. Pettipher, E. D. Salter, L. Contillo, and H. J. Showell. 1996. Inhibition of leukotriene B₄-receptor interaction suppress eosinophil infiltration and disease pathology in a murine model of experimental allergic encephalomyelitis. *J. Exp. Med.* **183**:1893–1898.
- Griffiths, R. J., E. R. Pettipher, K. Koch, C. A. Farrell, R. Breslow, M. J. Conklyn, M. A. Smith, B. C. Hackman, D. J. Wimberly, A. J. Milici, D. N. Scampoli, J. B. Cheng, J. S. Pillar, C. J. Pazoles, N. S. Doherty, L. S. Melvin, L. A. Reiter, M. S. Biggars, F. C. Falkner, D. Y. Mitchell, T. E. Liston, and H. J. Showell. 1995. Leukotriene B₄ plays a critical role in the progression of collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* **92**:517–521.
- Komatsu, H., M. Amano, S. Yamaguchi, and K. Sugahara. 1999. Inhibition of activation of human peripheral blood eosinophils by Y-24180, an antagonist to platelet-activating factor receptor. *Life Sci.* **65**:171–176.
- Levy, B. D., C. B. Clish, B. Schmidt, K. Gronert, and C. N. Serhan. 2001. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* **2**:612–619.
- Machado, F. S., G. A. Martins, J. C. S. Aliberti, F. L. A. Mestriner, F. Q. Cunha, and J. S. Silva. 2000. *Trypanosoma cruzi*-infected cardiomyocytes produce chemokines and cytokines that trigger potent nitric oxide-dependent trypanocidal activity. *Circulation* **102**:3003–3008.
- McDonald, P. P., S. R. McColl, P. Braquet, and P. Borgeat. 1994. Autocrine enhancement of leukotriene synthesis by endogenous leukotriene B₄ and platelet-activating factor in human neutrophils. *Br. J. Pharmacol.* **111**:852–860.
- Melo, R. C., and Z. Brener. 1978. Tissue tropism of different *Trypanosoma cruzi* strains. *J. Parasitol.* **64**:475–482.
- Moilanen, E., A. L. Kirkkola, H. Kankaanranta, M. M. Nieminen, and H. Vapaatalo. 1993. Interactions between synthesis of platelet-activating factor and leukotriene B₄ in isolated human polymorphonuclear leukocytes. *Inflammation* **17**:705–714.
- Moncada, S., and A. Higgs. 1993. The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* **30**:2002–2012.
- Munoz-Fernandez, M. A., M. A. Fernandez, and M. Fresno. 1992. Synergism between tumor necrosis factor- α and interferon- γ on macrophage activation for the killing of intracellular *Trypanosoma cruzi* through a nitric oxide-dependent mechanism. *Eur. J. Immunol.* **22**:301–307.
- Munoz-Fernandez, M. A., M. A. Fernandez, and M. Fresno. 1992. Activation of human macrophages for the killing of intracellular *Trypanosoma cruzi* by TNF- α and IFN- γ through a nitric oxide-dependent mechanism. *Immunol. Lett.* **33**:35–40.
- Nathan, C., and Q. W. Xie. 1994. Nitric oxide synthases: roles, tools and controls. *Cell* **78**:915–918.
- Petray, P., M. E. Rottemberg, S. Grinstein, and A. Orn. 1994. Release of nitric oxide during the experimental infection with *Trypanosoma cruzi*. *Parasite Immunol.* **16**:193–199.
- Rediske, J. J., J. C. Quintavalla, W. O. Haston, M. M. Morrissey, and B. Seligman. 1992. Platelet activating factor stimulates intracellular calcium transients in human neutrophils: involvement of endogenous 5-lipoxygenase products. *J. Leukoc. Biol.* **51**:484–489.
- Showell, H. J., E. R. Pettipher, J. B. Cheng, R. Breslow, M. J. Conklyn, C. A. Farrell, G. P. Hingorani, E. D. Salter, B. C. Hackman, D. J. Wimberly, N. S. Doherty, J. R. Melvin, L. A. Reiter, M. S. Biggars, and K. Koch. 1995. The in vitro and in vivo pharmacologic activity of the potent and selective leukotriene B₄ receptor antagonist CP-105696. *J. Pharmacol. Exp. Ther.* **273**:170–184.
- Showell, H. J., R. Breslow, M. J. Conklyn, G. P. Hingorani, and K. Koch. 1996. Characterization of the pharmacological profile of the potent LTB₄ antagonist CP-105,696 on murine LTB₄ receptors in vitro. *Br. J. Pharmacol.* **117**:1127–1132.
- Silva, J. S., G. N. R. Vespa, M. A. G. Cardoso, J. C. S. Aliberti, and F. Q. Cunha. 1995. Tumor necrosis factor α mediates resistance to *Trypanosoma cruzi* infection in mice by inducing nitric oxide production in infected gamma interferon-activated macrophages. *Infect. Immun.* **63**:4862–4867.
- Silva, J. S., P. J. Morrissey, K. H. Grabstein, K. M. Mohler, D. Anderson, and S. G. Reed. 1992. Interleukin 10 and interferon gamma regulation of experimental *Trypanosoma cruzi* infection. *J. Exp. Med.* **175**:169–174.

29. **Silva, J. S., D. R. Twardzik, and S. G. Reed.** 1991. Regulation of Trypanosoma cruzi infections in vitro and in vivo by transforming growth factor beta (TGF-beta). *J. Exp. Med.* **74**:539-545.
30. **Souza, D. G., D. C. Cara, G. D. Cassali, S. F. Coutinho, M. R. Silveira, S. P. Andrade, S. Poole, and M. M. Teixeira.** 2000. Effects of the PAF receptor antagonist UK74505 on local and remote reperfusion injuries following ischemia of the superior mesenteric artery in the rat. *Br. J. Pharmacol.* **131**:1800-1808.
31. **Vespa, G. N. R., F. Q. Cunha, and J. S. Silva.** 1994. Nitric oxide is involved in control of *Trypanosoma cruzi*-induced parasitemia and directly kills the parasite in vitro. *Infect. Immun.* **62**:5177-5182.
32. **Wirth, J. J., and F. Kierszenbaum.** 1985. Stimulatory effects of leukotriene B4 on macrophage association with an intracellular destruction of Trypanosoma cruzi. *J. Immunol.* **134**:1989-1993.
33. **Yokomizo, T., T. Izumi, and T. Shimizu.** 2001. Leukotriene B4: metabolism and signal transduction. *Arch. Biochem. Biophys.* **385**:231-241.

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