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*J Immunol* 2006; 177:3201-3208; doi: 10.4049/jimmunol.177.5.3201
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Leukotrienes Are Essential for the Control of Leishmania amazonensis Infection and Contribute to Strain Variation in Susceptibility

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Leukotrienes (LTs) are known to be produced by macrophages when challenged with Leishmania, but it is not known whether these lipid mediators play a role in host defense against this important protozoan parasite. In this study, we investigated the involvement of LTs in the in vitro and in vivo response to Leishmania amazonensis infection in susceptible (BALB/c) and resistant (C3H/HePAS) mice. Pharmacologic or genetic deficiency of LTs resulted in impaired leishmanicidal activity of peritoneal macrophages in vitro. In contrast, addition of LTB4 increased leishmanicidal activity and this effect was dependent on the BLT1 receptor. LTB4 augmented NO production in response to L. amazonensis challenge, and studies with a NO synthesis inhibitor revealed that NO was critical for the enhancement of macrophage leishmanicidal activity. Interestingly, macrophages from resistant mice produced higher levels of LTB4 upon L. amazonensis challenge than did cells from susceptible mice. In vivo infection severity, as assessed by footpad swelling following s.c. promastigote inoculation, was increased when endogenous LT synthesis was abrogated either pharmacologically or genetically. Taken together, these results for the first time reveal an important role for LTB4 in the protective response to L. amazonensis, identify relevant leishmanicidal mechanisms, and suggest that genetic variation in LTB4 synthesis might influence resistance and susceptibility patterns to infection. The Journal of Immunology, 2006, 177: 3201–3208.

Infection by protozoan parasites of the Leishmania genus represents an important public health problem in >80 countries around the world, with 2 million new cases each year (1). Leishmania amazonensis is a member of the Leishmania mexicana complex. In South American countries, it can cause a broad spectrum of clinical manifestations, ranging from single cutaneous lesions to multiple disfiguring nodules and even visceral complications. At present, the molecular and genetic basis for the development of different clinical diseases following infection with L. amazonensis is undefined.

Murine models of infection with the Old World species Leishmania major demonstrate that outcome of disease is determined by the nature (i.e., Th1 or Th2 cells) and magnitude of the T cell and cytokine responses early in infection. In infected inbred mice (such as C57BL/6 or C3H/HePas), the production of IFN-γ by Th1 cells and NK cells mediates resistance, whereas production of Th2 cell-derived cytokines confers susceptibility (2). However, outcomes in infection with the New World species L. amazonensis are less clearly related to Th1/Th2 polarization (3–6). This led us to speculate whether differences in the synthesis of lipid mediators involved in the early phases of infection might influence patterns of resistance and susceptibility to L. amazonensis.

Although best known for their participation in inflammatory diseases such as asthma (7) and atherosclerosis (8), there is increasing recognition that leukotrienes (LTs) are also important in protective host responses to infection. They have been shown to be critical for the in vivo clearance of various types of microbes and in mediating the phagocytic and microbicidal capacities of phagocytes (9). LTs are derived from the metabolism of the cell membrane fatty acid arachidonic acid via the enzyme 5-lipoxygenase (5-LO), in concert with its helper protein 5-LO-activating protein (FLAP) (10). The two principal bioactive classes of LTs include LTB4 and the cysteinyl-LTs (cysLTs), LTC4, LTD4, and LTE4 (10).

LTs are also involved in the control of protozoan infections. Wirth et al. (11, 12) reported that both LTB4 and LTC4 increased the phagocytosis and killing of Trypanosoma cruzi by peritoneal macrophages. The IFN-γ-mediated killing of Toxoplasma gondii by human monocytes was shown to be dependent on LT biosynthesis (13). In addition, Talvani et al. (14) showed that during T. cruzi infection, LTB4 is able to promote NO release and thereby kill this parasite.

It has been shown that 5-LO products are produced during in vivo and in vitro infection with Leishmania donovani (15, 16). However, there is no information on whether LTs participate in the host response to leishmanial infection. In this work, we sought to determine the role of specific LTs in leishmanicidal activity of macrophages in vitro, and in the control of infection in vivo, by studying both susceptible and resistant mouse strains.

3 Abbreviations used in this paper: LT, leukotriene; 5-LO, 5-lipoxygenase; FLAP, 5-LO-activating protein; cysLT, cysteinyl LT; iNOS, inducible NO synthase; KO, knockout; WT, wild type.

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Materials and Methods

Reagents

M199, RPMI 1640, and thiglycollate were purchased from Invitrogen Life Technologies. t-NAME (NO synthase inhibitor), t-glutamine, penicillin, streptomycin, and peroxidase-labeled monoclonal anti-rabbit IgG were all purchased from Sigma-Aldrich. LTB4, U75302 (BLT1 receptor antagonist), and MK571 (cysLT1 antagonist) were purchased from BIOMOL. MK0591 (FLAP inhibitor) was donated from Merck-Frost. The rabbit antiserum to inducible NO synthase (iNOS) was from Cayman Chemical. Compounds requiring reconstitution were dissolved in either ethanol or DMSO. Required dilutions of all compounds were prepared immediately before use, and equivalent quantities of vehicle were added to the appropriate controls.

Cell viability

All compounds and vehicles used in the experiments showed no adverse effects on macrophage or L. amazonensis viability as determined by a cell-based MTT assay (data not shown).

Parasite

Promastigotes of L. amazonensis (MHOM/BR/73/M2269) were derived from amastigotes isolated from the infected footpad of BALB/c mice and resuspended in M199 plus 10% FBS for a maximum of six passages. The experiments were performed with parasites in stationary phase (5 days in culture).

Animals

Eight-week-old female 5-LO knockout (KO) (129-Alox5tm1FlpF1) (17) and strain-matched wild-type (WT) sv129 mice were obtained from The Jackson Laboratory and kept at our own animal facilities (Institute of Biomedical Science Animal House). Eight-week-old female BALB/c and C3H/HePas mice were bred and kept at our own facilities. Animals were kept under conventional conditions with free access to food and water. Animal protocols were approved by the University of São Paulo Committee on Use and Care of Animals.

Cell harvest

Macrophages were harvested from the peritoneal cavities of the mice by lavage with PBS 4 days after the injection of 1 ml of 3% thiglycollate as described (18). Contaminating RBC were lysed with H2O and the cells were washed twice with PBS. The percentage of macrophages was determined microscopically using a modified Wright-Giemsa stain and a typical experiment yielded ~80% macrophages.

Macrophage leishmanicidal activity

Approximately 2–3 × 10^5 cells were allowed to attach for 60 min to round, 13-mm-diameter glass coverslips placed in 24-well plates (Costar) containing 0.5 ml of RPMI 1640. The nonadherent cells were removed by three washings in warm medium. The adherent cells were incubated in RPMI 1640 supplemented with 10% FCS, penicillin (100 U/ml), and streptomycin (0.1 mg/ml) for 18 h at 37°C in 5% CO2. The cells were pretreated with 10 min with 50 μl of ice-cold lysis buffer (10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 2% Nonidet P-40, 1 mM 4-(2-aminophenyl)-benzenesulfonyl fluoride, and 5 μM leupeptin). The lysed cell preparation was centrifuged at 10,000 × g for 5 min at 4°C. Protein content in the supernatant was determined using the BCA protein assay kit (Pierce) according to the manufacturer’s protocols and was adjusted to 20 μg/ml.

SDS-PAGE and immunoblotting

Cell lysate was mixed with 4 μl of 5X loading buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 100 mM DTT, 1% glycerol, and 0.1% bromphenol blue). Heated samples of equal amounts of protein (20 μg/20 μl) were applied to 8% SDS-polyacrylamide gels and subjected to electrophoresis. The separated proteins were transferred to nitrocellulose membranes in Trans-blot SD-Semidyry Transfer Cells (Bio-Rad; 15 min at 15 μA). After transfer, the membranes were incubated in TBST buffer (150 mM NaCl, 20 mM Tris, 0.01% Tween 20 (pH 7.4)) containing 5% fat-free dry milk. The blot was treated with a 1/1000 dilution of rabbit polyclonal Ab to iNOS for 1 h at room temperature, then washed three times with TBST, and incubated with 1/5000 dilutions of peroxidase-conjugated monoclonal anti-rabbit IgG for 1 h at room temperature. The immunocomplexed peroxidase-labeled Abs were visualized by an ECL chemiluminescence kit following the manufacturer’s instruction (Amersham Biosciences).

Measurement of nitrite levels

To evaluate NO production, nitrite concentration in the supernatants of macrophage cultures was measured using the standard Griess reaction (18). Briefly, 50 μl of the culture supernatant was reacted with 50 μl of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, and 2.5% H3PO4) for 10 min at room temperature. The absorbance was measured at 540 nm by using a 620-nm reference filter in a Dynatech microplate reader and the nitrite concentration was calculated using a standard curve of sodium nitrite. All tests were done at least in triplicate.

Measurement of LTs

Macrophages from BALB/c or C3H/HePas mice (2 × 10^5 cells/well) were cultured in 96-well plates in RPMI 1640. Cultures were then incubated for 2, 4, 8, and 24 h at a ratio of 5:1 L. amazonensis:macrophage. Supernatants were collected and LTB4 and cysLT levels were quantified by enzyme immunoassay according to the manufacturer (Cayman Chemical). The limit of assay detection for LTB4 and cysLTs is 3.9 and 7.8 pg/ml, respectively.

In vivo treatment and infection

The mice were treated with 1 mg/kg zileuton i.p. 1 h before infection and once daily for 7 days thereafter. A total of 1 × 10^6 stationary phase promastigotes of L. amazonensis was inoculated s.c. into the left hind footpad of 8-wk-old BALB/c, C3H/HePas, 5-LO KO, and the counterpart WT female mice (at least five mice per group). The evolution of the disease was monitored biweekly over the next 10 wk by measuring footpad thickness with a pachymeter (Mitutoyo). Results are expressed as the difference in thickness between the infected and the noninfected contralateral footpad.

Statistical analysis

Data are represented as mean ± SEM and were analyzed with the Prism 3.0 statistical program (GraphPad Software). Comparisons between two experimental groups were performed using Student’s t test. Comparisons among more than or equal to three experimental groups were performed by ANOVA followed by the Bonferroni test. Differences were considered significant if p ≤ 0.05. All experiments were performed on more than or equal to three separate occasions unless otherwise specified.

Results

5-LO metabolites increase macrophage leishmanicidal activity

Infection index of macrophages from susceptible BALB/c mice was generally higher than that from resistant C3H/HePas mice, as expected. Under all conditions, the infection index was higher at 24 than at 4 h of incubation (data not shown). Changes in infection index observed with experimental treatments were qualitatively similar at both time points, but only 24 h data will be presented.

Pharmacologic inhibition of LT synthesis with the FLAP inhibitor MK0591 increased the infection index of macrophages from both susceptible and resistant mice (Fig. 1, A and B). Although these results were obtained with thiglycollate-elicited macrophages, a similar decrease in leishmanicidal activity was also observed with MK0591 treatment of resident peritoneal macrophages from BALB/c mice (data not shown). We verified the importance
of endogenous LTs in leishmanicidal activity by using macrophages from 5-LO-deficient mice. As can be observed in Fig. 1C, macrophages from 5-LO KO mice showed impaired leishmanicidal activity (\(-112\%\) increase in the infection index) when compared with macrophages from WT mice. These results suggest that LTs produced by macrophages following infection with *L. amazonensis* promastigotes support their capacity to kill the parasite. We have also observed the same effects of LT biosynthesis inhibition in macrophage infection by the Old World parasite *L. major* (data not shown).

To investigate which LTs are responsible for the increased leishmanicidal activity, we pretreated macrophages with the BLT1 antagonist U75302 or the cysLT1 antagonist MK571. Antagonism of BLT1 in macrophages from both BALB/c (Fig. 2A and B) and C3H/HePas (Fig. 2B) mice increased the infection index when compared with the untreated control. However, antagonism of cysLT1 with MK571 had no effect on BALB/c macrophage infection (Fig. 2C).

Because these data suggested a role for endogenous LTB\(_4\) in macrophage leishmanicidal activity, we wished to confirm that exogenous LTB\(_4\) was capable of directly enhancing it. Indeed, the addition of LTB\(_4\) dose-dependently increased leishmanicidal activity in both susceptible and resistant mice. However, this effect was more pronounced in macrophages from BALB/c than from C3H/HePas mice (Fig. 3, A and B). Importantly, the large difference in infection index between macrophages from susceptible and resistant mice under control conditions was abolished by 100 nM LTB\(_4\) treatment of cells from susceptible animals. Although our data showed no effect of endogenous cysLTs on the infection index, we wished to determine whether the BALB/c macrophages were able to respond to exogenous cysLTs. As can be observed in Fig. 3C, the addition of 100 nM LTD\(_4\) enhanced macrophage leishmanicidal activity when compared with untreated control, though not to the same degree as did LTB\(_4\) (Fig. 3A).

**Macrophage LT synthesis in response to *L. amazonensis* infection**

We next sought to verify that LTB\(_4\) was indeed generated upon macrophage challenge with promastigotes in vitro, and compare the responses of cells from susceptible and resistant strains. Fig. 4 shows the time course of LTB\(_4\) production by infected macrophages. Significant increases in LTB\(_4\) production over the uninfected control level (measured at 24 h) were observed by both strains. C3H/HePas macrophages produced significantly higher levels of LTB\(_4\) than BALB/c cells at all time points tested. After a plateau in synthesis reached at 8 h in both strains, a further increment in LTB\(_4\) accumulation at 24 h was noted only in the C3H/HePas cells (Fig. 4). The levels of cysLTs in macrophage culture supernatant were below the detection limit of the assay (7.9 pg/ml) at all time points tested (data not shown). This result is in accordance with data in Fig. 2C showing that the cysLT1 antagonist had no effect on macrophage leishmanicidal activity.
LTB₄ enhances in vitro leishmanicidal activity through the induction of NO formation

NO is well-established as a mediator involved in the control of *Leishmania* infection. The importance of NO in the control of *L. amazonensis* infection in vitro was verified by the fact that treatment with the NO synthesis inhibitor L-NAME (1 mM) enhanced the infection index of BALB/c macrophages. We next wished to determine whether NO was the microbicidal molecule responsible for the ability of LTB₄ to enhance killing. BALB/c macrophages incubated with or without LTB₄ (100 nM) were pretreated or not with L-NAME 30 min before infection. The ability of exogenous LTB₄ to enhance leishmanicidal activity was abolished by the inhibitor of NO synthesis (Fig. 5). We further evaluated the ability of exogenous LTB₄ to induce NO secretion and iNOS expression. As can be observed in Fig. 6, A and B, macrophages from both strains induced significant levels of nitrite (a final product derived from NO production) after treatment with 100 nM LTB₄, when compared with untreated macrophages. Of note, the cells from BALB/c mice manifested a greater response and did so at a lower LTB₄ concentration as compared with cells from C3H/HePas mice. In addition, iNOS expression in macrophages from BALB/c mice also increased dose-dependently after LTB₄ stimulation (Fig. 6C).

**FIGURE 3.** Exogenous LTB₄ increases leishmanicidal activity of macrophages. Macrophages from BALB/c (A and C) or C3H/HePas (B) mice were infected with the promastigote form of *L. amazonensis* after 5 min pretreatment with or without the indicated concentrations of LTB₄ (A and B) or 100 nM LTD₄ (C). After 24 h, the infection index was determined. Data are expressed as the mean ± SE of one experiment representative of a total of three. *, *p* < 0.05 vs control.

**FIGURE 4.** LTB₄ production in macrophages from BALB/c and C3H/HePas mice infected with *L. amazonensis*. LTB₄ levels were measured as described in Materials and Methods in BALB/c or C3H/HePas macrophage supernatants at different time points following infection with the promastigote form of *L. amazonensis*. Data are expressed as the mean ± SE from two independent experiments, each performed in triplicate. *, *p* < 0.05 vs control; #, *p* < 0.05 vs the uninfected control by ANOVA. LTB₄ level of uninfected control supernatant were 2.73 ± 0.63 (C3H/HePas) and 3.20 ± 0.94 (BALB/c) after 24 h of culture.

**FIGURE 5.** LTB₄-induced leishmanicidal activity is dependent on NO production. Macrophages from BALB/c were infected with the promastigote form of *L. amazonensis* after 30 min pretreatment with or without the NO synthase inhibitor L-NAME (1 mM) or LTB₄ (100 nM). After 24 h, the infection index was determined. Data are expressed as the mean ± SE of one experiment representative of a total of three. *, *p* < 0.05 vs control; #, *p* < 0.05 vs L-NAME by ANOVA.
before the infection. While *L. amazonensis* infection induced NO release, treatment with LTB₄ dose-dependently increased NO levels (Fig. 6).

**LTs modulate the in vivo *L. amazonensis* infection**

The in vivo infection of susceptible BALB/c mice with *L. amazonensis* promastigotes resulted in a greater degree of footpad swelling after 10 wk than in the resistant C3H/HePas mice. Of note, the degree of footpad swelling in the WT sv/129 animals was less than that of other mouse strains at all time points observed. To verify the importance of LTs in the control of *L. amazonensis* infection in vivo, we used pharmacological and genetic approaches. First, BALB/c or C3H/HePas mice were treated daily with the LT synthesis inhibitor zileuton (1 mg/kg) for the first 7 days following inoculation in the left hind footpad with 1 × 10⁶ promastigotes. The footpad swelling was measured every 2 wk. Both mouse strains exhibited an increase in lesional size with zileuton treatment which was apparent at 4 wk and maximal at 8 wk post infection (Fig. 7, A and B). The time course curves for zileuton were left shifted as compared with those for vehicle. By 10 wk of infection, the lesion in zileuton-treated C3H/HePas mice was no longer different from that in untreated mice (Fig. 7B).

**Discussion**

This study for the first time establishes the importance of LTs in both in vivo and in vitro leishmanicidal activity and identifies LTB₄ as the macrophage-derived species involved in this phenomenon. The relevant findings are: 1) endogenous and exogenous LTs increase in vitro leishmanicidal activity of macrophages obtained from susceptible and resistant mice; 2) LTB₄ appears to be the major molecule that mediates resistance and its effects are exerted via the BLT1 receptor; 3) after *L. amazonensis* infection, macrophages from resistant mice produce higher levels of LTB₄ when compared with macrophages from susceptible mice; 4) the enhanced leishmanicidal activity induced by LTs is dependent on NO production; 5) LTs are relevant mediators for the in vivo control of *L. amazonensis* infection in both susceptible and resistant mice.

It has been demonstrated in different models of infection that LTs increase phagocyte effector functions, including phagocytosis, microbicidal activity, generation of reactive oxygen and nitrogen species, and a myriad of proinflammatory cytokines (9). Our group
has demonstrated that LTs increase phagocytosis of IgG- and complement-opsonized targets as well as microbicidal activity, and have identified a number of the relevant signal transduction events which are amplified (19–22). Because infection with the promastigote form of the Leishmania parasite does not require opsonization, we sought to determine the importance of LTs in a model of protozoan infection. Thus, we evaluated whether LTs would influence the outcome of L. amazonensis infection in vitro and in vivo.

The immune mechanisms that underpin resistance/susceptibility to Leishmania infections and particularly to L. amazonensis infection are as yet uncertain. It is becoming increasingly apparent that the nature of the immune response which dictates outcomes are variable and dependent on both the mouse strain and the Leishmania species (3, 4, 23, 24).

With respect to L. amazonensis infection, it is now clear that BALB/c mice develop tumor-like lesions, followed by the dissemination of the parasite, while the CH3/HePas animals develop a small local lesion which resolves spontaneously (2, 5, 25–28). With this in mind, we asked whether LTs might influence the susceptibility phenotype. Both pharmacological and genetic approaches indicated that LTs are important mediators in the control of leishmanicidal activity in macrophages from both susceptible BALB/c and resistant C3H/HePas mice. We also performed experiments in macrophages from WT sv/129 or 5-LO KO mice. There are no reports in the literature regarding infection of L. amazonensis in sv/129 mice. We found that this strain resembles the resistant C3H/HePas mice. Indeed, WT sv/129 did not exhibit any increase in the infection index between 4 and 24 h of infection (data not shown). However, macrophages from 5-LO-deficient mice were unable to control leishmanial infection at 24 h of infection. Our in vitro experiments indicated that LTB4 is the major LT involved in the leishmanicidal activity of macrophage, because unlike cysteine LTs, it was produced and antagonism of its high-affinity BLT1 receptor increased infection index. These results are in line with our previous work showing that LTB4 was the major LT involved in the bactericidal activity of alveolar macrophages (22). It has been demonstrated that exogenous LTB4 and LTC4 enhanced phagocytosis and killing of T. cruzi (11, 12). This is in accordance with our findings that exogenous LTD4 was able to enhance macrophage leishmanicidal activity. In our model, LTs promote killing but did not influence the uptake of unopsonized L. amazonensis (data not shown). Promastigotes can attach to the macrophage via the mannose-fucose receptor, which binds to mannan residues of the lipophosphoglycan in the promastigote forms (29–31). It is not known whether LTs can modulate the signal through the mannose receptor.

The mechanisms that underlie resistance and susceptibility to L. amazonensis infection are still elusive. Differences in the generation of IL-10 (32, 33), TGF-β (34), and NO (35), and in the response to IL-12 (5) and IFN-γ (36, 37), have all been suggested. However, no previous reports have considered the role of lipid mediators in the resistance and susceptibility to infection. Kuroda et al. (38) showed that BALB/C c mice were more sensitive to the suppressive effect of PGE2 as compared with C3H/HePas and C57BL/6 mice and this effect was due to a higher number of PGE2-binding sites than those of other mouse strains. The fact that BALB/c macrophages tended to exhibit a greater increase in the leishmanicidal activity (Fig. 3) and NO generation (Fig. 6) in response to lower concentrations of LTB4 might be consistent with a similar difference in BLT1 expression. However, we found that C3H/HePas macrophages produced 3-fold more LTB4 than BALB/c macrophages when challenged with L. amazonensis. The levels of LTB4 found were rather low and this might in part be explained by the well-known attenuated eicosanoid synthetic capacity of thioglycolate-elicited macrophages (39, 40). Steil et al. (41) previously demonstrated that immune complex-induced peritonitis was associated with greater macrophage generation of LTB4 in the peritoneal cavity of C3H/HePas mice than BALB/c mice. This suggests that the higher capacity for LTB4 production of C3H/HePas is not specific for L. amazonensis infection, but it extends to other stimuli. The mechanisms responsible for the differences in LTB4 production among different strains are currently under investigation.

It is well-established that NO is involved in the control of L. major and L. donovani infection. However, it has been reported that NO inhibition did not modify the course of L. amazonensis infection in vitro (42) or in vivo (24). Our results with L-NAME implicates NO as a major mediator of leishmanial activity of LTB4. However, some persistent leishmanicidal activities of LTB4-treated macrophages even in the presence of L-NAME suggest that other mechanisms independent of NO may be operative. Talvani et al. (14) demonstrated that NO is the molecule involved...
in LTB4-mediated T. cruzi killing. However, the authors did not evaluate the relative importance of LTB4 on NO production. We showed an enhanced production of NO in LTB4-treated macrophages that were infected with Leishmania. Moreover, treatment of macrophages with LTB4 induces iNOS expression and NO production in both strains of mice. Our results are in line with the findings of Talvani et al. (14) that showed a synergism between infection with T. cruzi and treatment with LTB4.

Our findings in vivo confirmed the involvement of LTs in the control of L. amazonensis infection because treatment of mice with zileuton increased the footpad swelling of resistant and susceptible mice when compared with untreated control animals. This is the first report showing in vivo and in vitro L. amazonensis infection of sv129 mice. We found that the outcome of infection in this strain is similar to the resistant C5H/HePas strain. Interestingly, the sv/129 strain is also resistant to L. major infection (43–45). The importance of endogenous LTs in the in vivo control of infection has been demonstrated in different models of infection in vivo. Our group was the first to show that 5-LO-deficient mice are unable to control Klebsiella pneumoniae infection (46). In another model of protozoan infection, the treatment of BALB/c mice with a BLT1 antagonist increased T. cruzi parasitism but not lethality (14). In our model, both pharmacological inhibition and genetic deficiency in LT biosynthesis increased the footpad swelling after L. amazonensis infection.

Deficiency of LT synthesis has been described in malnutrition (47, 48) and HIV infection (49–51). Those conditions are also known to predispose to reactivation of latent leishmaniasis (1, 52–54). Those conditions are also known to predispose to reactivation of latent leishmaniasis (1, 52–54) and HIV infection (49–51). Those conditions are also known to predispose to reactivation of latent leishmaniasis (1, 52–54) and HIV infection (49–51). Those conditions are also known to predispose to reactivation of latent leishmaniasis (1, 52–54) and HIV infection (49–51). Those conditions are also known to predispose to reactivation of latent leishmaniasis (1, 52–54) and HIV infection (49–51).

In summary, our results show that LTB4 plays a role in the in vivo and in vitro control of L. amazonensis in both susceptible and resistant mouse strains and its effect is mediated by the increase of iNOS expression and NO generation. In addition, we also observed an increase in LTB4 generation by macrophages of resistant mice when compared with cells from a more susceptible strain. Our data implicate LTB4 as a mediator involved in the pattern of resistance/susceptibility to infection with Leishmania.

Acknowledgments
We acknowledge Richard Landgraf for technical contributions and Karina Bastos, Daniel Mucida, and David Aronoff for helpful discussions.

Disclosures
The authors have no financial conflict of interest.

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