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Original article

Role of interleukin-4 and prostaglandin E_2 in *Leishmania amazonensis* infection of BALB/c mice

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Abstract

The role of cytokines in *Leishmania amazonensis* experimental infection has not been as well studied as in *Leishmania major* infection model. Here we investigated the role of interleukin (IL)-4 and PGE_2 in *L. amazonensis* infection of susceptible BALB/c mice. IL-4 deficient (−/−) or wild-type (+/+) BALB/c mice were infected with different inocula of *L. amazonensis*. Two weeks after infection with 5×10^6 promastigotes/footpad, the production of interferon (IFN)-gamma upon *L. amazonensis* antigen stimulation was significantly higher in lymph node cell cultures of IL-4−/− mice than in IL-4+/+ mice. The levels of anti-leishmania IgG2a antibodies were also significantly higher in serum from IL-4−/− mice. In contrast, the levels of IgG1 antibodies were increased in IL-4+/+ mice and almost undetectable in IL-4−/− mice. Despite the increased Th1 response, lesions of IL-4−/− BALB/c mice progressed similarly to those of IL-4+/+ mice upon infection with the 5×10^6 inoculum. However, IL-4−/− mice developed smaller lesions upon infection with 10^5, 10^4 or 10^3 parasites than IL-4+/+ mice. The resistance of IL-4−/− correlated with higher Th1 response, compared to IL-4+/+ upon infection with 10^4 *L. amazonensis*. IL-4+/+ mice treated with indomethacin, an inhibitor of PGE_2 synthesis, during the first 3 weeks of infection developed smaller lesions and lower parasitic load when compared to the control group. The lesions of indomethacin-treated groups contained mostly macrophages without vacuoles and small or absent necrotic areas. These results indicate that IL-4 and PGE_2 are susceptibility factors to *L. amazonensis* infection.

Keywords: *Leishmania amazonensis*; BALB/c mice; Interleukin-4; Prostaglandin E_2; Indomethacin

1. Introduction

Leishmaniasis represents a serious public health problem in many tropical and sub-tropical countries, including Brazil. About 12 million people suffer from this disease, and approximately 2 million individuals become infected annually [1]. The use of experimental parasitic infections for the study of the disease has facilitated not only the understanding of how the immune processes are induced and regulated, but also the mechanisms used by the parasites to escape immune surveillance [2].

The experimental infection of mice with *Leishmania major* is one of the best characterized models for the study of the immune response associated with the differentiation of T lymphocytes [3]. Many studies demonstrated the role of the IFN-γ in polarized type 1 responses and resistance to *Leishmania* infection, whereas the cytokine IL-4 is the main factor determining type 2 responses and disease susceptibility [4–9]. Recent studies, however, yielded contradictory observations regarding the role of IL-4 in susceptibility to *Leishmania* infection. Depending on the strain of *L. major* used, BALB/c IL-4 and IL-4Rα deficient mice remain susceptible to the infection [2]. When present in the period of activation of T cells, IL-4 inhibits the expression of β2 chain of IL-12 receptor, leading to the development of a Th2 response and susceptibility. IL-4 can also induce resistance in BALB/c mice infected

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with *L. major* when present in the initial phase of antigen present cell activation that precedes the stimulation of T cells by instructing dendritic cells to produce IL-12, which will cause the maturation of Th1 cells and resistance [10].

BALB/c mice develop resistance to infection by *L. major* when infected with a small number of parasites [11,12]. When infected with 1000 promastigotes, BALB/c mice develop a stable Th1 response and resist to a subsequent challenge, whereas the infection with higher numbers of promastigotes results in a polarization for a Th2 response and susceptibility [12]. This resistance is associated with a state of memory that generates an active Th1 response during a posterior challenge. Therefore, depending on the species of parasite, the inoculum size used and the strain of mice studied, a different response regarding the susceptibility may be observed. In addition to IL-4, other factors which act together or independently of IL-4 can lead to susceptibility, such as IL-10, IL-13, TGF-β and PGE_2 [13–19]. PGE_2 inhibits Th1 response and increases the production of cytokines Th2 such as IL-4 [20,21].

Despite the fact that *Leishmania amazonensis* is responsible for a wide variety of clinical manifestations in South America, few studies have investigated the response of the host during experimental infection by this parasite [22]. In the present study we investigated the role of inhibitory soluble mediators IL-4 and PGE_2 and their associations with the mechanisms of parasite elimination during infection of BALB/c mice by *L. amazonensis.* The correlations between histopathological and immunological aspects were carried out in the murine model, in order to understand some of the mechanisms involved in the susceptibility profile during infection with this parasite.

2. Materials and methods

2.1. Mice

Specific-pathogen-free, 8-week-old female wild-type and IL-4−/− BALB/c mice were maintained at the animal facilities at the Gonçalo Moniz Research Center-FIOCRUZ, and provided with rodent diet and water ad libitum. IL-4−/− breeder pairs, generated on a pure BALB/c genetic background, were purchased from The Jackson Laboratory (Bar Harbor, ME) and kindly donated by Dr João S. Silva (University of São Paulo, Ribeirão Preto). All procedures described here had prior approval from the local animal ethics committee.

2.2. Parasites and soluble leishmania antigen preparation

L. amazonensis promastigotes (MHOM/BR88/BA-125 Leila strain) were grown to the stationary phase in axenic liver infusion-tryptose (LIT) medium supplemented with 10% fetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), 5% sterile human urine and 50 μg/ml gentamycin (Sigma, St. Louis, MO, USA). The viability of the parasites was maintained by periodic passages in BALB/c mice. Freeze-thawed antigen (FTAg) was prepared using stationary-phase *L. amazonensis* promastigotes re-suspended in PBS and submitted to four cycles of freezing (−196 °C) and thawing (37 °C). After protein determination by fluorescence [23], FTAg was aliquoted and stored at −70 °C.

2.3. Infection and indomethacin treatment

Stationary-phase promastigotes (5×10^6, 10^5, 10^4 or 10^3) were used to infect groups of IL-4+/+ and IL-4−/− BALB/c mice, subcutaneously, in 25 μl of saline in the right footpad. The footpad swelling was monitored weekly with a digital caliper (Mitutoyo, Yokohama, Japan) and was calculated by subtracting the thickness of the uninfected contra-lateral footpad from the thickness of the infected footpad. In some experiments, mice were treated with indomethacin (INDO; Sigma, St. Louis, MO, USA) dissolved in absolute ethanol at 10 mg/ml, and diluted to a final concentration of 20 μg/ml in saline by intraperitoneal injection, once a day during the first 3 weeks of infection. Control groups received saline and ethanol in the same concentration. In one experiment, groups of IL-4−/− mice were treated with INDO administered in drinking water (20 μg/ml in 0.4% ethanol) or with vehicle (0.4% ethanol).

2.4. Parasite quantification

The number of parasites in the lymph node was estimated by limiting dilution assay. The popliteal lymph nodes draining the infected footpad were removed and used to prepare a cell suspension in PBS. After centrifugation at 1500 rpm for 10 min, the pellet was resuspended in LIT medium. The suspension was then serially diluted in 10-fold dilutions and distributed in 96-well culture plates. After 5–10 days of incubation at 26 °C, the wells were examined in an inverted microscope for the presence or absence of promastigotes. Results were expressed as −log of the highest cell suspension dilution with wells positive for leishmania.

2.5. Histopathologic evaluation

Infected footpads of BALB/c IL-4+/+ or IL-4−/− were removed after 5 weeks of infection and immediately placed in 10% formaldehyde. After 12–24 h of fixation, bones were decalcified in 7% nitric acid. Tissues were washed, embedded in paraffin, and sections 3–5 μm thick were stained with conventional hematoxylin and eosin (H&E).

2.6. Immunohistochemistry

To demonstrate the presence of parasite in the site of infection, immunostaining for *Leishmania* was performed in 3–5 μm thick sections obtained from formalin-fixed and paraffin-embedded tissue. The indirect immunoperoxidase technique was applied using a rabbit polyclonal antibody against *Leishmania* [24]. The antibody was diluted 1:1000 in PBS containing 1% bovine serum albumin (BSA). For negative control, non-related rabbit IgG replaced the specific antibody. The primary antibody was detected by using biotinylated goat

anti-rabbit antibody (American Qualex, San Clemente, CA, USA) at a dilution of 1:200. Streptavidin-conjugated peroxidase was used for secondary antibody detection, and revealed with 3,3′diaminobenzidine (DAB) (Sigma). Tissue sections were then counterstained with Harris's hematoxylin.

2.7. Cytokine, nitric oxide and antibody detection assays

For determination of cytokine production, draining lymph node cells were prepared in RPMI medium supplemented with 10% FBS (Cultilab), 7.5% sodium bicarbonate (Sigma), 1 M Hepes (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1 mM sodium pyruvate (Sigma), 2 mM L-glutamine (Sigma), 0.05 μM 2-ME, 50 μg/ml gentamycin (Sigma) and cultured in 24-well plates (5×10^6 cells in 1 ml/well) in the absence or in the presence of concanavalin A (Con A; 1 μg/ml) or FTAg (100 μg/ml) for 48 h. IFN-γ and IL-10 levels in supernatants were determined using Duoset ELISA kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. The assay sensitivities for IFN-γ and IL-10 were 16 and 8 pg/ml, respectively. *Leishmania*-specific IgG1 and IgG2a antibodies were determined by ELISA. Microtiter plates were sensitized overnight with 50 μl/well of 3 μg/ml SLA solution in carbonate-bicarbonate buffer, pH 9.6. Serum samples were diluted (1:50) and incubated for 2 h at room temperature. Bound antibodies were detected using biotinylated rat anti-mouse IgG1 or IgG2a (Pharmingen, Minneapolis, MN, USA), followed by avidin-conjugated peroxidase. The plates were developed with a solution containing 3,5,3′,5′-tetramethylbenzidine tablets (Sigma, St. Louis, MO, USA) in 10 ml citrate–phosphate buffer and 2 μl of H_2O_2, and the reaction was stopped by adding 2 M H_2SO_4. The plates were read at a 450 nm in an ELISA reader (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA). The serum concentration of nitrite (NO_2^-) was determined by the Griess reaction, as an indicator of NO production [25]. Nitrate was reduced to nitrite by an enzymatic process [26,27]. The absorbance of the reaction product at 570 nm was measured using an ELISA reader. The NO_2^- concentration was determined by using sodium nitrite as standard.

2.8. Statistical analyses

Differences of means between groups were analyzed using Student's *t* test or Wilcoxon's rank sum test when parametric testes were not recommended. Differences in proportion were analyzed using Fisher's exact probability test, as indicated in the text. The level of rejection of the null hypothesis was fixed at $P < 0.05$.

3. Results

3.1. Course of L. amazonensis infection in BALB/c IL-4+/+ and IL-4−/− mice

To determine whether IL-4 plays a role in the susceptibility during infection by *L. amazonensis*, the course of infection of IL-4−/− BALB/c mice was compared to that of wild-type mice upon infection with different inocula of this parasite. Lesions of IL-4+/+ and IL-4−/− mice were similar upon infection with 5×10^6 *L. amazonensis* (Fig. 1A). In contrast, when lower inocula (10^3, 10^4 and 10^5 promastigotes) were used for infection, IL-4−/− mice developed smaller lesions than IL-4+/+, although they remained incapable of healing the infection (Fig. 1B–D).

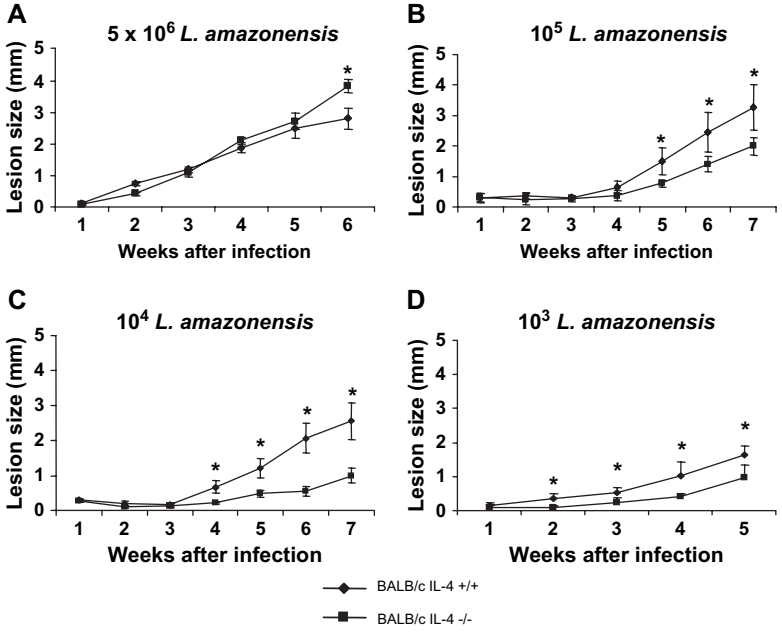

Fig. 1. Course of *L. amazonensis* infection in BALB/c mice challenged with different inocula. IL-4−/− (■) and IL-4+/+ (◆) BALB/c mice were infected in the right footpad with 10^3, 10^4, 10^5 or 5×10^6 *L. amazonensis* and lesion development was monitored weekly by measurement of footpad thickness during 5–7 weeks. One representative of three independent experiments is shown. Data represent the means ± SE of individual mice ($n = 5$). *$P < 0.05$, Student's *t* test.

The parasite load correlated with lesion progression. With 2 weeks of infection, parasites could be detected only in lymph nodes of mice infected by 5×10^6 *L. amazonensis*, although no significant differences in the parasitic loads of IL-4+/+ and IL-4−/− mice were found (Fig. 2A). In contrast, after 5 weeks of infection with 10^3, 10^4 and 10^5 promastigotes of *L. amazonensis*, IL-4−/− mice had significantly smaller numbers of parasites compared to IL-4+/+ (Fig. 2B−D). To confirm these results, immunohistochemistry analysis was performed in footpad sections for detection of parasites in the lesion. Lesions of IL-4+/+ mice infected by 10^4 *L. amazonensis* had many macrophages with vast vacuoles containing innumerous parasites, whereas lesions of IL-4−/− mice had only isolated foci of parasite predominantly non-vacuolated macrophages (Fig. 3A and B).

3.2. Increased Th1 responses in the absence of IL-4 during infection by L. amazonensis

To evaluate the profile of the immune response in IL-4−/− BALB/c mice infected by *L. amazonensis*, IFN-γ production by lymph node cells stimulated in vitro with Con A or *L. amazonensis* antigen was determined. After 2 weeks of infection by 5×10^6 *L. amazonensis*, IFN-γ production was higher in IL-4−/− mice compared to IL-4+/+ when stimulated by Con A or FTAg (Fig. 4A). A similar result was observed in cells supernatant of mice infected by 10^4 *L. amazonensis* after 7 weeks of infection (Fig. 4B). The levels of anti-leishmania IgG2a antibodies were also significantly higher in serum from IL-4−/− mice during 5×10^6 *L. amazonensis* infection. In contrast, the levels of IgG1 antibodies were increased in IL-4+/+ mice and almost undetectable in IL-4−/− mice 2 weeks after infection by 5×10^6 *L. amazonensis* (Fig. 4C). Similarly, 5 weeks after infection by 10^4 *L. amazonensis*, IL-4+/+ mice had higher levels of IgG1 antibodies, whereas IL-4−/− mice had predominantly IgG2a antibodies (Fig. 4D).

Nitric oxide (NO) is a critical mediator in *Leishmania* destruction by murine macrophages and is induced by IFN-γ. The levels of NO in sera of IL-4+/+ and IL-4−/− BALB/c mice infected by *L. amazonensis* were estimated by measurement of the oxidative products, nitrate and nitrite. The levels of NO were low in both groups of mice upon infection with 5×10^6 *L. amazonensis* (Fig. 4E). When infection was performed using 10^4 promastigotes of *L. amazonensis*, however, the levels of NO production were higher in sera from IL-4−/− than IL-4+/+ mice (Fig. 4F).

3.3. Effects of indomethacin treatment in mice infected with L. amazonensis

To investigate the participation of PGE_2 during *L. amazonensis* infection, IL-4−/− and IL-4+/+ BALB/c mice were infected with this parasite and treated with indomethacin, a potent PGE_2 inhibitor, during the first 3 weeks of infection. After 5 weeks of infection, there was a significant reduction in lesion development in IL-4+/+ and IL-4−/− mice when compared to the control group (Fig. 5A). IL-4+/+ mice treated with indomethacin had significantly reduced levels of parasites in lymph nodes when compared to those in control mice (Fig. 5B). Increased resistance after indomethacin treatment correlated with higher production of IFN-γ or decreased IL-10 production in IL-4−/− and IL-4+/+ mice, respectively (Fig. 5C).

After 5 weeks of infection, histological changes in control and indomethacin treated mice were observed. IL-4+/+ mice treat only with vehicle presents extensive and monomorphic collection of many vacuolated and heavily parasitized macrophages. This monotonous aspect was only altered by the necrotic areas. There were also neutrophils and eosinophils

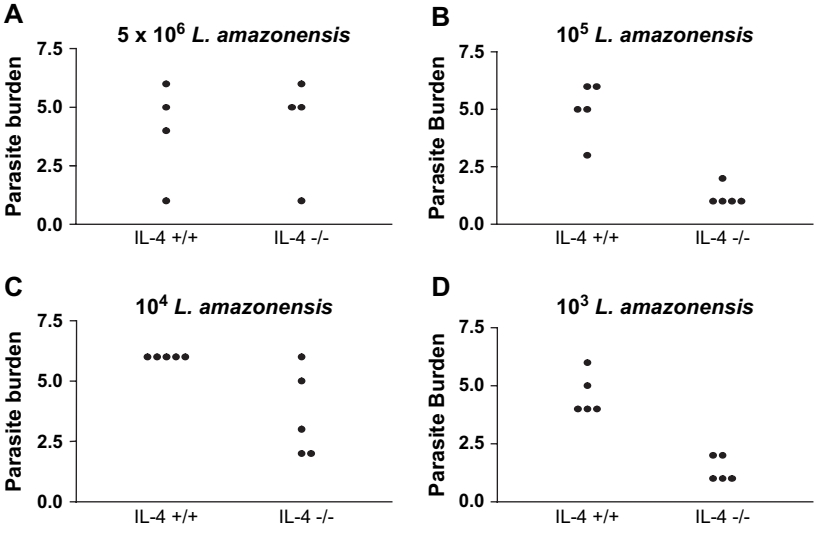

Fig. 2. Decreased parasite load in IL-4−/− mice infected with *L. amazonensis*. Parasite load in lymph nodes was determined by limiting-dilution assay of IL-4−/− and IL-4+/+ BALB/c mice infected by 10^3, 10^4, 10^5 and 5×10^6 *L. amazonensis*, as described in Section 2. The numbers of viable parasites in the lymph node of individual mice was determined 2 (A), 5 (D) and 7 weeks (B and C) after infection. Data show the results of one representative experiment of three performed. Differences observed in (B), (C) and (D) are statistically significant (Student's *t* test, $P < 0.05$).

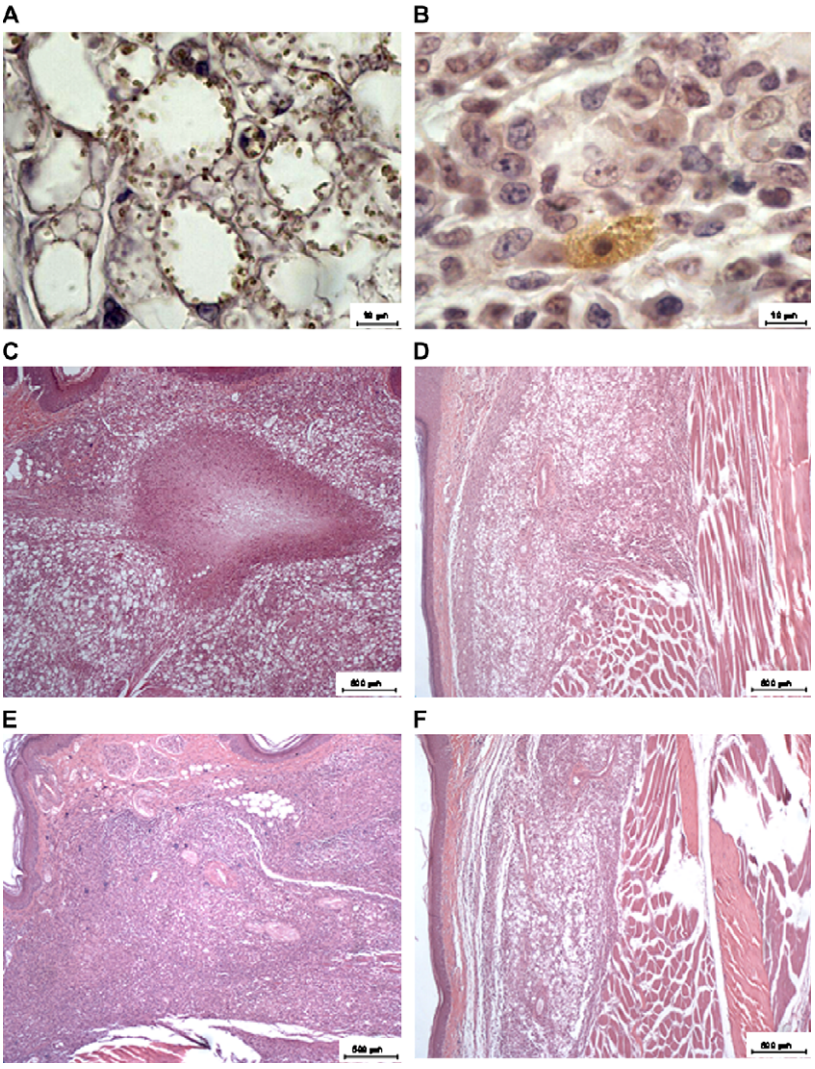

Fig. 3. Histology of the lesions in BALB/c mice infected with *L. amazonensis*. (A and C) Wild type mice. Monomorphic inflammatory infiltrate of vacuolated, parasite-containing macrophages (A), with extensive necrotic areas surrounded by neutrophils (C). (B and E) IL-4−/− mice. Pleomorphic inflammatory infiltrate with non-vacuolated and vacuolated, parasite-containing macrophages (B). Necrotic areas were small or absent (E). Wild type animals treated with indomethacin (D) present monomorphic infiltrate with less frequent necrotic areas. IL-4−/− animals treated with indomethacin present mixed inflammatory infiltrate. Necrotic areas are absent (F). (A and B) Immunohistochemistry using a polyclonal anti-leishmania antibody (bar = 10 μm). (C, D, E and F) H&E (bar = 500 μm).

around an area of microabscess (Fig. 3C). In IL-4+/+ mice treated with indomethacin the footpad lesion had a similar monomorphic macrophagic inflammatory infiltrate, and only few parasitized macrophage could be found among these cells. Necrotic areas were however observed only in two out of five animals (Fig. 3D). The lesions in untreated IL-4−/− mice, a mixed-cell vacuolated and non-vacuolated macrophage inflammatory reaction was observed. A small necrotic area and a microabscess were found only in the lesion of one out of five animals (Fig. 3E). IL-4−/− mice treated with indomethacin had small lesions represented by a mixed-cell inflammatory reaction, with absence of necrotic areas (Fig. 3F).

Although lesions in indomethacin-treated IL-4−/− mice were smaller and less severe, they progressed especially after the end of treatment (Fig. 5D). To investigate whether a prolonged treatment with this drug would render the mice resistant to *L. amazonensis* infection, IL-4−/− mice were infected by 10^3 promastigotes and treated with indomethacin or vehicle for 7 weeks. As shown in Fig. 5D, the lesions progressed in the two groups of mice, although they were significantly smaller in the group treated with indomethacin.

4. Discussion

Infection of inbred mice with *L. amazonensis* and *L. major*, two species causative of cutaneous leishmaniasis, may progress differently, indicating the influence of the parasite in the host's immune response. Although the mechanisms involved in the control of *L. major* are very well studied, little is known about the infection with *L. amazonensis*. In this study, we

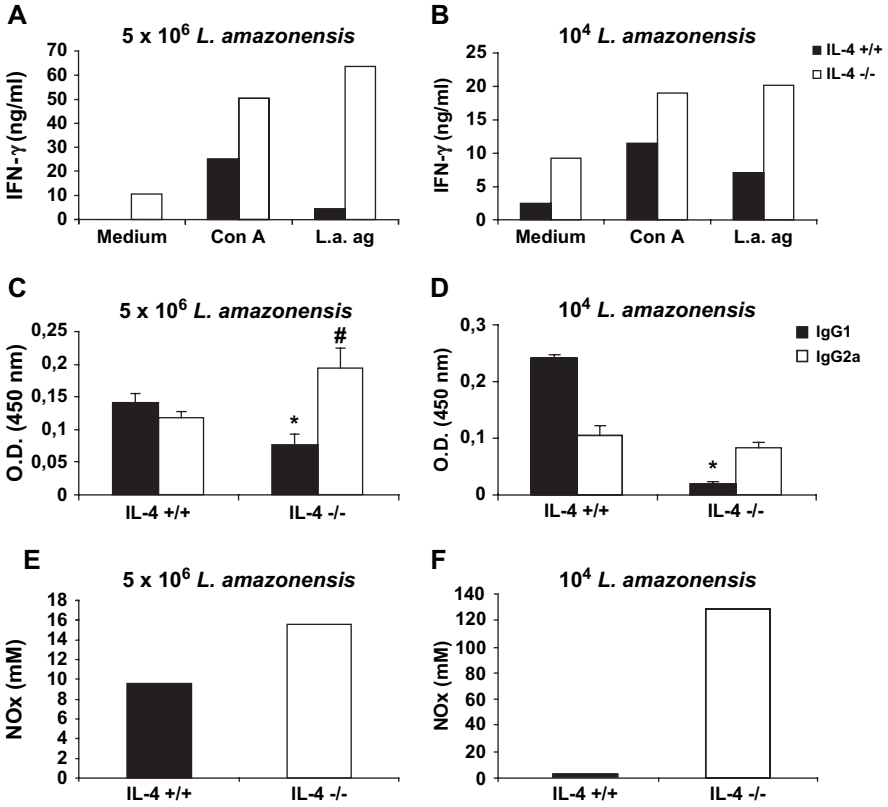

Fig. 4. Increased Th1 response and NO production against leishmania antigens in IL-4−/− mice. (A and B) Detection of IFN-γ in lymph node cells of IL-4−/− and IL-4+/+ BALB/c mice infected by 10^4 and 5×10^6 *L. amazonensis* promastigotes. Lymph node cells (five mice per group) were pooled and stimulated in vitro by Con A or FTAg for 48 h. IFN-γ production was evaluated in the supernatant by ELISA. (C and D) Levels of parasite-specific IgG1 and IgG2a antibodies in the serum of IL-4−/− and IL-4+/+ BALB/c mice infected by 10^4 and 5×10^6 *L. amazonensis* were determined by ELISA. The data are representative of two independent experiments. $^*P < 0.05$ and $\#P < 0.05$, Student's *t* test. (E and F) Nitric oxide production in sera of IL-4−/− and IL-4+/+ BALB/c mice infected by 10^4 and 5×10^6 *L. amazonensis*. NO production was detected in the pooled sera (five mice per group) using the Griess reagent.

investigated the ability of IL-4 and PGE_2 to modify the course of infection with different inocula of *L. amazonensis*.

We observed that IL-4−/− mice develop smaller lesions than IL-4+/+ when infected by 10^3, 10^4 and 10^5 *L. amazonensis*, although these mice are not capable of healing the infection. When these inocula are used to infect, the parasite load of IL-4−/− mice was about 1000 times smaller when compared with IL-4+/+ mice. These results indicate that IL-4 is indeed a susceptibility factor to *L. amazonensis* infection, since the lack of this cytokine leads to partial resistance.

Other authors observed similar lesion progression in C57BL/6 IL-4+/+ and IL-4−/− mice infected with *L. amazonensis*, and concluded that the susceptibility to this parasite was independent of IL-4 [28]. However, the number of parasites used to infect the mice was high (5×10^6 promastigotes). This is in accordance with our work, in which lesions and parasitic load were similar in IL-4+/+ and IL-4−/− mice upon infection with 5×10^6 parasites.

IL-4−/− mice produced higher levels of IFN-γ when compared to IL-4+/+ mice, even during the infection by 5×10^6 *L. amazonensis*. Although lesions and parasitic load were similar in IL-4+/+ and IL-4−/− mice when this inoculum was used, IL-4−/− mice had increased Th1 response compared to IL-4+/+ mice. The lack of difference in lesion size using such a high inoculum could be explained by the reduced levels of NO when compared to those of IL-4−/− mice infected with 10^4 *L. amazonensis*. The high number of parasites used in the initial inoculum could be one of the inhibitory factors of macrophage activation, causing suppression of the effector mechanisms of parasite elimination. The suppression of NO synthesis is probably an initial escape mechanism leading to the propagation of *L. amazonensis* in the host, and may be related to induction of PGE_2 production, since indomethacin treatment caused a significant reduction in lesion size and parasite load in IL-4+/+ mice. Exogenous administration of IFN-γ in the culture of macrophages of CBA mice does not reduce the infection by *L. amazonensis*, whilst this treatment causes a significant reduction of the number of parasitic cells by *L. major* [29]. These data strengthen the hypothesis that *L. amazonensis* interferes in the immune response of the host, particularly at the level of macrophage activation, influencing its ability to control the infection.

The fact that IL-4−/− mice were not completely resistant to the infection suggests that other factors contribute to the disease progression, in addition to IL-4. Thus, it was evaluated whether the association between the lack of IL-4 and PGE_2 (by indomethacin treatment) would render the mice fully resistant to infection by *L. amazonensis*. IL-4+/+ mice treated with indomethacin during the first 3 weeks of infection developed

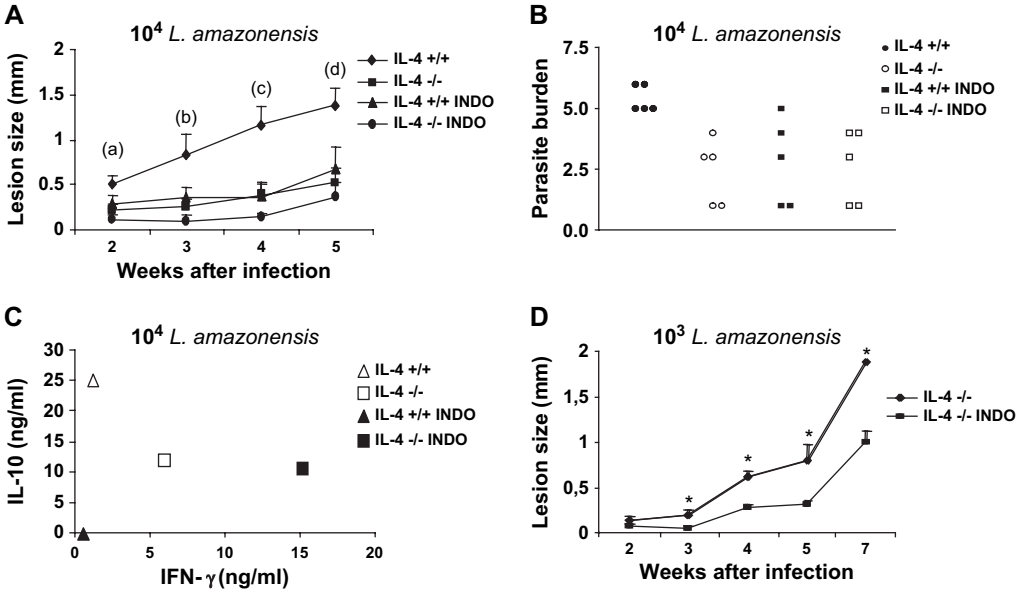

Fig. 5. Indomethacin treatment decreases lesion size and parasite load in *L. amazonensis*-infected BALB/c+/+ mice. IL-4+/+ and IL-4−/− BALB/c mice were treated with indomethacin (20 μg/ml in 0.4% ethanol) or 0.4% ethanol, intraperitoneally, once a day, during the first 3 weeks after infection by 10^4 *L. amazonensis* promastigotes. (A) Lesion size was determined by weekly evaluation of the difference in thickness between the infected and the uninfected contralateral footpads. (a) IL-4+/+ vs. IL-4+/+ INDO: $P < 0.05$, (b) IL-4+/+ vs. IL-4+/+ INDO: $P < 0.05$; IL-4−/− vs. IL-4−/− INDO: $P < 0.05$, (c) IL-4+/+ vs. IL-4+/+ INDO: $P < 0.05$; IL-4−/− vs. IL-4−/− INDO: $P < 0.05$, (d) IL-4+/+ vs. IL-4+/+ INDO: $P < 0.05$; IL-4−/− vs. IL-4−/− INDO: $P < 0.05$ using one-way ANOVA test. Values represent the means ± SE of five mice per group from one experiment of two performed. (B) Parasite burden in lymph nodes at week 5 of infection in mice infected by 10^4 *L. amazonensis* and treated with indomethacin. Numbers of parasites were determined by limiting dilution assay as described in Section 2 ($P < 0.05$, Student's *t* test). (C) Cytokine production in the pool lymph node cells. IL-10 and IFN-γ levels were measured in cultures by ELISA after in vitro stimulation of cells with FTAg for 48 h. (D) Indomethacin prolonged treatment in IL-4−/− mice slows disease progression but does not cure the infection. Error bars represent the mean ± SE of five mice per group. *$P < 0.05$, Student's *t* test.

smaller lesions and lower parasitic load when compared to the control group. However, although indomethacin treatment caused a significant reduction in lesion size of IL-4−/− mice, no difference in the parasitic load was observed. Prolonged treatment with indomethacin in IL-4−/− mice also did not cure the lesions of *L. major*. Freitas et al. (1999) demonstrated that indomethacin treatment in BALB/c mice infected by *L. major* diminishes the lesion size and the parasitic load, although the effects of this drug were insufficient to promote the cure of the infection. The authors suggested that indomethacin treatment only delayed the infection during a period in which the drug was incapable of blocking a Th2 response already established and to induce cure.

IL-4+/+ mice had decreased IL-10 production after indomethacin treatment. This indicates that PGE_2 may stimulate directly or indirectly the production of this anti-inflammatory cytokine, contributing to macrophage deactivation and parasite dissemination. This is consistent with the shown ability of prostaglandins to induce elevation of cyclic AMP and the consequent production of IL-10 in several cell types [30]. Thus, our work reinforces the results in which IL-10−/− mice develop lower lesions upon infection with *L. amazonensis* [31]. In addition, our work indicates that IL-10 production is also stimulated in the presence of IL-4, since IL-4−/− mice produced lower levels of IL-10 when compared to wild-type mice.

Correlating with lesion progression, in the absence of indomethacin BALB/c mice developed greater lesions of monotonous aspect, with many vacuolated macrophages containing parasites, and moderate or extensive areas of necrosis, characteristics that are typical of the infection by *L. amazonensis* in BALB/c mice [32,33]. The lesions of indomethacin-treated groups contained mostly macrophages without vacuoles and small or absent necrotic areas. Similar histological alterations had been observed in the immunization of BALB/c mice by soluble antigen of leishmania [33] and after the treatment with indomethacin in BALB/c mice infected by *L. major* [17].

In conclusion, our results suggest that *L. amazonensis* can interfere with the process of macrophage activation. By modulating the production of mediators, such as IL-4 and PGE_2, *L. amazonensis* appears to suppress effector mechanisms responsible for the destruction of parasites. Despite the important role of IL-4 and PGE_2 in the susceptibility of mice to *L. amazonensis* infection, the lack of these two factors is not enough to promote the cure of the infection, suggesting a direct inhibitory action in macrophages by the parasite and/or the participation of other factors in mediating susceptibility/resistance to *L. amazonensis* infection. Among the possible candidates are IL-10, IL-13 and TGF-β. The understanding of the signaling pathways involved in regulation of the production soluble mediators involved in macrophage deactivation and susceptibility to *L. amazonensis* infection may contribute to a rational design of molecular tools for treatment of infection by this parasite.

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