A trial of immunotherapy against *Leishmania amazonensis* infection 
*in vitro* and *in vivo* with Z-100, a polysaccharide obtained from *Mycobacterium tuberculosis*, alone or combined with meglumine antimoniate

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**Objectives:** To determine the efficacy and the immunomodulatory function of Z-100 alone or combined with meglumine antimoniate on *Leishmania amazonensis* infection.

**Methods:** The effect of the compounds was evaluated by microscopic counting of intracellular amastigotes in macrophages stained with Giemsa, or axenic promastigotes, and IC50 was determined by linear regression. The antileishmanial effect of the compounds was assessed in infected BALB/c mice by a limiting dilution analysis and the production of gamma interferon (IFN-γ), interleukin 10 (IL-10), IL-4, IgG1 and IgG2a was measured by ELISA.

**Results:** *In vitro*, Z-100 showed antileishmanial activity against intracellular amastigotes of *L. amazonensis* with an IC50 of 13 mg/L. Moreover, infected macrophages treated with Z-100 (12 mg/L) showed smaller parasitophorous vacuoles with fewer parasites than the control. In addition, the efficacy of Z-100 plus meglumine antimoniate [14 mg/L pentavalent antimony (Sb5+)] was higher (46% inhibition) than either Z-100 or meglumine antimoniate alone. Nevertheless, no effect of Z-100 on axenic promastigotes was observed. Infected BALB/c mice treated with Z-100 (100 μg/kg) alone did not show any antileishmanial effects in comparison with the control group, and IFN-γ, as well as IL-10 and IL-4, was up-regulated by the treatment. In addition, both IgG1 and IgG2a were also increased by the Z-100 treatment. Although Z-100 plus meglumine antimoniate (14 or 28 mg/kg Sb5+) controlled both the parasite load and the footpad swelling in comparison with control mice, no significant differences were found with meglumine antimoniate alone.

**Conclusions:** *In vitro*, Z-100 alone or combined with meglumine antimoniate showed an antileishmanial effect on *L. amazonensis*. However, no effect was observed in infected BALB/c mice treated with Z-100, suggesting that the up-regulation of IL-10 and IL-4 production by the treatment could be interfering with the development of a protective Th1-type response. For further understanding of the effects of Z-100 *in vivo*, another strain of mice such as C57BL/6 should be tested in future.

**Keywords:** *L. amazonensis*, IL-10, IL-4

**Introduction**

The first line of treatment for all clinical forms of leishmaniasis has been pentavalent antimonials such as sodium stibogluconate and meglumine antimoniate. However, these drugs have shown various disadvantages such as side effects, parenteral administration and the appearance of resistance to antimony. Immunotherapies have been applied in the treatment of leishmaniasis in order to improve chemotherapy, as the parasite can establish the infection through host immunosuppression.
Several immunomodulators have been studied alone or combined with chemotherapy either in cutaneous or visceral leishmaniasis; for example, endogenous biological gamma interferon (IFN-γ), interleukin 12 (IL-12), glucan, CP-46,665-1, a liposomal amine, tucarsen and OX40L-Fc, a member of the tumour necrosis factor receptors.7

Z-100 (also called Ancer-20), an immunomodulator polysaccharide composed mainly of arabinomannan, was obtained from Mycobacterium tuberculosis strain Aoyama B.8 This drug has been approved for years in Japan at a commercial level, and it has been extensively used in patients for stimulating growth of blood cells after cancer therapy.8 In addition, Z-100 has been shown to induce the production of some cytokines such as IL-1, IL-3 and mitogenic factors and also to inhibit others such as IL-4 and IL-10.9 It was reported that Z-100 restored the balance of Th1/Th2 cell response in tumour-bearing mice through up-regulation of IL-12 production from macrophages and of IFN-γ production from CD4+ T cells.10 Antimetastatic11 and antitumour12 effects in experimental animals and protective activity against Pseudomonas aeruginosa infection and LP-BM5 murine leukaemia and herpes virus10 have also been reported. In addition, Z-100 suppressed human immunodeficiency virus type 1 replication by the induction of IFN-β in macrophages through the activation of the p38 mitogen-activated protein kinase (p38 MAPK) signalling pathway.12 Until recently, a protective immunity against all species of Leishmania mediated by an IL-12-driven Th1 response had been widely believed to result in an increased IFN-γ production, which induced protection through nitric oxide synthase 2 (iNOS) expression and nitric oxide (NO) production by macrophages.13,14 However, a recent study in IL-12−/− mice has revealed that resistance to Leishmania mexicana (a parasite closely related to Leishmania amazonensis) infection is related to an IL-12-independent, but IFN-γ-, STAT4- and iNOS-dependent pathway.15 In contrast, the role of the Th2 response in leishmaniasis and the functions of Th2 cytokines (IL-10 and IL-4) are still unclear.15 However, it has been reported that IL-10 function in L. amazonensis infection is related to limiting the macrophage activation by inhibiting the NO production required for parasite killing.16 It was also shown that IL-4 could mediate susceptibility to L. amazonensis in BALB/c mice as monoclonal antibody (11B11) treatment against IL-4 induced a reduction in the parasite burden.13

Considering that Z-100 has been extensively used in humans, that it showed immunomodulatory activity through the up-regulation of IFN-γ and also the down-modulation of IL-10 and IL-4 production and that the Th1 cell response is necessary to induce a protective immunity against Leishmania, in the present study, we assessed the effects of immunotherapy with Z-100 alone or combined with meglumine antimoniate on L. amazonensis infection in vitro as well as in vivo. In addition, the immunomodulatory activity of Z-100 in L. amazonensis-infected BALB/c mice and its relation to the course of the disease are discussed.

Materials and methods

Drugs

Commerically available Z-100 (or Ancer-20) and meglumine antimoniate were purchased from Zeria Pharmaceutical Co. Ltd, Tokyo, Japan and from Rhône-Poulenc, Paris, France, respectively. The structural analyses of Z-100 were described previously.17

Parasites

In order to recover the infectivity of the stock L. amazonensis WHO reference strain (MHOM/BR/73/M2269), the parasites were passed four times by inoculating them to BALB/c mice and culturing at 23°C in RPMI 1640 medium (Nissui Pharmaceutical Co. Ltd) supplemented with 10% heat-inactivated fetal bovine serum, 200 mM L-glutamine, 50 mg/L streptomycin and 100 U/mL penicillin. In order to assess the effects of Z-100 on promastigotes cultures, the parasites in the exponential growth phase were harvested. For studying the activity of Z-100 and meglumine antimoniate in a macrophage model, intracellular amastigotes were used as the infective form. Amastigotes were obtained from a cell line of mouse macrophages, J774.1 (RCB No. 0434, Riken BioResource Center, Saitama, Japan), as described previously,18 with some modifications. Briefly, the macrophages were infected with promastigotes of the stationary phase of culture at a ratio of 10/1 (parasites/macrophage) and plated in culture flasks. After 12 days, the macrophages showed huge vacuoles with a large number of amastigotes. The intracellular parasites were released from the host cells by three cycles of mixing in a vortex followed by centrifugation at 3500 rpm for 10 min. Finally, the amastigotes were separated from the debris with 28% Percoll (Sigma Chemical Co.) in phosphate-buffered saline (PBS), layered on 1 mL of 100% Percoll and centrifuged at 7000 rpm for 30 min at 4°C. For in vivo studies, stationary phase promastigotes were washed twice with saline solution before they were used to infect mice.

Leishmania antigen

Leishmania antigen was prepared to stimulate the lymph node cells to produce cytokines and to sensitize the ELISA plate for detecting parasite-specific IgG1 and IgG2a. The parasites derived from in vitro cultivation were resuspended in PBS and disrupted by sonication on ice once for 5 min using a sonicator (Bioruptor). Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Inc.) and the final concentration was adjusted to 50 or 1.5 mg/L for cytokine and immunoglobulin detection, respectively.

Intracellular amastigotes

J774.1 macrophages (5 × 10⁶ cells/mL) were cultured in complete RPMI medium and placed in Lab-Tek eight chamber slides for 3 h at 37°C in a 5% CO2/95% air mixture. Adherent macrophages were infected with L. amazonensis amastigotes at a ratio of 4 or 2 amastigotes/macrophage and incubated at 34°C in a 5% CO2/95% air mixture overnight. After infection, the cells were washed with pre-warmed PBS to remove free parasites. New medium with Z-100 (4, 8 and 12 mg/L), meglumine antimoniate [14, 21 and 35 mg/L penta-valent antimony (SbV)] or Z-100 (8 mg/L) plus meglumine antimoniate (14 mg/L SbV) was added to each well; control culture was only incubated with the medium. The chambers were returned to the CO2 incubator for an additional 48 h at 34°C. After staining with Giemsa, the drug activity was determined by counting the number of intracellular amastigotes in 100−200 macrophages in treated and control cultures. In addition, the size of the parasitophorous vacuoles (PVs) was measured in 30 infected macrophages (control) and 30 infected macrophages treated with Z-100 (12 mg/L). In a preliminary experiment, the toxicity of Z-100 and meglumine antimoniate...
on J774.1 macrophages was determined using Trypan Blue (data not shown).

Promastigotes assay
The antileishmanial activity of Z-100 against *L. amazonensis* promastigotes was also assessed: \(1 \times 10^6\) parasites/mL of an exponential growth phase culture were placed in 96-well plates with 4, 8 and 12 mg/L Z-100 and incubated for 48 h at 23°C. Promastigotes in the control group were incubated with RPMI medium alone without the drug. Finally, the number of live promastigotes was recorded by counting with a Neubauer chamber.

Mice and treatment
Female BALB/c mice of 6 weeks of age were purchased from Japan SLC (Shizuoka, Japan) and all animal experiments were performed under institutional animal guidelines (approval number: 295). Mice were infected in their hind right footpad with the stationary phase of *L. amazonensis* promastigotes (\(2 \times 10^6\) in 50 μL of saline solution). The footpad swelling was measured weekly with a digital caliper and the treatment was initiated at week 6 post-infection (p.i.) when the lesions were detected. Meglumine antimoniate containing Sb\(^+\) 85 mg/mL, and Z-100 (20 mg/L) were diluted in saline solution and prepared before administration. The control groups received intralesional (il) saline solution alone over 2 weeks. The number of viable parasites was determined from the highest dilution at which the parasite could grow.

Parasite quantification
The parasite burden was determined by a limiting dilution analysis as described previously, with some modifications. In brief, hind right footpads were excised and homogenized using a glass grinder. The samples were incubated at 23°C for 18–24 h and the number of viable parasites was determined from the highest dilution at which the parasite could grow.

Measurement of IFN-γ, IL-10 and IL-4
Popliteal lymph node cells were obtained at week 8 or 12 p.i. The tissues were ground and washed several times with Hanks solution (Nissui Pharmaceutical Co. Ltd). About \(5 \times 10^6\) cells/mL were plated in a 96-well plate in complete RPMI medium; the cell culture medium was prepared as described previously. Then, the lymph node cells were stimulated with *Leishmania* antigens for 72 h. IFN-γ, IL-4 and IL-10 in the supernatants were assessed according to the manufacturer’s instruction (Bioscience) by using ELISA kits.

ELISA for parasite-specific IgG1 and IgG2a
Parasite-specific IgG1 and IgG2a from serum were measured by ELISA. Plates were sensitized with *Leishmania* antigens and were blocked and treated with 1/400 and 1/50 dilutions of mouse serum samples for IgG1 and IgG2a, respectively. Approximately 1/2000 and 1/1000 dilutions of peroxidase-labelled antibodies specific to mouse IgG1 (Boehringer Mannheim Corporation) and IgG2a (The Binding Site Limited, UK) were used. Reactions were developed with 1,2-phenylenediamine dihydrochloride (DAKO) and 5 μL of 30% H\(_2\)O\(_2\) for 12 mL of solution. Optical densities were read at 490 nm.

Statistical analysis
Data are expressed as mean ± SD. Statistical analysis was carried out using the Student’s t-test and analysis of variance. The data are representative of two or three experiments. The IC\(_{50}\) was determined by linear regression with the SPSS program (version 11.0, SPSS Inc., IL, USA). The statistical analysis of the data obtained by the limiting dilution assay was done using ELIDA\(^{\text{TM}}\) software, which analyses data through the Poisson distribution and by the \(\chi^2\) test.

Results

**In vitro, Z-100 alone showed antileishmanial activity against intracellular amastigotes and its effects were potentiated in combination with meglumine antimoniate**

The antileishmanial effect of Z-100 (4, 8 and 12 mg/L) was studied in an amastigote–macrophage model for *L. amazonensis*. After 48 h of incubation with the drug, the number of intracellular parasites was reduced in a dose-dependent manner with an IC\(_{50}\) of 13 mg/L. About 8 and 12 mg/L of Z-100 inhibited the number of intracellular amastigotes to 7.47 ± 5.8 and 6.51 ± 6.2 parasites per macrophage, respectively (\(P < 0.001\) (Table 1)). Although the number of parasites per host cell in the cultures treated with 4 mg/L Z-100 was slightly lower than the controls, the degree of the reduction was not significant (Table 1). In addition, we also observed that infected macrophages treated with Z-100 (12 mg/L) showed smaller PVs than untreated cultures (\(P < 0.001\) (Table 1)). The antileishmanial effect of meglumine antimoniate on intracellular amastigotes was also assessed. Doses of 14, 21 and 35 mg/L Sb\(^+\) inhibited the amastigote growth (\(P < 0.001\)) in a dose-dependent manner (Table 2) as did Z-100, and no activity was detected with doses lower than 7 mg/L Sb\(^+\) (data not shown). In addition, the inhibition in the number of intracellular parasites reached 46% (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/L)</th>
<th>Amastigotes/macrophage ± SD</th>
<th>PV size (μm(^2)) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>0</td>
<td>11.3 ± 10</td>
<td>180.5 ± 103</td>
</tr>
<tr>
<td>Z-100</td>
<td>4</td>
<td>9.54 ± 8.1</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.47 ± 5.8*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.51 ± 6.2*</td>
<td>91.5 ± 55*</td>
</tr>
</tbody>
</table>

ND, not done.

\(*P < 0.001, \text{ compared with medium.} \)
in cultures treated with Z-100 (8 mg/L) in combination with meglumine antimoniate (14 mg/L Sb\textsuperscript{V}), compared with the control cultures, and it was even more effective than either Z-100 or meglumine antimoniate alone (Table 2).

**No effect of Z-100 was observed on promastigote cultures**

We also assessed the antileishmanial effect of Z-100 (4, 8 and 12 mg/L) on the promastigote form. However, no reduction in the number of viable parasites was observed after 48 h of incubation with the drug.

**In BALB/c mice, Z-100 alone did not affect the parasite burden in the lesions and no strong potentiation was seen in combination with meglumine antimoniate when it was compared with antimony alone**

We investigated the antileishmanial activity of Z-100 (100 μg/kg) on infected BALB/c mice at week 8 p.i., but no marked inhibition in the parasite burden was observed (data not shown). At week 12 p.i., a slight inhibition of 44% (not significant) was observed in Z-100-treated mice showing a similar course of infection as the control group (Figure 1). However, a significant reduction in the number of parasites in the lesions (99% of inhibition) was observed in mice treated with Z-100 plus either 14 or 28 mg/kg Sb\textsuperscript{V} (Table 3) along with a pronounced diminution in the footpad swelling at weeks 8 and 12 p.i. (P < 0.05) (Figure 1). The next experiment was designed in order to compare the effects of Z-100 (100 μg/kg) with one scheme of antimony treatment (14 mg/kg Sb\textsuperscript{V}), and we found a significant diminution (P < 0.01) in the parasite burden in both treated groups in comparison with control mice (Table 4). However, the parasite burden in mice treated with Z-100 plus meglumine antimoniate was almost half (not statistically significant) of the parasite burden in mice treated with antimony alone (Table 4). Both treated groups showed a similar course of infection (Figure 2) and the footpad swelling was significantly different (P < 0.05) when compared with that of control mice at week 8 p.i.

**Z-100 treatment induced IFN-γ, IL-10 and IL-4 cytokine production in infected BALB/c mice**

When we assessed the antigen-specific stimulation in lymph node cells of Z-100-treated mice at week 8 p.i., an increased production of IFN-γ (a Th1 cytokine) was found, and it was nine times higher than that in the control group (P < 0.05). Th2 cytokines such as IL-10 and IL-4 were also stimulated by this treatment (Figure 3) (P < 0.05). In addition, at week 12 p.i. (4 weeks after the end of treatment), the level of IFN-γ was still higher in mice treated with Z-100 alone or combined with meglumine antimoniate (28 mg/kg Sb\textsuperscript{V}) than in the control group (Figure 4). However, similar levels of IL-10 between control and Z-100-treated mice were observed (Figure 4). In contrast, the levels of IFN-γ (P < 0.001), IL-10 and IL-4 (P < 0.05) cytokines were lower in mice treated with different doses of meglumine antimoniate administered by ip or il route, respectively, over 2 weeks in *L. amazonensis*-infected mice.

**Table 3. Effects of treatment with either Z-100 alone or combined with different doses of meglumine antimoniate administered by ip or il route, respectively, over 2 weeks in *L. amazonensis*-infected mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Percentage suppression of parasite burden in lesion</th>
<th>Mean no. of parasites per footpad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>—</td>
<td>0</td>
<td>2.5 × 10\textsuperscript{8}</td>
</tr>
<tr>
<td>Z-100 (100 μg/kg)</td>
<td>ip</td>
<td>44</td>
<td>1.4 × 10\textsuperscript{8}</td>
</tr>
<tr>
<td>Meglumine antimoniate (14 mg/kg of Sb\textsuperscript{V})</td>
<td>il</td>
<td>99.7</td>
<td>6.5 × 10\textsuperscript{5}∗</td>
</tr>
<tr>
<td>+ Z-100 (100 μg/kg)</td>
<td>ip</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meglumine antimoniate (28 mg/kg of Sb\textsuperscript{V})</td>
<td>il</td>
<td>99.5</td>
<td>1.2 × 10\textsuperscript{6}∗</td>
</tr>
<tr>
<td>+ Z-100 (100 μg/kg)</td>
<td>ip</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

∗P < 0.05, compared with control (saline).
meglumine antimoniate (14 mg/kg Sb) than in the control group (Figure 5).

L. amazonensis-infected mice treated with Z-100 alone showed a tendency to induce the Th1 and Th2 immune responses At week 8 p.i., after the end of treatment, the specific IgG1 and IgG2a levels were slightly increased in Z-100-treated mice, but this increase was not significant in comparison with the control group (Figure 6).

Discussion
The combination of chemotherapy with immunomodulators has offered a new path for exploration, since it may improve the treatment increasing the efficacy of antimicrobial drugs through a Th1 immune response and/or decreasing the toxicity of drugs with relatively low doses.

In the present study, the antileishmanial activity of Z-100 against intracellular amastigotes of L. amazonensis in vitro was shown. This inhibition in the number of amastigotes was in a dose-dependent manner, reaching 44.3% with 12 mg/L Z-100. The formation of PVs may indicate intracellular amastigote survival. Small PVs with fewer parasites were observed in infected macrophages treated with Z-100 (12 mg/L), suggesting that the viability of the amastigotes inside the PVs might be affected directly or indirectly by the drug (Table 1). It was reported that Z-100 inhibited HIV-1 replication in macrophage culture through p38 MAPK activation. The protein kinase activation may play an essential role during Leishmania infection, because its activation could activate a component of NADPH oxidase (p47phox) catalysing the oxidative burst and consequently generating oxygen radicals, which can kill intracellular amastigotes in an activated macrophage. However, Z-100 did

Table 4. Effects of treatment with Z-100 combined with meglumine antimoniate and antimony alone on the parasite burden of L. amazonensis in the footpad lesions of BALB/c mice after week 4 of finishing the dosing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Percentage suppression of parasite burden in lesion</th>
<th>Mean no. of parasites per footpad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>—</td>
<td>0</td>
<td>1 × 10^8</td>
</tr>
<tr>
<td>Meglumine antimoniate (14 mg/kg Sb)</td>
<td>il</td>
<td>97.4</td>
<td>2.6 × 10^6*</td>
</tr>
<tr>
<td>+ Z-100 (100 µg/kg)</td>
<td>ip</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meglumine antimoniate (14 mg/kg Sb)</td>
<td>il</td>
<td>95.1</td>
<td>4.9 × 10^6*</td>
</tr>
</tbody>
</table>

ip, intraperitoneal; il, intraleSIONAL.

*P < 0.05, compared with control (saline).
**P > 0.05 (not statistically significant), compared with meglumine antimoniate.

L. amazonensis-infected mice treated with Z-100 alone showed a tendency to induce the Th1 and Th2 immune responses

At week 8 p.i., after the end of treatment, the specific IgG1 and IgG2a levels were slightly increased in Z-100-treated mice, but this increase was not significant in comparison with the control group (Figure 6).

Figure 2. Course of L. amazonensis infection in BALB/c mice treated with Z-100 plus meglumine antimoniate and antimony alone. The control group received il saline solution alone daily over 2 weeks. Mice treated with either Z-100 plus meglumine antimoniate or meglumine antimoniate alone showed a drastic diminution in the footpad swelling. Each point represents the mean ± SD of the mean difference in size between infected and uninfected footpads of four to five mice per group (P < 0.05).

Figure 3. Production of cytokines in popliteal lymph node cells from mice infected with L. amazonensis and treated with Z-100 (100 µg/kg) at week 8 p.i. Lymph node cells were cultured and stimulated with Leishmania antigen. After 72 h, the supernatants were collected and stored at −20°C until they were examined for IFN-γ, IL-10 and IL-4. *P < 0.05. ND, not detected.

Figure 4. Production of cytokines in popliteal lymph node cells from mice infected with L. amazonensis and treated with Z-100 or Z-100 plus meglumine antimoniate. The production of both cytokines IFN-γ and IL-10 was in a dose-dependent manner. *P < 0.05, compared with control (saline).
a slight increase in both IgG1 and IgG2a levels at week 8 p.i.

In our in vivo model, at week 8 p.i., Z-100 treatment induced IFN-γ cytokine production as was reported previously, but conversely, IL-10 and IL-4 were also stimulated (Figure 3), and no inhibition of the parasite burden in the lesions was observed (data not shown). In addition, a tendency to increase both IgG1 and IgG2a levels was seen in mice treated with Z-100 (Figure 6). Four weeks after finishing the treatment (at week 12 p.i.), antigen-stimulated lymph node cells from Z-100-treated mice were even able to produce a high level of IFN-γ, but a slight reduction in the parasite burden was observed (Table 3 and Figure 4). The absence of antileishmanial effects of Z-100 in the presence of a high level of IFN-γ could be due to the IL-10 cytokine, which plays an important role in regulating the development of a protective Th1 immune response through the suppression of IFN-γ action. Therefore, an effective elimination of L. amazonensis parasites in BALB/c mice needs a neutralization of both IL-10 and IL-4 cytokines. Interestingly, the up-regulation of IFN-γ cytokine by Z-100 (Figure 4) was also observed when the Z-100 immunomodulator was combined with either 14 or 28 mg/kg Sbv; moreover, this immunomodulation was correlated with a drastic diminution in the parasite burden (Table 3) and with a marked diminution in the footpad swelling (Figure 1) in comparison with the control group after 2 weeks of treatment. However, the additional therapeutic effect of Z-100 was very low (not statistically significant), as the parasite burden was half of the parasite burden quantified in mice treated with meglumine antimoniate alone (Table 4). The high effectiveness of il meglumine antimoniate treatment observed in mice could be related to the amount of Sbv administered, the route of administration of the drug (direct toxic effect on the parasites), and/or to the treatment regimen, suggesting that doses lower than 14 mg/kg Sbv and/or a treatment regimen that does not include daily il injection of antimony could be helpful to better see the additional effect induced by Z-100. It is important to mention that in meglumine antimoniate-treated mice, low levels of IFN-γ, IL-10 and IL-4 cytokines were observed (Figure 5), suggesting that the number of responsive T cells could be decreased by the reduction of the parasite burden, as was reported previously. In opposition to this, mice treated with Z-100 plus meglumine antimoniate (28 mg/kg Sbv) displayed an increased amount of IFN-γ (Figure 4), suggesting that Z-100 might stimulate T cells even with a decreased parasite burden.

In conclusion, our in vitro L. amazonensis model suggests the antileishmanial effects of Z-100 and meglumine antimoniate could be related to a macrophage-mediated activity and to the direct toxic effect of the trivalent antimonite, respectively. In addition, in L. amazonensis-infected BALB/c mice, Z-100 is able to increase IFN-γ production, but is not able to reduce the parasite burden in the lesions. This lack of antileishmanial activity of Z-100 in BALB/c mice could be related to the high levels of IL-10 and IL-4 induced by Z-100. In further studies, the antileishmanial effects of Z-100 using another strain of mice was combined with meglumine antimoniate, the treatment was more effective against intracellular amastigotes than either Z-100 or meglumine antimoniate alone, suggesting that Z-100 increased the leishmanicidal activity of meglumine antimoniate. The toxic effect of antimonial compounds such as meglumine antimoniate could be due to the trivalent form of the antimonial compound, which is created through the conversion of the pentavalent antimonite by the glutathione or a related thiol in the phagolysosome of macrophage.

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such as C57BL/6, which is also susceptible to L. amazonensis infection, could reveal a better response to immunotherapy with Z-100, because the susceptibility to L. amazonensis is due to the absence of a Th1 cell response, rather than to the presence of a Th2 immune response.

Considering the extensive use of Z-100 in humans, further studies should be performed for a better understanding of role(s) of the drug in the treatment of leishmaniasis.

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Transparency declarations

None to declare.

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