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Insulin-like growth factor-I serum levels and their biological effects on *Leishmania* isolates from different clinical forms of American tegumentary leishmaniasis

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Abstracts

Background: American tegumentary leishmaniasis (ATL) in Brazil is mostly caused by *Leishmania (Viannia) braziliensis*, with known forms of the disease being cutaneous (CL), mucosal (ML) and disseminated (DL) leishmaniasis. The development of the lesion in ATL is related both to the persistence of the *Leishmania* in the skin and to the parasite-triggered immune and inflammatory responses that ensue lesions. In this context one factor with expected role in the pathogenesis is insulin-like growth factor (IGF)-I with known effects on parasite growth and healing and inflammatory processes. In the present study, we addressed the effect of IGF-I on intracellular amastigote isolates from CL, ML and DL patients within human macrophage and we evaluated the IGF-I and IGF-binding protein-3 (IGFBP3) serum levels in patients presenting different clinical forms and controls from the endemic area.

Methods: We evaluated biological variability in the responses of intracellular amastigotes of *Leishmania* isolates derived from CL, ML, and DL patients from an area for ATL in response to IGF-I. Intracellular amastigote growth was evaluated using the human macrophage cell line THP-1. Arginase activity in infected cells was evaluated quantifying the generated urea concentration. Serum samples from patients and controls were assayed using chemiluminescent immunometric assay to determine IGF-I and IGFBP3 levels.

Results: We observed an increase in intracellular parasitism upon IGF-I stimulus in 62.5 % of isolates from CL, in 85.7 % from ML and only 42.8 % from DL cases. In DL, the basal arginase activity was lower than that of CL. We then evaluated the IGF-I and IGFBP3 serum levels in patients, and we observed significantly lower levels in ML and DL than in CL and control samples.

Conclusions: The data suggest that IGF-I is modulated distinctly in different clinical forms of tegumentary leishmaniasis. IGF-I seemingly exerts effect on parasite growth likely contributing to its persistence in the skin in earlier phase. In addition the decreased IGF-I serum levels may affect the modulation of inflammation and lesion healing in chronic phase. In view of potential role of IGF-I in the pathogenesis of ATL we can speculate on therapeutic procedures taking into account the local IGF-I level.

Keywords: *Leishmania braziliensis*, Tegumentary leishmaniasis, THP-1, IGF-I level

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Background

Human leishmaniasis caused by *Leishmania* (*Viannia*) *braziliensis* infection has a broad spectrum of manifestations ranging from an asymptomatic infection to severe destructive ulcerated and inflammatory forms. The main clinical forms are cutaneous leishmaniasis (CL), severe destructive mucosal leishmaniasis (ML) and disseminated leishmaniasis (DL) [1]. Some studies relate the different disease manifestations to the intraspecific variability of *L. (V.) braziliensis* not only in Brazil but also in Colombia [2–4] but not in another study [5]. Besides, the presence of *Leishmania* RNA virus 1 in *L. (V.) braziliensis* has been associated with the development of ML but not in all ML cases [6]. Therefore, the wide spectrum of manifestations caused by *L. (V.) braziliensis* are still poorly understood.

It is known that the development of the lesion in tegumentary leishmaniasis is related both to the persistence of the *Leishmania* in the skin and to the parasite-triggered immune and inflammatory responses that result in lesion development [7, 8]. The Th1-type immune response controls the parasite growth [7] but in chronic and severe lesions such as in mucosal leishmaniasis maintenance of the supposedly beneficial immune response turn into chronic and harmful process [9, 10].

In this context one of elements that may have role in the pathogenesis is insulin-like growth factor (IGF)-I since this factor has effect on both parasite growth [11] contributing to its persistence and on the healing and inflammatory processes [12–14].

We have been studying the effect of IGF-I on *Leishmania* and in leishmaniasis, demonstrating its impact on parasite growth and lesion development. IGF-I in cases of *L. (L.) amazonensis* showed effects increasing the parasite growth and cutaneous leishmaniasis lesion development [11]. In *Leishmania*-macrophage interaction, IGF-I exerts effect on *Leishmania* and host cells, mainly inducing arginase expression and activity, leading to alternative activation of the macrophages interfering with inducible nitric oxide synthase expression [11, 15, 16]. Because biological effects may differ in other species and American tegumentary leishmaniasis is mostly caused by *L. (V.) braziliensis*, we analyzed the effect of this growth factor on this parasite species. Then, we observed complex results related to the source of the parasite isolates. IGF-I induced higher basal arginase activity in promastigote isolates derived from patients with CL and DL; however, arginase activity was already increased under basal conditions in isolates from ML with no further increase upon IGF-I stimulus [17]. In the present study, we addressed the effect of IGF-I on amastigote forms of isolates from CL, ML and DL patients within the THP-1 human macrophage lineage. We used the macrophage lineage rather than macrophages derived from peripheral blood monocytes to avoid variations in cellular response in different individuals. We observed an

increase in intracellular parasitism upon IGF-I stimulus in 62.5 % of isolates from CL, in 85.7 % from ML and only 42.8 % from DL cases. Although we observed differences among *Leishmania* isolates, they do not clearly explain their different clinical manifestations.

Since in addition to the effect on parasite growth, IGF-I has a pleiotropic effect on cell migration [18], wound healing [12, 13] and inflammatory process [14], in the present study, we evaluated the IGF-I and IGF-binding proteins-3 (IGFBP3) serum levels in patients presenting different clinical forms, CL, ML and DL and controls from the endemic area. In this analysis, both IGF-I and IGFBP3 levels decreased in ML and DL compared with CL and controls. The data as a whole suggest that IGF-I is modulated in different clinical forms of tegumentary leishmaniasis, and IGF-I seemingly exerts effects on both parasite growth likely contributing to its persistence in the skin and its lower level affecting the modulation of inflammation and lesion healing process. In view of potential role of IGF-I in pathogenesis of tegumentary lesion development we can speculate on therapeutic procedures considering local IGF-I level.

Methods

Study design and subjects

The patients included in this study were from Corte de Pedra municipality, Bahia state, Northeastern Brazil, an area with endemic tegumentary leishmaniasis mostly caused by *L. (V.) braziliensis*. The patients were approached at the Corte de Pedra Health Post from 2002 through 2007. All participants were volunteers and provided their individual informed consent.

A leishmaniasis diagnosis was made based on a clinical feature of these forms of leishmaniasis along with one of the following criteria: parasite isolation or a positive skin test for *Leishmania* soluble antigen and the presence of histopathological findings suggestive of leishmaniasis. Patients with CL had a typical ulcerative lesion in the skin; ML patients had a metastatic mucosal nasal lesion that was not contiguous with the primary cutaneous lesion, and DL patients had polymorphic lesions (acneiform, papular, nodular and/or ulcerated) on two or more parts of their body. The exclusion criteria included HIV infection, diabetes mellitus and pregnancy. All patients were evaluated before receiving therapy and during the active disease. The ages ranged from 21 through 65 years. One-hundred fourteen patients were studied, suffering from CL ($n = 65$), ML ($n = 20$), DL ($n = 29$) and 14 endemic control subjects living in the same municipality. The sera were stored at -70°C until they were analyzed.

Isolates of *L. (V.) braziliensis*

The 22 isolates of *Leishmania* were obtained from patients presenting with the following different clinical forms of the

disease: cutaneous leishmaniasis ($n = 8$), mucosal leishmaniasis ($n = 7$) and disseminated leishmaniasis ($n = 7$). The isolates were obtained by aspirating the lesion, and the samples were grown in tubes with biphasic medium (LIT/NNN) supplemented with 10 % heat inactivated fetal calf serum (FCS) (Cripion Biotechnology, Brazil) at 26 °C. The parasite isolates were cryopreserved and expanded in Schneider's insect medium (Sigma), pH 7.2, supplemented with 10 % FCS. Most of the isolates were characterized as *L. (V.) braziliensis* by isoenzyme electrophoresis and monoclonal antibodies in the Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil [19]. The promastigotes used in the experiments were in the stationary phase of growth and had no more than four passages in culture.

THP-1 monocytic cell line and cell culture

The monocytic cell line THP-1 (ATCC) was grown and replicated every 4 days in RPMI 1640 supplemented with 5 % FCS, 100 UI/ml streptomycin, 2 mM L-glutamine, 11 mM sodium bicarbonate and maintained in a humidified atmosphere at 37 °C with 5 % CO₂. The viability was assessed by a dye exclusion test using 0.02 % trypan blue in phosphate-buffered saline 0.01 M, pH 7.2 (PBS); to test for infection, 2×10^5 cells in 500 μ l in RPMI 1640 with 2 % FCS were distributed in each well of 24-well culture plates in triplicate with sterile, round 13 mm coverslips.

THP-1 monocytes were submitted to 20 ng/ml phorbol 12-myristate 13 acetate (PMA) for 24 h at 37 °C, 5 % CO₂ for differentiation into macrophages [20]. The non-adherent cells were removed, the medium was replaced, and the cells were maintained for 24 h in the same conditions. Infection with *Leishmania*/macrophage (ratio of 10:1), using isolates from CL, ML and DL of *L. (V.) braziliensis*, was performed with or without stimulation with IGF-I (50 ng/ml) and incubated at 33 °C, 5 % CO₂. After 4 h, the plates were washed with warm PBS to remove non-internalized parasites, and the RPMI 1640 medium with 2.0 % FCS and IGF-I was replaced. After different incubation times, the supernatant was collected and stored at -70 °C for evaluation of arginase activity, and the coverslips were taken for intracellular parasite counting.

Evaluation of intracellular parasite load

The coverslips removed from the plates were stained with Giemsa dyes, and intracellular parasites were counted under a light microscope (Carl Zeiss, Germany). For each experimental condition, 600 cells and intracellular parasites were counted by two independent observers, blinded for experimental conditions. The data are presented as the number of parasites per 100 cells.

Arginase activity

The cells and promastigotes were removed from the cultures, lysed and submitted to arginase activity determination [21]. Briefly, to activate the arginase, 50 μ l of lysate was treated with the same volume of 5 mM MnCl₂, 25 mM Tris-HCl pH 7.4 at 56 °C for 10 min. Then, 25 μ l of 0.5 M L-arginine pH 9.7 was added to 25 μ l of the activated lysate and incubated at 37 °C for 60 min. The reaction was stopped with 400 μ l of H₂SO₄/H₃PO₄/H₂O (1/3/7, v/v/v). The urea concentration was measured at 540 nm in a spectrophotometer Multiskan MCC/340 P version 2.20 plate reader (Labsystems, Vantaa, Finland) after the addition of 25 μ l of 9 % α -isonitrosopropiophenone in 100 % methanol and submitted to 100 °C for 45 min. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1 μ mol of urea per minute.

Analyzes of circulation IGF-I and IGFBP3 levels

IGF-I and IGFBP3 assays were performed using the IMMULITE® 2000 (DPC-Diagnostics Products Corporation, Los Angeles, CA, USA). This procedure is an automated 2-site sandwich immunoassay with a chemiluminescent immunometric assay. It was configured and calibrated on an instrument at Laboratório de Hormônio do Hospital das Clínicas da Universidade de São Paulo, according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism5 (GraphPad Software, Inc., San Diego, CA, USA). The isolates results were submitted to ANOVA with *post-hoc* Student-Newman Keuls test. An alpha error of 5 % ($P = 0.05$) was considered to determine statistical significance. The IGF-I and IGFBP3 assay results were expressed as medians and percentiles (25–75) and submitted to the Kruskal-Wallis test with the Student Newman-Keuls *post-hoc* test to statistically compare the groups.

Results

In this study, we initially evaluated the parasitism and the arginase activity of the isolates of *L. (V.) braziliensis* derived from patients presenting CL ($n = 8$), ML ($n = 7$) or DL ($n = 7$).

We evaluated the parasites as amastigotes within human monocytic cell line THP-1 in the presence or the absence of the growth factor IGF-I. This cell line can be activated by IGF-I [22]. Having perceived differences in the biological parameters in this approach, we then evaluated the IGF-I and IGFBP3 serum levels in patients and in controls living in the same endemic area.

The percentage of infected cells in basal conditions (i.e. no IGF-I stimulus) indicated that the parasitism tended to be lower with isolates of *Leishmania* coming

from DL than from CL and ML cases (Fig. 1a). The parasite load (number of amastigotes/100 infected cells) in the basal conditions tended to be greater with parasites from CL than ML and DL cases (Fig. 1b and Table 1). Evaluating the effect of IGF-I on the isolates as a whole in each group, we observed no significant differences in the percentage of infection (Fig. 1a) or in the number of amastigotes/100 cells (Fig. 1b and Table 1).

However, to further assess the effect of IGF-I in infections and have a better view of the behavior of individual isolates, we calculated the ratio of the individual parasite load in cultures stimulated with IGF-I in relation to its basal culture in which a ratio greater than 1.0 would indicate the positive effect on parasite growth (Fig. 2). After a culture period of

Table 1 Effect of IGF-I on parasitism in THP-1 with isolates of *Leishmania*

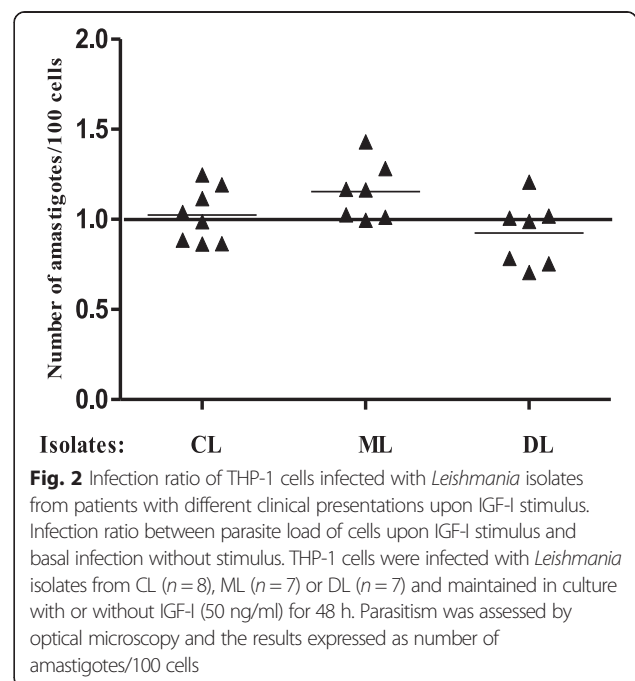
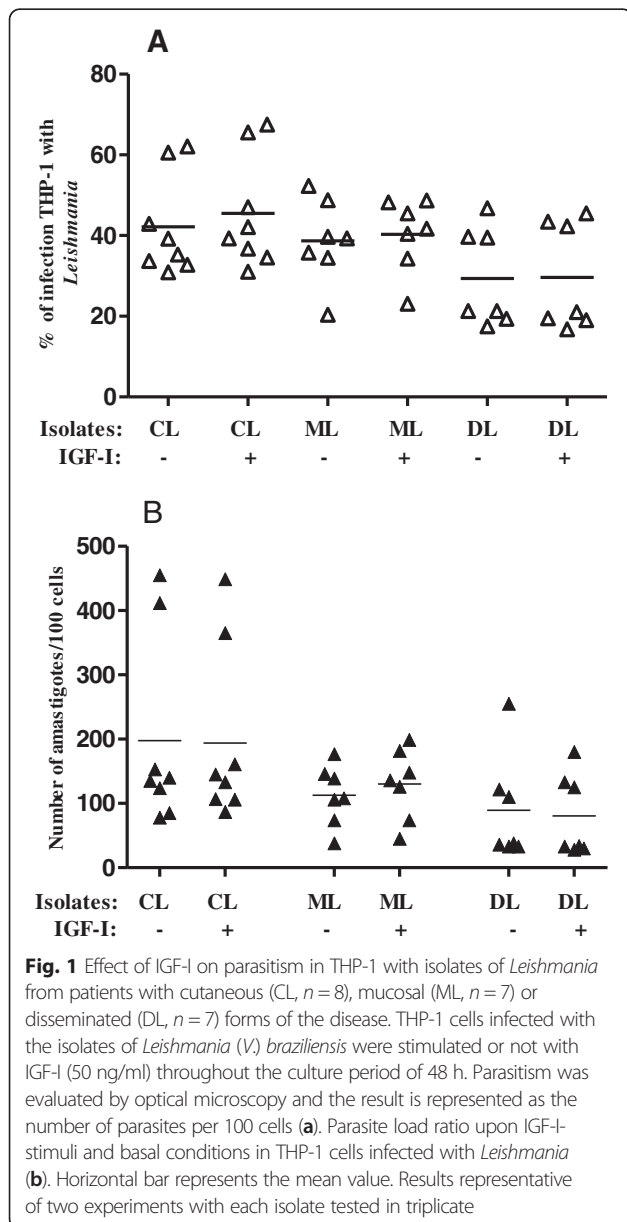
<i>Leishmania</i> from	CL		ML		DL	
	-	+	-	+	-	+
IGF-I (50 ng/ml)	-	+	-	+	-	+
Parasite isolates	124	107	139	199	255	180
	78	87	146	148	110	133
	449	455	73	73	33	33
	140	145	177	182	36	28
	135	161	108	126	122	125
	412	365	38	45	33	34
	85	106	106	136	38	30
	153	133				

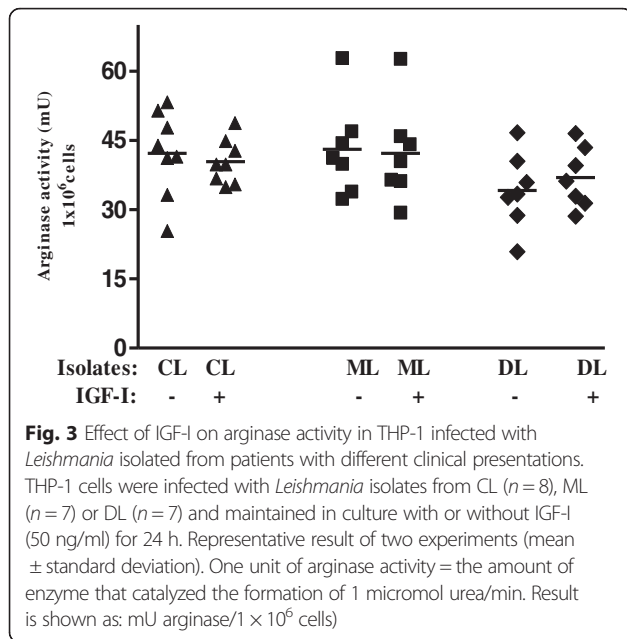
THP-1 cells were infected with the isolates of *L. (V.) braziliensis* from patients with cutaneous (CL, *n* = 8), mucosal (ML, *n* = 7) or disseminated (DL, *n* = 7) forms of the disease and stimulated or not with IGF-I (50 ng/ml) throughout the culture period of 48 h. Parasitism was evaluated by optical microscopy and the result is represented as the number of parasites per 100 cells. Results representative of two experiments with each isolate tested in triplicate

48 h, we observed an increase in the parasitism ratio upon IGF-I stimulus in 62.5 % (5 of 8) of the isolates from CL cases, in 85.7 % (6 of 7) of the isolates from ML cases and in only 42.8 % (3 of 7) from DL patients. Notably, the isolates from the DL cases showed reduced growth in the presence of IGF-I.

In the analysis of arginase activity, both basal and IGF-I-stimulated levels were lower in those cells infected with isolates from DL than in those from CL and ML (Fig. 3).

The above results suggest that IGF-I is beneficial or deleterious depending on the isolates; therefore, we analyzed whether IGF-I serum levels were altered in





leishmaniasis patients presenting with different clinical forms of the disease.

The clinical features of the studied patients are presented in Tables 2 and 3. There were no significant differences in the group distributions according to age (Table 2) and sex (Table 3). The higher percentage of men than women in all groups of patients likely reflects the epidemiological characteristics of the transmission of infection with higher exposure of the male to the insect bite due to their agricultural activity, although sex-related biological factors cannot be discarded.

The IGF-I and IGFBP3 serum levels were analyzed, and the fact that the groups do not differ in age distribution was important for these evaluations because these levels vary considerably in different age ranges [23]. While patients with CL showed similar average levels of IGF-I compared with control subjects, patients with ML and DL had significantly lower levels than patients with CL (Fig. 4). These results were similar when analyzing men and women

Table 2 Clinical profile of study patients with Leishmaniasis

Parameters	Clinical Form			
	Cutaneous L	Mucosal L	Disseminated L	Controls
Number	65	20	29	14
Age (years)	34 \pm 10	43 \pm 10	38 \pm 12	30 \pm 8

The age was expressed as mean \pm standard deviation. See Methods for further details. ANOVA test with the Student Newman-Keuls *post-hoc* test for statistical comparison among groups. $P > 0.05$

separately (data not shown). In contrast, IGFBP3 levels in the serum of patients were similar between the studied groups (data not shown).

Discussion

The pathogenesis of American tegumentary leishmaniasis currently is considered multifactorial, due to not only specific immune response [24] but also parasite diversity [4, 17] and other factors related to the host [25, 26]. In this scenario, we have been studying the role of IGF-I in leishmaniasis mainly *in vitro* and in experimental models of leishmaniasis. The role of IGF-I in human leishmaniasis has not been established.

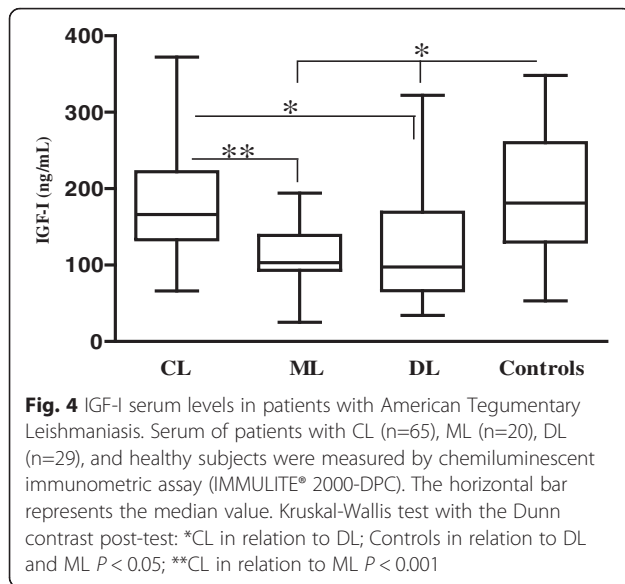
Based on our previous experimental data, IGF-I can play a role in the progression of *Leishmania (Leishmania) amazonensis* infection by (i) decreasing NO production in macrophages and allowing parasite multiplication, (ii) modulating immune responses by increasing TGF- β and decreasing IFN γ production in promastigote infected macrophages, and (iii) inducing apoptotic mimicry characterized by exposure of phosphatidyl serine on amastigotes but without leading to death [15, 16]. However, it is unknown whether similar effects of IGF-I would occur in other *Leishmania* species.

Because tegumentary leishmaniasis in Brazil is mostly caused by *L. (V.) braziliensis* [1] and different biological effects may occur upon IGF-I stimulus, we initially studied its effect on promastigotes. Focusing on the increase of arginase activity of the parasite, which was seen as main effect of IGF-I [16], we confronted more complex outcomes that were dependent on *Leishmania* isolates,

Table 3 Gender distribution of American Tegumentary Leishmaniasis (ATL) patients

Clinical Form	Sex		OR	95 % CI	P-value*
	Male	Female			
Controls ^{1,2,4}	12	2			
Cutaneous L ^{1,3,5}	55	10	1.1	0.21–5.63	¹ 0.9172
Mucosal L ^{2,3,6}	13	7	3.2	0.55–18.72	² 0.1779
Disseminated L ^{4,5,6}	26	2	3.0	0.95–9.26	³ 0.0551
			0.5	0.06–3.68	⁴ 0.4572
			0.4	0.09–2.07	⁵ 0.2768
			0.1	0.02–0.79	⁶ 0.0148

*P value analyzed by the χ^2 test. Note: Odds ratios (OR) and χ^2 tests were performed with groups that bear the same number in superscript; the respective odds ratio, 95 % confidence interval (CI), and P-value for each comparison has the same number in superscript. No statistically significant differences were found



whether coming from patients with CL, ML or DL. IGF-I induced higher basal arginase activity in *L. (V.) braziliensis* promastigote isolates from CL and DL but not in ML. Conversely, in *Leishmania* isolate from ML the arginase activity was already increased in basal conditions [17]. In the present study, we analyzed the effect of IGF-I on intracellular amastigotes in sequence.

The intracellular amastigote growth was evaluated here using human macrophage cell line THP-1 rather than macrophages derived from peripheral blood monocytes to avoid the variation in cellular response that would occur if obtained from different individuals. IGF-I induced an increase in parasitism in CL and ML isolates but a decrease in parasitism in DL isolates. In CL and ML these data suggest that the presence of IGF-I may contribute to the persistence of the parasite in the skin. In DL isolates, basal arginase activity was also lower than that of CL. However, arginase activity was similar with and without IGF-I stimulus, leaving mechanisms that need to be further explored.

To proceed with the study of the involvement of IGF-I in different clinical manifestations, IGF-I and IGFBP3 serum levels were evaluated in patients with CL, ML and DL. In CL, both of these levels were similar to those in healthy controls, while in ML and DL, both levels were decreased. Altogether, these data indicate that IGF-I cannot be interpreted as having similar effects in patients presenting different clinical manifestations and that the effects may encompass healing and inflammatory processes beyond parasite growth.

American tegumentary leishmaniasis is characterized by an intense inflammation with a high production of tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ),

molecules that are necessary to protect the host against *Leishmania* but can also cause tissue damage even with a scarce number of parasites in the lesions [25, 27]. In the early phase of the disease when patients have a small cutaneous lesion or when is evolving to heal [28], this T helper type 1 immune response is known to be down modulated [29]. IGF-I is known to be increased in the initial phase of skin injury because this molecule is necessary for epidermis maintenance [30, 31], which would occur also in CL. In CL, because IGF-I is suggested by the present findings to have a parasite growth promoting effect on *Leishmania* isolates and has its serum level maintained in the level of controls, IGF-I would probably contribute to the progression of parasite growth and establishment of *Leishmania* in the host skin.

Cutaneous ulcer is the first lesion of tegumentary leishmaniasis that later can be complicated by the appearance of mucosal or disseminated disease. After the initial phase of *L. (V.) braziliensis* infection, a very strong Th1 immune response occurs that is associated with a decrease in IL-10 production [32]. IFN- γ and TNF- α are also produced in even higher concentrations in ML patients [33], and this increased production of cytokines may relate to the lower level of IGF-I in patients with ML observed in the present study.

In DL patients, a slight decrease in IFN- γ production was observed when analyzing peripheral blood mononuclear cells, which may result from the migration of activated T cells to the multiple lesions and to the mucosal tissue because up to 40 % of DL patients have mucosal disease [34]. In DL, IFN- γ production is still high that may relate to the decreased IGF-I serum levels found here in this form of the disease.

IGF-I was shown to decrease vascular inflammatory process in mouse model of atherosclerosis [14]. Because the pathogenesis of American tegumentary leishmaniasis is based on an exaggerated immune response [35, 36], a decrease in IGF-I in ML and DL may contribute to the persistence of the inflammatory response and further on the delayed healing of the lesion.

Having pathogenic mechanism involving IGF-I in mind we can speculate the use of IGF-I to stimulate healing and control of inflammation in chronic and severe cases of ATL where IGF-I level is decreased. There are studies on IGF-I use in other dermatological lesions. In diabetes mellitus ulcer it is suggested that the healing is delayed due to a decrease in IGF-I level in the skin [12] and some approaches have been tried. In diabetes mellitus patients the skin ulcer healed with an increase in local IGF-I level upon the use of hyperbaric oxygen therapy [37]. In experimental diabetes model local use of IGF-I cream has also improved the healing [38]. In ATL IGF-I may increase the parasite growth but it would be in the tissue environment where strong Th1-type immune

response is present and thus the parasite growth would be restrained.

Conclusions

In the present study, analyzing the effect of IGF-I on amastigotes of *Leishmania* isolates from patients and evaluating the IGF-I serum levels, we observed results that suggest IGF-I affecting both parasites and healing and inflammatory processes with IGF-I being modulated differently in distinct clinical forms of ATL. We suggest that in the initial phase of the disease, there are maintained levels of IGF-I that contribute to downregulation of the immune response and for parasite growth/persistence. In the late phase of the infection, with the decrease in IGF-I levels it would allow mainly the persistence of a tissue damage.

In view of potential role of IGF-I in the pathogenesis of ATL we can speculate on therapeutic procedures taking into account the local IGF-I level.

Abbreviations

ATCC, American type culture collection; ATL, American tegumentary leishmaniasis; CI, confidence interval; CL, cutaneous leishmaniasis; DL, disseminated leishmaniasis; FCS, fetal calf serum; HIV, human immunodeficiency virus; IFN- γ , interferon gamma; IGF, insulin-like growth factor; IGFBP3, IGF-binding proteins-3; IL-10, interleukin 10; ML, mucosal leishmaniasis; OR, odds ratios; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13 acetate; RPMI, Roswell park memorial institute medium; TGF- β , transforming growth factor beta; TNF- α , tumor necrosis factor alpha

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Availability of data and material

All data are disclosed as tables and figures in the main document.

Authors' contributions

LDS, CMW, ARJ, EMC and HG conceived and designed distinct parts of studies. AM cultivated the isolates and organized the samples; EMC, AS and LHG participated in the field work approaching individuals in endemic area of ATL, selecting and obtaining samples, organizing the data. LDS, CMW and MDTC performed the experiments; LDS, CMW, MDTC and HG analyzed and interpreted the data; HG conceived whole study, coordinated the project; LDS, CMW, EMC and HG drafted and revised the article. All authors read and approved the final manuscript.

Authors' information

LDS and CMW had equal participation in this study.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All patients were volunteers and provided their individual informed consent, and the study was approved by the Conselho Nacional de Ética e Pesquisa (Ethical Certificate 1583/2002). The use of parasite isolates from patients were approved by the Ethics Committee of Faculdade de Medicina da Universidade de São Paulo (protocol 0792/09).

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References

- Goto H, Lauletta Lindoso JA. Cutaneous and mucocutaneous leishmaniasis. *Infect Dis Clin North Am.* 2012;26(2):293–307.
- Saravia NG, Segura I, Holguin AF, Santrich C, Valderrama L, Ocampo C. Epidemiologic, genetic, and clinical associations among phenotypically distinct populations of *Leishmania* (*Viannia*) in Colombia. *AmJTrop Med Hyg.* 1998;59(1):86–94.
- Indiani de Oliveira C, Teixeira MJ, Teixeira CR, Ramos de Jesus J, Bomura Rosato A, Santa da Silva J, et al. *Leishmania braziliensis* isolates differing at the genome level display distinctive features in BALB/c mice. *Microbes and infection/Institut Pasteur.* 2004;6(11):977–84.
- Schriefer A, Schriefer AL, Goes-Neto A, Guimaraes LH, Carvalho LP, Almeida RP, et al. Multiclonal *Leishmania braziliensis* population structure and its clinical implication in a region of endemicity for American tegumentary leishmaniasis. *Infect Immun.* 2004;72(1):508–14.
- Oliveira FS, Valet-Rosalino CM, Pacheco SJ, Costa FA, Schubach AO, Pacheco RS. American tegumentary leishmaniasis caused by *Leishmania* (*Viannia*) *braziliensis*: assessment of parasite genetic variability at intra- and inter-patient levels. *Parasit Vectors.* 2013;6:189.
- Cantanhede LM, da Silva Junior CF, Ito MM, Felipin KP, Nicolette R, Salcedo JM, et al. Further evidence of an association between the presence of *Leishmania* RNA virus 1 and the mucosal manifestations in tegumentary leishmaniasis patients. *PLoS Negl Trop Dis.* 2015;9(9), e0004079.
- Da-Cruz AM, Bittar R, Mattos M, Oliveira-Neto MP, Nogueira R, Pinho-Ribeiro V, et al. T-cell-mediated immune responses in patients with cutaneous or mucosal leishmaniasis: long-term evaluation after therapy. *Clin Diagn Lab Immunol.* 2002;9(2):251–6.
- Silveira FT, Lainson R, De Castro Gomes CM, Laurenti MD, Corbett CE. Immunopathogenic competences of *Leishmania* (*V.*) *braziliensis* and *L. (L.) amazonensis* in American cutaneous leishmaniasis. *Parasite Immunol.* 2009;31(8):423–31.
- Carvalho LP, Passos S, Bacellar O, Lessa M, Almeida RP, Magalhaes A, et al. Differential immune regulation of activated T cells between cutaneous and mucosal leishmaniasis as a model for pathogenesis. *Parasite Immunol.* 2007; 29(5):251–8.
- Faria DR, Gollob KJ, Barbosa Jr J, Schriefer A, Machado PR, Lessa H, et al. Decreased in situ expression of interleukin-10 receptor is correlated with the exacerbated inflammatory and cytotoxic responses observed in mucosal leishmaniasis. *Infect Immun.* 2005;73(12):7853–9.
- Goto H, Gomes CM, Corbett CE, Monteiro HP, Gidlund M. Insulin-like growth factor I is a growth-promoting factor for *Leishmania* promastigotes and amastigotes. *Proc Natl Acad Sci U S A.* 1998;95(22):13211–6.
- Blakytyn R, Jude EB, Martin Gibson J, Boulton AJ, Ferguson MW. Lack of insulin-like growth factor 1 (IGF1) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. *J Pathol.* 2000;190(5):589–94.
- Kratz G, Lake M, Gidlund M. Insulin like growth factor-1 and -2 and their role in the re-epithelialisation of wounds; interactions with insulin like growth factor binding protein type 1. *Scandinavian journal of plastic and reconstructive surgery and hand surgery/Nordisk plastikkirurgisk forening [and] Nordisk klubb for handkirurgi.* 1994;28(2):107–12.
- Sukhanov S, Higashi Y, Shai SY, Vaughn C, Mohler J, Li Y, Song YH, Titterton J, Delafontaine P. IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol.* 2007;27(12):2684–90.
- Vendrame CM, Carvalho MD, Rios FJ, Manuli ER, Petitto-Assis F, Goto H. Effect of insulin-like growth factor-I on *Leishmania amazonensis* promastigote

- arginase activation and reciprocal inhibition of NOS2 pathway in macrophage in vitro. *Scand J Immunol.* 2007;66(2–3):287–96.
16. Vendrame CM, Carvalho MD, Tempone AG, Goto H. Insulin-like growth factor-1 induces arginase activity in *Leishmania amazonensis* amastigote-infected macrophages through a cytokine-independent mechanism. *Mediat Inflamm.* 2014;2014:475919.
 17. Vendrame CM, Souza LD, Carvalho MD, Salgado K, Carvalho EM, Goto H. Insulin-like growth factor-I induced and constitutive arginase activity differs among isolates of *Leishmania* derived from patients with diverse clinical forms of *Leishmania braziliensis* infection. *Trans R Soc Trop Med Hyg.* 2010;104(8):566–8.
 18. Gomes CM, Goto H, Ribeiro Da Matta VL, Laurenti MD, Gidlund M, Corbett CE. Insulin-like growth factor (IGF)-I affects parasite growth and host cell migration in experimental cutaneous leishmaniasis. *Int J Exp Pathol.* 2000;81(4):249–55.
 19. Cupolillo E, Grimaldi Jr G, Momen H. A general classification of New World *Leishmania* using numerical zymotaxonomy. *AmJTrop Med Hyg.* 1994;50(3):296–311.
 20. Tsuchiya S, Kobayashi Y, Goto Y, Okumura H, Nakae S, Konno T, Tada K. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res.* 1982;42(4):1530–6.
 21. Corraliza IM, Campo ML, Soler G, Modolell M. Determination of arginase activity in macrophages: a micromethod. *J Immunol Methods.* 1994;174(1–2):231–5.
 22. Heller NM, Qi X, Junntila IS, Shirey KA, Vogel SN, Paul WE, Keegan AD. Type I IL-4Rs selectively activate IRS-2 to induce target gene expression in macrophages. *Sci Signal.* 2008;1(51):ra17.
 23. Elmlinger MW, Kuhnel W, Weber MM, Ranke MB. Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). *Clinical chemistry and laboratory medicine : CCLM/FESCC.* 2004;42(6):654–64.
 24. Cunningham AC. Parasitic adaptive mechanisms in infection by *Leishmania*. *Exp Mol Pathol.* 2002;72(2):132–41.
 25. Carvalho LP, Passos S, Schriefer A, Carvalho EM. Protective and pathologic immune responses in human tegumentary leishmaniasis. *Front Immunol.* 2012;3:301.
 26. Soong L, Henard CA, Melby PC. Immunopathogenesis of non-healing American cutaneous leishmaniasis and progressive visceral leishmaniasis. *Semin Immunopathol.* 2012;34(6):735–51.
 27. Gollob KJ, Viana AG, Dutra WO. Immunoregulation in human American leishmaniasis: balancing pathology and protection. *Parasite Immunol.* 2014;36(8):367–76.
 28. Pereira-Carvalho R, Mendes-Aguiar CO, Oliveira-Neto MP, Covas CJ, Bertho AL, Da-Cruz AM, Gomes-Silva A. *Leishmania braziliensis*-reactive T cells are down-regulated in long-term cured cutaneous leishmaniasis, but the renewal capacity of T effector memory compartments is preserved. *PLoS One.* 2013; 8(11), e81529.
 29. Rocha PN, Almeida RP, Bacellar O, De Jesus AR, Filho DC, Filho AC, et al. Down-regulation of Th1 type of response in early human American cutaneous leishmaniasis. *J Infect Dis.* 1999;180(5):1731–4.
 30. Tavakkol A, Varani J, Elder JT, Zouboulis CC. Maintenance of human skin in organ culture: role for insulin-like growth factor-1 receptor and epidermal growth factor receptor. *Arch Dermatol Res.* 1999;291(12):643–51.
 31. Sharp LL, Jameson JM, Cauvi G, Havran WL. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat Immunol.* 2005;6(1):73–9.
 32. Bacellar O, Lessa H, Schriefer A, Machado P, Ribeiro De Jesus A, Dutra WO, Gollob KJ, Carvalho EM. Up-regulation of Th1-type responses in mucosal leishmaniasis patients. *Infect Immun.* 2002;70(12):6734–40.
 33. Ribeiro-de-Jesus A, Almeida RP, Lessa H, Bacellar O, Carvalho EM. Cytokine profile and pathology in human leishmaniasis. *Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica.* 1998;31(1): 143–8.
 34. Turetz ML, Machado PR, Ko AI, Alves F, Bittencourt A, Almeida RP, Mobashery N, Johnson WD, Jr, Carvalho EM. Disseminated leishmaniasis: a new and emerging form of leishmaniasis observed in northeastern Brazil. *J Infect Dis.* 2002;186(12):1829–34.
 35. Castellucci L, Cheng LH, Araujo C, Guimaraes LH, Lessa H, Machado P, Almeida MF, Oliveira A, Ko A, Johnson WD. Familial aggregation of mucosal leishmaniasis in northeast Brazil. *Am J Trop Med Hyg.* 2005;73(1):69–73.
 36. Leopoldo PT, Machado PR, Almeida RP, Schriefer A, Giudice A, de Jesus AR, Ho JL, Guimaraes LH, Bacellar O, Carvalho EM. Differential effects of antigens from *L. braziliensis* isolates from disseminated and cutaneous leishmaniasis on in vitro cytokine production. *BMC Infect Dis.* 2006;6:75.
 37. Aydin F, Kaya A, Karapinar L, Kumbaraci M, Imerci A, Karapinar H, Karakuzu C, Incesu M. IGF-1 Increases with hyperbaric oxygen therapy and promotes wound healing in diabetic foot ulcers. *J Diabetes Res.* 2013;2013:567834.
 38. Achar RA, Silva TC, Achar E, Martines RB, Machado JL. Use of insulin-like growth factor in the healing of open wounds in diabetic and non-diabetic rats. *Acta cirurgica brasileira/Sociedade Brasileira para Desenvolvimento Pesquisa em Cirurgia.* 2014;29(2):125–31.

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