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**Fator de crescimento insulina-símile I (IGF-I) e sua interação com citocinas na
infecção *in vitro* de macrófago murino por *Leishmania (L.) infantum***

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Dissertação apresentada ao Instituto de Medicina Tropical da Universidade de São Paulo para obtenção do título de Mestre em Ciências.

Área de Concentração: Doenças Tropicais e Saúde Internacional.

Orientadora: Profa. Dra. Hiro Goto

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Descritores: 1. LEISHMANIA INFANTUM. 2. FATOR DE CRESCIMENTO INSULIN-
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RESUMO

USCATA BA. Fator de crescimento insulina-símile I (IGF-I) e sua interação com citocinas na infecção *in vitro* de macrófago murino por *Leishmania (L.) infantum*. São Paulo: Instituto de Medicina Tropical de São Paulo da Universidade de São Paulo; 2019.

O desenvolvimento da leishmaniose visceral (LV), causada por *Leishmania (Leishmania) infantum*, envolve fatores específicos e inespecíficos, como o fator de crescimento insulina-símile I (IGF-I). Na LV experimental o papel das citocinas Th1 e Th2 ainda não está esclarecido, nem o seu efeito associado ao IGF-I. Este projeto teve como objetivo elucidar a participação do IGF-I na infecção por *L. infantum*, como também o efeito das citocinas Th1 e Th2 sobre o parasitismo, produção de óxido nítrico (NO), atividade de arginase, expressão de mRNA de *Nos2*, *Arg1* e *Cat-2B*, em macrófagos derivados de medula óssea de camundongos BALB/c infectados com promastigotas de *L. infantum* e tratados com IGF-I recombinante (rIGF-I) ou citocinas IL-4, IL-13 e IFN- γ . Na análise do efeito de rIGF-I, verificamos uma diminuição do parasitismo em 24 horas e um aumento em 48h nas células tratadas com rIGF-I em relação ao controle sem rIGF-I. Observamos uma diminuição do parasitismo nos grupos tratados com as citocinas Th1 e Th2 quando comparado com o grupo controle. Foi observada uma diminuição na expressão de mRNA de *Igf-I* sob estímulo de IFN- γ e um aumento com citocinas Th2. Observamos um aumento na expressão de mRNA de *Nos2* e *Cat-2B*, mas um aumento de *Arg1* nas células infectadas quando comparado com o controle. Nos grupos infectados e estimulados com as citocinas, todos os grupos apresentaram aumento na expressão de mRNA de *Arg1*, *Nos2* e *Cat-2B*. No nosso modelo, as citocinas do perfil Th2, IL-4 e IL-13, apresentaram um efeito diferente no parasitismo e no metabolismo de L-arginina, assim como IFN- γ , quando comparado com a infecção por *L. major*. Na infecção por *L. infantum* as citocinas IL-4, IL-13, IFN- γ e o IGF-I têm efeitos diferentes dos observados com as cepas dermatrópicas.

Descritores: *Leishmania infantum*. Fator de crescimento insulina-símile I. Parasitismo. RNA. Óxido nítrico. Expressão Gênica.

ABSTRACT

USCATA BA. Insulin-like growth factor I (IGF-I) and its interaction with cytokines in murine macrophage infection in vitro by *Leishmania (L.) infantum*. São Paulo: Instituto de Medicina Tropical de São Paulo da Universidade de São Paulo; 2019.

The development of visceral leishmaniasis (VL), caused by *Leishmania (Leishmania) infantum* involves specific and nonspecific factors such as insulin-like growth factor I (IGF-I). In experimental VL the role of Th1 and Th2 cytokines is still unclear, nor is their effect associated with IGF-I. This project aimed to elucidate the participation of IGF-I in *L. infantum* infection, as well as the effect of Th1 and Th2 cytokines on parasitism, nitric oxide (NO) production, arginase activity and *Nos2*, *Arg1* and *Cat-2B* mRNA expression, in bone marrow-derived macrophages of BALB/c mice infected with *L. infantum* promastigotes and treated with recombinant IGF-I (rIGF-I) or cytokines IL-4, IL-13 and IFN- γ . In the analysis of the effect of rIGF-I, we observed a decrease in parasitism at 24 hours and an increase at 48h in rIGF-I-treated cells compared to control without rIGF-I. We observed a decrease in parasitism in the groups treated with cytokines Th1 and Th2 when compared with the control group. A decrease in *Igf-I* mRNA expression was observed under IFN- γ stimulation and an increase with Th2 cytokines. We observed an increase in *Nos2* and *Cat-2B* mRNA expression, but an increase in *Arg1* in infected cells compared to the control. In groups infected and stimulated with cytokines, all groups showed increased mRNA expression of *Arg1*, *Nos2* and *Cat-2B*. In our model, Th2 profile cytokines, IL-4 and IL-13, had a different effect on parasitism and metabolism of L-arginine as well as IFN- γ when compared to *L. major* infection. In *L. infantum* infection, cytokines IL-4, IL-13, IFN- γ and IGF-I have different effects than those observed with dermatotropic strains.

Descriptors: *Leishmania infantum*. Insulin-like growth factor I. Parasitism. RNA. Nitric Oxide. Gene expression.

CONCLUSÕES

Com estudos em macrófagos derivados de medula óssea de camundongos BALB/c *in vitro*, infectados com promastigotas de *L. (L.) infantum*, sob estímulos de IGF-I recombinante ou citocinas IFN- γ , IL-4 e IL-13 observamos :

- a) O IGF-I favorece a proliferação do parasito mais tardiamente;
- b) Quando analisamos a expressão de IGF-I sob efeito de citocinas, foi observada uma diminuição da expressão de mRNA de *Igf-I* quando as células são estimuladas com IFN- γ , e um aumento da expressão quando as células são estimuladas com IL-4 e IL-13;
- c) Na avaliação do parasitismo, a citocina IFN- γ leva uma diminuição do parasitismo associado a um aumento da produção de óxido nítrico (NO);
- d) No tratamento com citocinas Th2, observou-se uma diminuição do parasitismo sob estímulo de IL-4 e também de IL-13;
- e) A produção de NO e da expressão de mRNA de *Nos2* apresentaram um aumento nas células infectadas e estimuladas com citocinas do perfil Th1 quanto Th2;
- f) A atividade da arginase, expressão de mRNA de *Arg1* e *Cat-2B* apresentaram aumento nos grupos estimulados com as citocinas do perfil Th1 e Th2;
- g) Os resultados encontrados neste trabalho demonstraram algumas semelhanças entre as infecções por *Leishmania infantum* e as espécies dermatrópicas, como também significantes diferenças entre esses modelos, sugerindo um papel diferente dessas citocinas e do IGF-I na infecção por *L. infantum*.

REFERÊNCIAS

1. Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, et al. Correction: A Historical Overview of the Classification, Evolution, and Dispersion of Leishmania Parasites and Sandflies. PLoS Negl Trop Dis. 2016;10(6):e0004770.
2. World Health Organization. Leishmaniasis . 2019 [citado 2019 maio 24]. Available from: <https://www.who.int/leishmaniasis/en/>
3. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet. 2018;392(10151):951–70.
4. McCall L-I, Zhang W-W, Matlashewski G. Determinants for the Development of Visceral Leishmaniasis Disease. PLoS Pathog. 2013;9(1):e1003053.
5. Grimaldi G, Tesh RB. Leishmaniasis of the New World: current concepts and implications for future research. Clin Microbiol Rev. 1993 Jul;6(3):230–50.
6. Balanco JM, Pral EM, da Silva S, Bijovsky AT, Mortara RA, Alfieri SC. Axenic cultivation and partial characterization of *Leishmania braziliensis* amastigote-like stages. Parasitology. 1998;116(2):103–13.
7. Hoare CA, Wallace FG. Developmental Stages of Trypanosomatid Flagellates: a New Terminology. Nature. 1966;212(5068):1385–6.
8. Akopyants NS, Matlib RS, Bukanova EN, Smeds MR, Brownstein BH, Stormo GD, et al. Expression profiling using random genomic DNA microarrays identifies differentially expressed genes associated with three major developmental stages of the protozoan parasite *Leishmania major*. Mol Biochem Parasitol. 2004;136(1):71–86.
9. Rey L. O complexo *Leishmania braziliensis* e a leishmaniose tegumentar americana. In: Parasitologia. 3rd ed. Rio de Janeiro: Guanabara Koogan; 2001. p. 180–92.
10. Ostyn B, Gidwani K, Khanal B, Picado A, Chappuis F, Singh SP, et al. Incidence of Symptomatic and Asymptomatic *Leishmania donovani* Infections in High-Endemic Foci in India and Nepal: A Prospective Study. PLoS Negl Trop Dis. 2011;5(10):e1284.
11. Herwaldt BL. Leishmaniasis. Lancet. 1999;354(9185):1191–9.
12. Lindoso JA, Goto H. Leishmaniose Visceral. In: Lopes AC, editor. Tratado de Clínica Médica. 3ª. Rio de Janeiro: Rio de Janeiro: Guanabara Koogan; 2016. p.

3172–6.

13. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis Worldwide and Global Estimates of Its Incidence. PLoS One. 2012;7(5):e35671.
14. Steverding D. The history of leishmaniasis. Parasit Vectors. 2017;10(1):82.
15. Killick-Kendrick R. The biology and control of Phlebotomine sand flies. Clin Dermatol. 1999;17(3):279–89.
16. Carvalho RW, Serra-Freire NM, Souza MB. Fauna de flebotomíneos da Ilha do Araújo, Município de Paraty-RJ. 1.- Diversidades e Aspectos do Comportamento. Parasitol Dia. 1995;19:104–12.
17. Costa FA, Prianti MG, Silva TC, Silva SM, Guerra JL, Goto H. T cells, adhesion molecules and modulation of apoptosis in visceral leishmaniasis glomerulonephritis. BMC Infect Dis. 2010;10:112.
18. Cupolillo E, Kuhls K, Giacoia-Gripp CBW, Oliveira-Neto MP, Santos-Oliveira JR, Da-Cruz AM, et al. Atypical Lesions as a Sign of Cutaneous Dissemination of Visceral Leishmaniasis in a Human Immunodeficiency Virus–Positive Patient Simultaneously Infected by Two Viscerotropic Leishmania Species. Am J Trop Med Hyg. 2011;85(1):55–9.
19. Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. Nat Rev Immunol. 2002;2(11):845–58.
20. Mosser DM, Brittingham A. Leishmania , macrophages and complement: a tale of subversion and exploitation. Parasitology. 1997;115(7):9–23.
21. Antoine JC, Prina E, Jouanne C, Bongrand P. Parasitophorous vacuoles of *Leishmania amazonensis*-infected macrophages maintain an acidic pH. Infect Immun. 1990;58(3):779–87.
22. Haidaris CG, Bonventre PF. A role for oxygen-dependent mechanisms in killing of *Leishmania donovani* tissue forms by activated macrophages. J Immunol. 1982;129(2):850–5.
23. Liew FY, Millott S, Parkinson C, Palmer RM, Moncada S. Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J Immunol. 1990;144(12):4794–7.
24. Green SJ, Meltzer MS, Hibbs JB, Nacy CA. Activated macrophages destroy intracellular *Leishmania major* amastigotes by an L-arginine-dependent killing mechanism. J Immunol. 1990;144(1):278–83.
25. Roberts SC, Tancer MJ, Polinsky MR, Gibson KM, Heby O, Ullman B. Arginase

- Plays a Pivotal Role in Polyamine Precursor Metabolism in *Leishmania*. *J Biol Chem*. 2004;279(22):23668–78.
26. Iniesta V, Carlos Gomez-Nieto L, Molano I, Mohedano A, Carcelen J, Miron C, et al. Arginase I induction in macrophages, triggered by Th2-type cytokines, supports the growth of intracellular *Leishmania* parasites. *Parasite Immunol*. 2002;24(3):113–8.
 27. Yeramian A, Martin L, Serrat N, Arpa L, Soler C, Bertran J, et al. Arginine transport via cationic amino acid transporter 2 plays a critical regulatory role in classical or alternative activation of macrophages. *J Immunol*. 2006;176(10):5918–24.
 28. Bogdan C, Rölinghoff M. The immune response to *Leishmania*: mechanisms of parasite control and evasion. *Int J Parasitol*. 1998;28(1):121–34.
 29. Hecker M, Nematollahi H, Hey C, Busse R, Racké K. Inhibition of arginase by NG-hydroxy-L-arginine in alveolar macrophages: implications for the utilization of L-arginine for nitric oxide synthesis. *FEBS Lett*. 1995;359(2–3):251–4.
 30. Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to Interferon- γ plus pentavalent antimony in Indian visceral leishmaniasis. *J Infect Dis*. 1997;176(4):1117–9.
 31. Saha S, Mondal S, Ravindran R, Bhowmick S, Modak D, Mallick S, et al. IL-10- and TGF- β -Mediated Susceptibility in Kala-azar and Post-kala-azar Dermal Leishmaniasis: The Significance of Amphotericin B in the Control of *Leishmania donovani* Infection in India. *J Immunol*. 2007;179(8):5592–603.
 32. Engwerda CR, Ato M, Kaye PM. Macrophages, pathology and parasite persistence in experimental visceral leishmaniasis. *Trends Parasitol*. 2004;20(11):524–30.
 33. Barcinski MA, Schechtman D, Quintao LG, Costa DA, Soares LR, Moreira ME, et al. Granulocyte-macrophage colony-stimulating factor increases the infectivity of *Leishmania amazonensis* by protecting promastigotes from heat-induced death. *Infect Immun*. 1992;60(9):3523–7.
 34. Bogdan C, Moll H, Solbach W, Rölinghoff M. Tumor necrosis factor- α in combination with interferon- γ , but not with interleukin 4 activates murine macrophages for elimination of *Leishmania major* amastigotes. *Eur J Immunol*. 1990;20(5):1131–5.
 35. Barral A, Barral-Netto M, Yong EC, Brownell CE, Twardzik DR, Reed SG.

- Transforming growth factor beta as a virulence mechanism for *Leishmania braziliensis*. Proc Natl Acad Sci. 1993;90(8):3442–6.
36. 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. Scand J Plast Reconstr Surg Hand Surg. 1994;28(2):107–12.
 37. Jones JJ, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev. 1995;16(1):3–34.
 38. Nagaoka I, Trapnell BC, Crystal RG. Regulation of insulin-like growth factor I gene expression in the human macrophage-like cell line U937. J Clin Invest. 1990;85(2):448–55.
 39. Arkins S, Rebeiz N, Biragyn A, Reese DL, Kelley KW. Murine macrophages express abundant insulin-like growth factor-I class I Ea and Eb transcripts. Endocrinology. 1993;133(5):2334–43.
 40. Daughaday WH, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev. 1989;10(1):68–91.
 41. Pedrini MT, Giorgino F, Smith RJ. cDNA cloning of the rat IGF I receptors: structural analysis of rat and human IGF I and insulin receptors reveals differences in alternative splicing and receptor-specific domain conservation. Biochem Biophys Res Commun. 1994;202(2):1038–46.
 42. Gomes CMC, Goto H, Corbett CEP, Gidlund M. Insulin-like growth factor-1 is a growth promoting factor for *Leishmania* promastigotes. Acta Trop. 1997;64(3–4):225–8.
 43. 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.
 44. Gomes C, Goto H, Matta VL, Gidlund M, Corbett CE. Insulin-like Growth Factor (IGF) -I affects parasite growth and host cell migration in experimental cutaneous leishmaniasis. Int J. 2000;81:249–55.
 45. Gomes CMC, Goto H, Magnanelli AC, Monteiro HP, Soares RPS, Corbett CEP, et al. Characterization of the receptor for insulin-like growth factor on *Leishmania* promastigotes. Exp Parasitol. 2001;99(4):190–7.
 46. 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.
47. Petitto-Assis F. Participação do fator de crescimento insulina símile (IGF) -I na imunidade específica na infecção por *Leishmania (L.) major*. Universidade de São Paulo; 2008.
 48. Reis LC, Ramos-Sanchez EM, Petitto-Assis F, Nerland AH, Hernandez-Valladares M, Selheim F, et al. Insulin-Like Growth Factor-I as an Effector Element of the Cytokine IL-4 in the Development of a *Leishmania major* Infection. Mediators Inflamm. 2018;2018:1–17.
 49. Wynes MW, Riches DWH. Induction of macrophage insulin-like growth factor-I expression by the Th2 cytokines IL-4 and IL-13. J Immunol. 2003;171(7):3550–9.
 50. Reis LC, Ramos-Sanchez EM, Goto H. The interactions and essential effects of intrinsic insulin-like growth factor-I on *Leishmania (Leishmania) major* growth within macrophages. Parasite Immunol. 2013;35(7–8):239–44.
 51. Sevillano O, Goto H, Ramos-Sanchez EM. Contribuição da expressão do fator de crescimento insulina-símile I (IGF) e do seu receptor no parasitismo de macrófagos humanos por *Leishmania (L) infatum*. Universidade de São Paulo; 2018.
 52. Murray HW, Stern JJ, Welte K, Rubin BY, Carriero SM, Nathan CF. Experimental visceral leishmaniasis: production of interleukin 2 and interferon-gamma, tissue immune reaction, and response to treatment with interleukin 2 and interferon-gamma. J Immunol. 1987;138(7):2290–7.
 53. Murphy ML, Wille U, Villegas EN, Hunter CA, Farrell JP. IL-10 mediates susceptibility to *Leishmania donovani* infection. Eur J Immunol. 2001;31(10):2848–56.
 54. Murray HW, Lu CM, Mauze S, Freeman S, Moreira AL, Kaplan G, et al. Interleukin-10 (IL-10) in experimental visceral leishmaniasis and IL-10 receptor blockade as immunotherapy. Infect Immun. 2002;70(11):6284–93.
 55. Goto H, Lindoso JAL. Immunity and immunosuppression in experimental visceral leishmaniasis. Brazilian J Med Biol Res. 2004;37(4):615–23.
 56. Zamboni DS, Rabinovitch M. Nitric Oxide Partially Controls Coxiella burnetii Phase II Infection in Mouse Primary Macrophages. Infect Immun.

- 2003;71(3):1225–33.
57. Englen MD, Valdez YE, Lehnert NM, Lehnert BE. Granulocyte/macrophage colony-stimulating factor is expressed and secreted in cultures of murine L929 cells. *J Immunol Methods*. 1995;184(2):281–3.
 58. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem*. 1982;126(1):131–8.
 59. Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J Immunol*. 1988;141(7):2407–12.
 60. 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.
 61. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*. 2001;29(9):45e – 45.
 62. Arkins S, Rebeiz N, Brunke-Reese DL, Biragyn A, Kelley KW. Interferon-gamma inhibits macrophage insulin-like growth factor-I synthesis at the transcriptional level. *Mol Endocrinol*. 1995;9(3):350–60.
 63. Arkins S, Rebeiz N, Brunke-Reese DL, Minshall C, Kelley KW. The colony-stimulating factors induce expression of insulin-like growth factor-I messenger ribonucleic acid during hematopoiesis. *Endocrinology*. 1995;136(3):1153–60.
 64. Marim FM, Silveira TN, Lima DS, Zamboni DS. A method for generation of bone marrow-derived macrophages from cryopreserved mouse bone marrow cells. *PLoS One*. 2010;5(12):e15263.
 65. Martins R.S. A, Goto H. Efeito das lipoproteínas plasmáticas no parasitismo de células monocíticas humanas infectadas com *Leishmania (Leishmania) infantum*. Universidade de São Paulo; 2017.
 66. Muleme HM, Reguera RM, Berard A, Azinwi R, Jia P, Okwor IB, et al. Infection with Arginase-Deficient *Leishmania major* Reveals a Parasite Number-Dependent and Cytokine-Independent Regulation of Host Cellular Arginase Activity and Disease Pathogenesis. *J Immunol*. 2009;183(12):8068–76.
 67. Boitz JM, Gilroy CA, Olenyik TD, Paradis D, Perdeh J, Dearman K, et al. Arginase Is Essential for Survival of *Leishmania donovani* Promastigotes but Not

- Intracellular Amastigotes. *Infect Immun.* 2017;85(1):1–14.
68. Stempin CC, Dulgerian LR, Garrido V V., Cerban FM. Arginase in parasitic infections: macrophage activation, immunosuppression, and intracellular signals. *J Biomed Biotechnol.* 2010;2010:683485.
 69. Iniesta V, Carcelén J, Molano I, Peixoto PM V, Redondo E, Parra P, et al. Arginase I induction during *Leishmania major* infection mediates the development of disease. *Infect Immun.* 2005;73(9):6085–90.
 70. Wanasen N, Soong L. L-arginine metabolism and its impact on host immunity against *Leishmania* infection. *Immunol Res.* 2008;41(1):15–25.
 71. Gregory DJ, Olivier M. Subversion of host cell signalling by the protozoan parasite *Leishmania*. *Parasitology.* 2005;130(S1):S27–35.