Chapter

L-arginine Metabolism in the Infection with *Trypanosoma cruzi*

Laila Gutiérrez-Kobeh and Arturo A. Wilkins-Rodríguez

Abstract

Trypanosoma cruzi is the causal agent of Chagas disease that affects 6–7 million people around the world, principally in Latin America. This disease is characterized for the presence of an acute phase in which the host immune response plays a central role in the elimination of the parasite. If the parasite is not efficiently eliminated, patients can remain asymptomatic or develop a chronic infection. One of the cells that are primarily infected with this intracellular parasite is macrophages (Mϕ). Mϕ present a wide array of activation states with classically activated macrophages in one pole (CAMϕ) and alternatively activated macrophages (AAMϕ) in the other. One of the most important differences between these two activation states is the presence of the inducible nitric oxide synthase (iNOS or NOS2) in CAMϕ and arginase 1 (Arg-1) in AAMϕ; both enzymes share the same substrate, L-arginine, and are reciprocally regulated by the action of Th1 cytokines in the case of NOS2 and Th2 cytokines in the case of Arg-1. The activation of CAMϕ permits the production of nitric oxide (NO), highly trypanotoxic, while the activation of AAMϕ allows the synthesis of polyamines, necessary for parasite duplication. L-arginine is a very important metabolite situated in the center between the elimination and perpetuation of *T. cruzi*.

Keywords: arginase-1, L-arginine, inducible nitric oxide synthase, macrophages, *trypanosoma cruzi*

1. Introduction

Trypanosoma cruzi is the causal agent of Chagas disease that affects 6–7 million people around the world, mainly in Latin America [1], although in the last years it has also become a potential public health problem in developed countries due to the constant migrations with cases reported in the USA, Canada, Europe, Japan, and Australia [2].

This intracellular obligate parasite enters the human host in the form of metacyclic promastigotes that are released from the triatomine feces during the blood meal, through damaged skin or mucosae. Alternatively, infection can occur through other routes such as oral, congenital, blood transfusions, or organ transplants. After entering the host, trypomastigotes are phagocytized mainly by macrophages, where they transform to amastigotes, the intracellular form that has the ability to replicate. In order to evade the host immune response and ensure its persistence inside macrophages, *Trypanosoma* has developed multiple strategies. One of these has as a target L-arginine metabolism. Macrophages can eliminate amastigotes or permit their survival depending on the balance of two inducible enzymes nitric

oxide synthase (iNOS or NOS2) and arginase-1 (Arg-1) that share the same substrate: L-arginine. During the activation of macrophages in the context known as classical activation, L-arginine is metabolized by iNOS giving rise to the production of nitric oxide (NO), one important trypanotoxic agent that permits these cells to destroy the parasite. On the other hand, during the activation of macrophages in the context known as alternative activation, L-arginine is metabolized by Arg-1 giving rise to the production of polyamines that favor multiplication and persistence of *Trypanosoma* in these cells. Thus, L-arginine is situated as a frontier between the elimination and survival of *Trypanosoma* in macrophages, and its metabolism is a determinant factor for the evolution of the disease.

2. Phases of the infection with *Trypanosoma cruzi*

The infection with *T. cruzi* presents an acute phase that is auto-limiting and can go unnoticed in many infected individuals. During this phase, parasites actively duplicate in different cells and tissues such as macrophages; muscular cells of smooth, striated, and cardiac muscles; adipocytes; and cells of the central nervous system [3]. While some patients succumb during the acute phase of the disease, the development of an adaptive immune generally permits the control of infection with *T. cruzi*. If the parasite is not completely eradicated, individuals remain infected for life, and a dynamic equilibrium is established with the parasite that results in different clinical outcomes. In this way, while many individuals chronically infected remain in an asymptomatic intermediate phase, a significant proportion (30–35%) of patients develop cardiac or digestive manifestations that can drive them to congestive cardiac failure, arrhythmias, and eventually death or develop colon or esophageal megasyndromes. All of these are irreversible pathologic changes that occur even though the presence of the parasite is scarce. One experimental model that recapitulates chagasic myocarditis is present in infected mice for long periods with different *T. cruzi* strains that develop chronic lesion in the myocardium [4, 5].

3. Generalities of L-arginine

L-arginine is one of the most versatile amino acids at the metabolic level. Besides serving as a precursor for protein synthesis, it is also a precursor of multiple compounds of great biologic importance such as urea, nitric oxide, polyamines, L-proline, glutamate, creatinine, and agmatine (**Figure 1**) [6, 7].

In adult mammals, L-arginine is a nonessential amino acid; nevertheless, during childhood and certain physiologic or pathologic conditions (e.g., pregnancy, sepsis, trauma, catabolic stress, intestinal or renal damage), it is considered as a semi-essential amino acid or conditioned nonessential, due to the fact that its consumption exceeds the capacity of being synthesized by the organism and has to be supplied exogenously [8–10]. In mammals, the provision of L-arginine depends on its procurement through the protein diet, endogenous synthesis (de novo synthesis), and its release during the process of protein replacement (**Figure 1**) [6]. Approximately 40% of the L-arginine that is obtained from the protein diet is catabolized in the intestine before entering the circulation [11]. In the absence of the contribution by the protein diet, approximately 80% of the L-arginine that enters the circulation derives from the protein replacement, and the remaining percentage is obtained through the novo synthesis [11]. L-arginine metabolism occurs basically in the liver and kidney; nevertheless, other tissues and cells also possess the required enzymes to metabolize it, including some cells of the immune response [12]. Regarding last point, it is

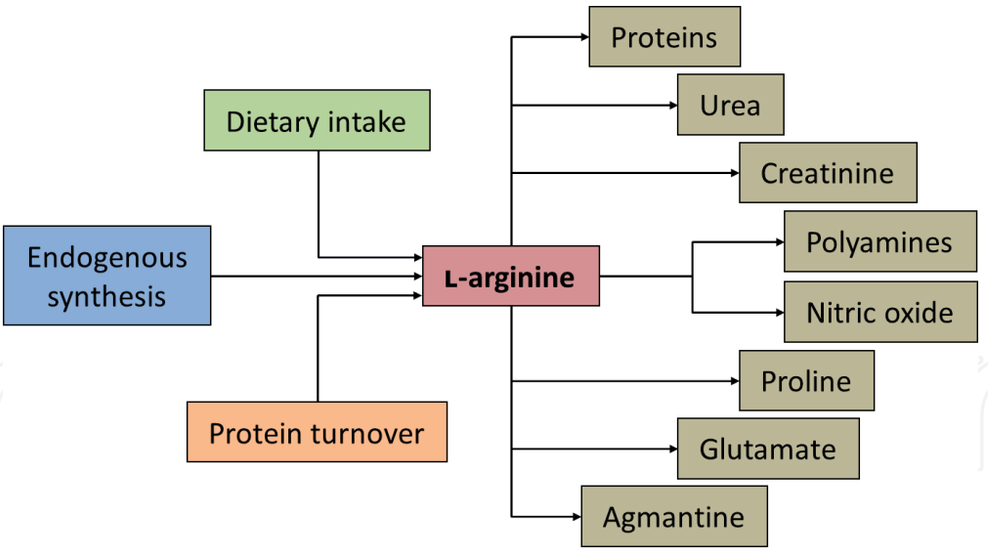

Figure 1.
Sources of L-arginine in mammals and its metabolic products.

interesting to note that a complete urea cycle has been described in macrophages [13]. Although only two enzymes directly involved in L-arginine synthesis have been identified (arginine succinate synthetase and arginine succinate lyase that are the third and fourth enzymes of the urea cycle), four enzymes utilize this amino acid as substrate: arginine decarboxylase, arginine/glycine aminotransferase, different isoforms of arginase (Arg), and the different isoforms of the nitric oxide synthases (NOS), the last two being the most studied and characterized [12]. In mammals two arginase isoforms exist, Arg-1 and Arg-2, that catalyze the same reaction but differ in cellular expression and subcellular localization. Arg-1 is cytosolic and is highly expressed in the liver and some cells of the immune response. Compared to Arg-1, Arg-2 is mitochondrial and is expressed in a great variety of peripheral tissues, mainly in the kidney, prostate, small intestine, and mammary glands during lactation [14]. Regarding NOS, this enzyme is present in three isoforms: neuronal NOS (nNOS or NOS1), inducible nitric oxide synthase (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). NOS 1 is expressed in specific neurons of the central nervous system (CNS), and NOS3 is mostly expressed in endothelial cells [15]. NOS 2 is not usually expressed in cells, but its expression can be induced by bacterial lipopolysaccharide, cytokines, and other agents. Although primarily identified in macrophages, the expression of this enzyme can be stimulated in almost any cell or tissue, provided that the appropriate inducing agents are present [16].

4. L-arginine metabolism in the immune response: special emphasis in macrophages

In the immune response, L-arginine metabolism through NOS2 and Arg-1 has a pivotal role in the regulation of the effector capabilities of macrophages, dendritic cells, and neutrophils [17–20] during infectious processes caused by a great variety of microorganisms: different species of *Mycobacterium*, *Leishmania*, *Trypanosoma*, *Schistosoma*, and *Salmonella*, among others [21, 22].

L-arginine metabolism in the immune response acquired great relevance with the discovery that murine macrophages express both NOS2 and Arg-1 and that their expression is reciprocally regulated by the action of Th1/proinflammatory cytokines (e.g., IFN-γ and TNF-α) and Th2/anti-inflammatory (e.g., IL-4, IL-10, and IL-13) that determine the activation state of macrophages [19, 23–27].

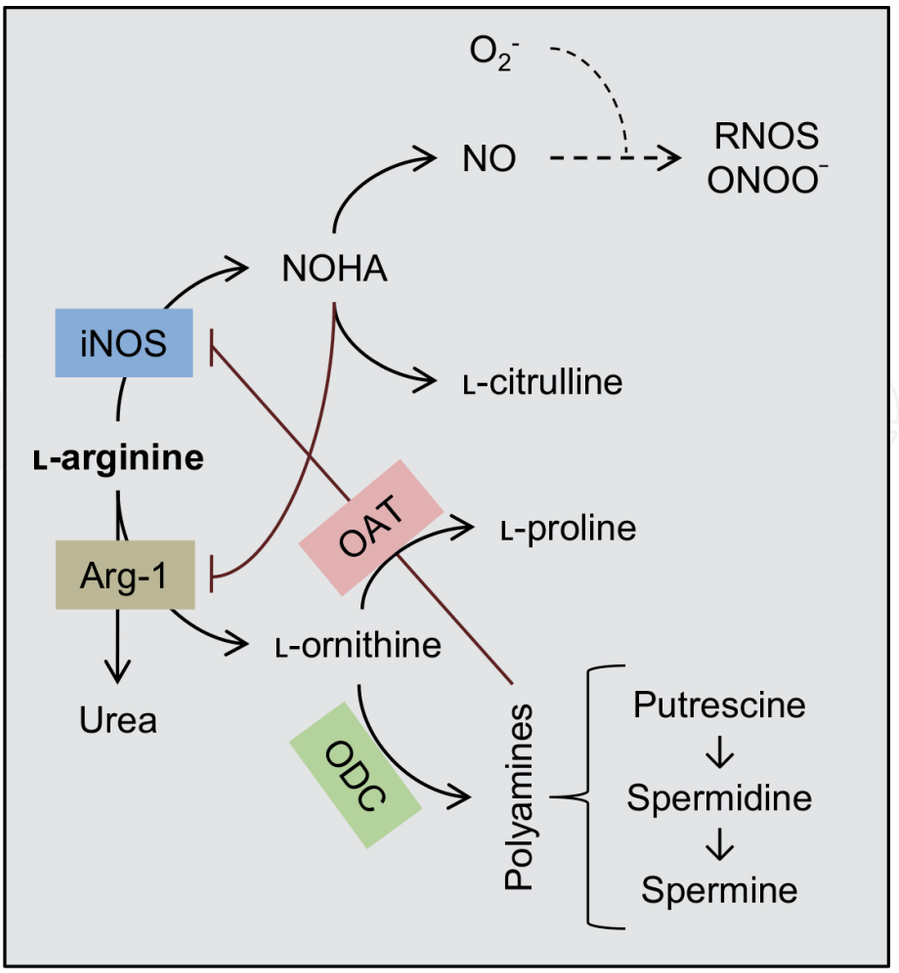

Figure 2.
L-arginine metabolism through iNOS and Arg-1. iNOS, inducible nitric oxide synthase; Arg-1, arginase 1; NOHA, N^{ω}–OH-L-arginine; OAT, ornithine-aminotransferase; ODC, ornithine-decarboxylase; NO•, nitric oxide; O_2^-, superoxide, RNOS, oxygen and nitrogen reactive species; ONOO-, peroxynitrite.

Th1 cytokines activate macrophages in a classical way (CAMΦ) and induce the expression and function of NOS2, while Th2 cytokines activate macrophages in an alternative way (AAMΦ) and induce the expression and function of Arg-1.

NOS2 or iNOS is an oxide-reductase responsible for the synthesis of L-citrulline and nitric oxide (NO•) from L-arginine in the presence of NADPH and oxygen. This reaction occurs through two successive reactions: the monooxygenation of L-arginine that drives to the production of the intermediary N^{ω}-OH- L-arginine (NOHA) and the subsequent hydrolysis of this last compound, thus producing L-citrulline and NO• (**Figure 2**). NOS2 generates both NO• and superoxide (O_2^-) that together react to form the radical peroxynitrite ($ONOO^-$) [28]. This last compound has been identified as a reactive species derived both from oxygen and nitrogen (RONS) that constitutes the principal cytostatic or cytotoxic mechanism of CAMΦ to fight the infections generated by virus, bacteria, fungi, and protozoan parasites [20, 23, 26, 29].

5. Immune response to *Trypanosoma cruzi*

Inside the mammalian host, macrophages represent an important site for the duplication of *T. cruzi*. One of the most important mechanisms in the protective immunity against *T. cruzi* is the activation of macrophages in order to achieve the elimination of parasites (**Figure 3**). CAMϕ are able eliminate *T. cruzi* thanks to

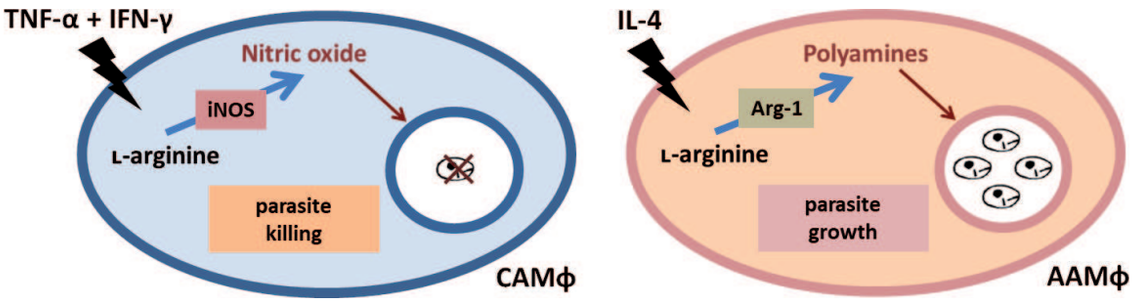

Figure 3.
L-arginine metabolism in macrophages during T. cruzi infection. In classically activated macrophages (CAMΦ), inducible nitric oxide synthase (iNOS) expression and function are induced. L-arginine metabolism through this enzyme entails the production of nitric oxide that possesses great trypanocidal capacity. In alternatively activated macrophages (AAMΦ), arginase 1 (Arg-1) expression and function are induced. L-arginine metabolism through this enzyme entails the production of polyamines that favor T. cruzi multiplication inside macrophages.

NOS2 and RONS that kill intracellular parasites by the modification of structural properties of *T. cruzi* molecules. On the other hand, the different forms of AAMϕ present high levels of mannose receptor (MR) and an overregulation of Arginase 1 [30]. Arg-1 hydrolyzes L-arginine in urea and L-ornithine; the latter is the principal intracellular source for the synthesis of polyamines and trypanothione. Polyamines are small cationic molecules required for cellular proliferation and macrophage homeostatic processes, besides being vital for the intracellular growth of *Trypanosoma* [19, 31]. Both inducible enzymes share L-arginine as substrate, and the expression and function of both enzymes are reciprocally regulated by the action of Th1 and Th2 cytokines. Thus, L-arginine is situated as a frontier between the elimination and survival of *Trypanosoma* in host cells, and its metabolism is a determinant factor in the evolution of the disease.

In response to the defense mechanisms of the host, parasites have developed several strategies in order to escape host immune response and take advantage of some host's molecules. In this way, parasites must reduce the production of toxic molecules, including nitric oxide and its derivatives, that are synthesized by the immune system, in particular by macrophages [32–34]. In addition, internalized parasites of different *T. cruzi* strains are able to escape from the parasitophorous vacuole of resident macrophages [35], a strategy that utilizes a variety of molecules with antioxidant properties [36, 37]. Nevertheless, as the infection progresses, the evasion strategies displayed by *T. cruzi* are widely surpassed by the development of a humoral specific immune response and the activation of macrophages by IFN-γ and other cytokines. As has been previously mentioned, the infection with *T. cruzi* can have an acute or a chronic phase. One of the possible causes of the passage from one phase to another is the fact that the effector immune response against the parasite is insufficient or inappropriate due to a deficient activation of the specific immune response or an excessive regulation of this response.

6. Role of Arg-1 in the infection with *Trypanosoma*

The induction of Arg-1 in macrophages promotes the infection of parasites of the genus *Trypanosoma* by providing nutrients derived from polyamines, since *Trypanosoma* parasites cannot generate their own source of ornithine through the activity of a functional arginase [38, 39]. The increase in arginase activity counteracts the host's immune response and favors parasite growth. It has been shown that in African trypanosomiasis caused by *Trypanosoma gambiense*, there is an increase in the serum level of Arg-1 that returns to basal values after the treatment

[40]. Similarly, in experimental murine trypanosomiasis caused by *Trypanosoma brucei*, macrophage Arg-1 activity represents a disease susceptibility marker [41]. In *T. brucei* Arg-1 activity is induced by excretion/secretion factors, particularly TbKHC1, kinesin H chain, and has been identified as an inductor factor of Arg-1 [38]. Other studies have demonstrated that the addition of an Arg-1 inhibitor reduces parasite growth, which is restored with L-ornithine supplementation. The essential requirement of L-ornithine is related with the absence of a functional arginase in *Trypanosoma* [39], which results in a dependence toward host's arginase for the synthesis of polyamines and trypanothione, which are essential for parasite survival, growth, and differentiation [42]. The difluoromethylornithine, structural analog of L-ornithine, has been used alone or in combination with nifurtimox as an effective drug against African trypanosomiasis [43]. Nevertheless, its administration is difficult and requires large amounts of i.v. injected fluids, which limits its use in remote areas. Thus, it is of utmost importance to find easier ways to select polyamine synthesis as a target against *Trypanosoma.* Alternatively, inhibitors of the route that conducts to arginase activity might reduce parasite loads in infected animals.

7. Conclusion

Trypanosoma cruzi is the causal agent of Chagas disease that affects 6–8 million people primarily in Latin America. It is an intracellular parasite that infects a variety of cells, among which macrophages are a very important target and thus transcendental for the immune response against the parasite. Macrophages can traverse through a gradient of stages of activation with classically activated macrophages in one end and alternatively activated macrophages in the other. These two phases of activation are characterized by the expression of two enzymes that are reciprocally regulated and share the same substrate: L-arginine. Classically activated macrophages express iNOS of NOS2 that is induced by Th1 cytokines and catalyze the conversion of L-arginine to L-citrulline and NO. Contrarily, alternatively activated macrophages express Arg-1 that is induced by Th2 cytokines. Thus, L-arginine metabolism is in the center of *Trypanosoma* elimination of survival. The better knowledge of this route during the different stages of *Trypanosoma* infection is of great importance for the better comprehension of disease progression and design of drugs.

Acknowledgements

This work was funded by project number IN218119 from Papiit, DGAPA, UNAM, to LGK.

Conflict of interest

Authors declare no conflict of interests.

DOI: http://dx.doi.org/10.5772/intechopen.85010

Author details

Laila Gutiérrez-Kobeh* and Arturo A. Wilkins-Rodríguez
Research Unit UNAM-INC, Research Division, School of Medicine, National Autonomous University of Mexico-National Institute of Cardiology "Ignacio Chávez", Mexico City, Mexico

*Address all correspondence to: lgutierr@unam.mx

References

[1] Coura JR, Vias PA. Chagas disease: A new worldwide challenge. Nature. 2010;**465**(7301):S6-S7. DOI: 10.1038/nature09221

[2] Tibayrenc M. Genetic subdivisions within *Trypanosoma cruzi* (discrete typing units) and their relevance for molecular epidemiology and experimental evolution. Kinetoplastid Biology and Disease. 2003;**2**(1):12. DOI: 10.1186/1475-9292-2-12

[3] Andrade SG, Andrade ZA. Pathology of prolonged experimental Chagas disease. Revista do Instituto de Medicina Tropical de Sao Paulo. 1968;**10**(3):180-187

[4] Laguens RP, Meckert PC, Gelpi RJ. Chronic Chagas disease in the mouse. I. Electrocardiographic and morphological patterns of the cardiopathy. Medicina (Buenos Aires). 1981;**41**(1):35-39

[5] Pinto Dias JC. The indeterminate form of human chronic Chagas disease. A clinical epidemiological review. Revista da Sociedade Brasileira de Medicina Tropical. 1989;**22**(3):147-156

[6] Morris SM Jr. Arginine: Beyond protein. American Journal of Clinical Nutrition. 2006;**83**(2):508S-512S. DOI: 10.1093/ajcn/83.2.508S

[7] Morris SM Jr. Arginases and arginine deficiency syndromes. Current Opinion in Clinical Nutrition and Metabolic Care. 2012;**15**(1):64-70. DOI: 10.1097/MCO.0b013e32834d1a08

[8] Bernard AC, Mistry SK, Morris SM Jr, O'Brian WE, Tsuei BJ, Maley ME, et al. Alterations in arginine metabolic enzymes in trauma. Shock. 2001;**15**(3):215-219

[9] Luiking YC, Hallemeesch MM, Vissers YL, Lamers WH, Deutz NE. In vivo whole body and organ arginine metabolism during endotoxemia (sepsis) is dependent on mouse strain and gender. Journal of Nutrition. 2004;**134**(10 Suppl):2768S-2774S; discussion 2796S–2797S. DOI: 10.1093/jn/134.10.2768S

[10] Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nature Reviews Immunolology. 2005;**5**(8):641-654. DOI: 10.1038/nri1668

[11] Wu G, Morris SM Jr. Arginine metabolism: Nitric oxide and beyond. Biochemical Journal. 1998;**336**:1-17. DOI: 10.1042/bj3360001

[12] Mori M, Gotoh T. Arginine metabolic enzymes, nitric oxide and infection. Journal of Nutrition. 2004;**134**(10 Suppl):2820S-2825S; discussion 2853S. DOI: 10.1093/jn/134.10.2820S

[13] Hofmann F, Kreusch J, Maier KP, Munder PG, Decker K. The urea cycle in different types of macrophages. Biochemical Society Transactions. 1978;**6**(5):990-993. DOI: 10.1042/bst0060990

[14] Munder M. Arginase: An emerging key player in the mammalian immune system. British Journal of Pharmacology. 2009;**158**(3):638-651. DOI: 10.1111/j.1476-5381.2009.00291.x

[15] Forstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. European Heart Journal. 2012;**33**:829-837, 837a-837d. DOI: 10.1093/eurheartj/ehr304

[16] Forstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, et al. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension. 1994;**23**:1121-1131

[17] Mayer AK, Bartz H, Fey F, Schmidt LM, Dalpke AH. Airway epithelial cells modify immune responses by inducing an anti-inflammatory microenvironment. European Journal of Immunology. 2008;**38**(6):1689-1699. DOI: 10.1002/eji.200737936

[18] Munder M, Mollinedo F, Calafat J, Canchado J, Gil Lamaignere C, Fuentes JM, et al. Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood. 2005;**105**(6):2549-2556. DOI: 10.1182/blood-2004-07-2521

[19] Munder M, Eichmann K, Morán JM, Centeno F, Soler G, Modolell M. Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. Journal of Immunology. 1999;**163**(7):3771-3777

[20] Wilkins-Rodriguez AA, Escalona-Montaño AR, Becker I, Gutiérrez-Kobeh L. Regulation of the expression of nitric oxide synthase by *Leishmania mexicana* amastigotes in murine dendritic cells. Experimental Parasitology. 2010;**126**(3):426-434. DOI: 10.1016/j.exppara.2010.07.014

[21] Wanasen N, Soong L. L-arginine metabolism and its impact on host immunity against *Leishmania* infection. Immunology Research. 2008;**41**(1):15-25. DOI: 10.1007/s12026-007-8012-y

[22] Das PA, Lahiri A, Chakravorty D. Modulation of the arginase pathway in the context of microbial pathogenesis: A metabolic enzyme moonlighting as an immune modulator. PLoS Pathogens. 2010;**6**(6):e1000899. DOI: 10.1371/journal.ppat.1000899

[23] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nature Reviews Immunology. 2008;**8**(12):958-969. DOI: 10.1038/nri2448

[24] Modolell M, Corraliza IM, Link F, Soler G, Eichmann K. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. European Journal of Immunology. 1995;**25**(4):1101-1104. DOI: 10.1002/eji.1830250436

[25] Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. Journal of Biological Chemistry. 1994;**269**(19):13725-13728

[26] MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annual Review of Immunology. 1997;**15**:323-350. DOI: 10.1146/annurev.immunol.15.1.323

[27] Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: Competitive regulation by $CD4^+$ T cells correlates with Th1/Th2 phenotype. Journal of Immunology. 1998;**160**(11):5347-5354

[28] Xia Y, Dawson VL, Dawson TM, Snyder SH, Zweier JL. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proceedings of the National Academy of Sciences USA. 1996;**93**(13):6770-6774

[29] Bogdan C. Nitric oxide and the immune response. Nature Immunology. 2001;**2**(10):907-916. DOI: 10.1038/ni1001-907

[30] Stempin C, Tanos TB, Coso OA, Cerbán FM. Arginase induction promotes *Trypanosoma cruzi* intracellular replication in cruzipain-treated J774 cells through the activation of multiple signaling pathways. European Journal of Immunology. 2004;**34**:200-209. DOI: 10.1002/eji.200324313

[31] Stempin C, Giordanengo L, Gea S, Cerbán F. Alternative activation and

increase of *Trypanosoma cruzi* survival in murine macrophages stimulated by cruzipain, a parasite antigen. Journal of Leukocyte Biology. 2002;**72**:727-734. DOI: 10.1189/jlb.72.4.727

[32] Vincendeau P, Daulouède S. Macrophage cytostatic effect on *Trypanosoma musculi* involves an L-arginine-dependent mechanism. Journal of Immunology. 1991;**146**:4338-4343

[33] Bogdan C. Nitric oxide synthase in innate and adaptive immunity: An update. Trends in Immunology. 2015;**36**:161-178. DOI: 10.1016/j.it.2015.01.003

[34] Nogueira N, Cohn Z. *Trypanosoma cruzi*: Mechanism of entry and intracellular fate in mammalian cells. Journal of Experimental Medicine. 1976;**143**(6):1402-1420. DOI: 10.1084/jem.143.6.1402

[35] Metz G, Carlier Y, Vray B. *Trypanosoma cruzi* upregulates nitric oxide release by IFN-γ-preactivated macrophages, limiting cell infection independently of the respiratory burst. Parasite Immunology. 1993;**15**:693-699. DOI: 10.1111/j.1365-3024.1993.tb00584.x

[36] Pakianathan DR, Kuhn RE. *Trypanosoma cruzi* affects nitric oxide production by murine peritoneal macrophages. Journal of Parasitology. 1994;**80**:432-437

[37] De Muylder G, Daulouède S, Lecordier L, Uzureau P, Morias Y, Van DenAbbeele J, et al. A *Trypanosoma brucei* kinesin heavy chain promotes parasite growth by triggering host arginase activity. PLoS Pathogens. 2013;**9**:e1003731. DOI: 10.1371/journal.ppat.1003731

[38] Hai Y, Kerkhoven EJ, Barrett MP, Christianson DW. Crystal structure of an arginase-like protein from *Trypanosoma brucei* that evolved without a binuclear manganese cluster. Biochemistry. 2015;**54**:458-471. DOI: 10.1021/bi501366a

[39] Namangala B, De Baetselier P, Noël W, Brys L, Beschin A. Alternative versus classical macrophage activation during experimental African trypanosomiasis. Journal of Leukocyte Biology. 2001;**69**:387-396. DOI: 10.1189/jlb.69.3.387

[40] Gobert AP, Daulouede S, Lepoivre M, Boucher JL, Bouteille B, Buguet A, et al. L-Arginine availability modulates local nitric oxide production and parasite killing in experimental trypanosomiasis. Infection and Immunity. 2000;**68**:4653-4657. DOI: 10.1128/IAI.68.8.4653-4657.2000

[41] Raes G, Brys L, Dahal BK, Brandt J, Grooten J, Brombacher FG, et al. Macrophage galactose-type C-type lectins as novel markers for alternatively activated macrophages elicited by parasitic infections and allergic airway inflammation. Journal of Leukocyte Biology. 2005;**77**:321-327. DOI: 10.1189/jlb.0304212

[42] Fairlamb AH, Cerami A. Metabolism and functions of trypanothione in the Kinetoplastida. Annual Reviews Microbiology. 1992;**46**:695-729. DOI: 10.1146/annurev.mi.46.100192.003403

[43] Priotto G, Kasparian S, Mutombo W, Ngouama D, Ghorashian S, Arnold U, et al. Nifurtimoxeflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: A multicentre, randomised, phase III, non-inferiority trial. Lancet. 2009;**374**:56-64. DOI: 10.1016/S0140-6736(09)61117-X