Axenic *Leishmania amazonensis* Promastigotes Sense both the External and Internal Arginine Pool Distinctly Regulating the Two Transporter-Coding Genes

Emerson A. Castilho-Martins, Maria Fernanda Laranjeira da Silva, Marcos G. dos Santos, Sandra M. Muxel, Lucile M. Floeter-Winter*

Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo São Paulo, Brazil

Abstract

*Leishmania (L.) amazonensis* uses arginine to synthesize polyamines to support its growth and survival. Here we describe the presence of two gene copies, arranged in tandem, that code for the arginine transporter. Both copies show similar Open Reading Frames (ORFs), which are 93% similar to the *L. (L.) donovani* AAP3 gene, but their 5′ and 3′ UTR′s have distinct regions. According to quantitative RT-PCR, the 5.1 AAP3 mRNA amount was increased more than 3 times that of the 4.7 AAP3 mRNA along the promastigote growth curve. Nutrient deprivation for 4 hours and then supplemented or not with arginine (400 μM) resulted in similar 4.7 AAP3 mRNA copy-numbers compared to the starved and control parasites. Conversely, the 5.1 AAP3 mRNA copy-numbers increased in the starved parasites but not in ones supplemented with arginine (p<0.05). These results correlate with increases in amino acid uptake. Both Meta1 and arginase mRNAs remained constant with or without supplementation. The same starvation experiment was performed using a *L. (L.) amazonensis* null knockout for arginase (arg) and two other mutants containing the arginase ORF with (arg/ARG) or without the glycosomal addressing signal (arg/argASKL). The arg and the arg/argASKL mutants did not show the same behavior as the wild-type (WT) parasite or the arg/ARG mutant. This can be an indicative that the internal pool of arginine is also important for controlling transporter expression and function. By inhibiting mRNA transcription or/and mRNA maturation, we showed that the 5.1 AAP3 mRNA did not decay after 180 min, but the 4.7 AAP3 mRNA presented a half-life decay of 32.6+/- 5.0 min. In conclusion, parasites can regulate amino acid uptake by increasing the amount of transporter-coding mRNA, possibly by regulating the mRNA half-life in an environment where the amino acid is not present or is in low amounts.

Introduction

Leishmaniasis is a complex parasitic disease that currently affects about 12 million people and an estimated 2 million new cases per year [1]. It is caused by protozoa in the *Leishmania* genus, which has two distinct phases in its life cycle: the promastigote, an extracellular flagellate present at the gut of sand flies, and the amastigote that lives inside mononuclear phagocytes, mainly macrophages, in a vertebrate host.

Arginine is a key amino acid for macrophages because, being the substrate for inducible nitric oxide synthase (iNOS) to produce nitric oxide (NO), it is involved in the macrophage-defense response against pathogen infections. [2–8]. This amino acid is also a substrate for arginase, which catalyzes the production of urea and ornithine, a product important for polyamine pathway. This pathway is used by *Leishmania* to replicate and is essential for the parasite to establish infection [9–12]. It has largely been reported that macrophage or *Leishmania* modulation of arginine is responsible for parasite survival or its killing in the mammal host [5,13–19].

Membrane transporters, present in both *Leishmania* and macrophages control arginine uptake [20–24], to sustain NO production, macrophages increase their expression of the main arginine transporter (CAT2B), which is indicative that the internal pool of arginine is not sufficient to supply arginine to iNOS [25–27]. On the other hand, a high-affinity arginine transporter has been described in *L. (L.) donovani*. This transporter is LdAAP3, and it has 480 amino acids and 11 predicted trans-membrane domains [22]. With this transporter, *Leishmania* seems to have mechanisms of sensing arginine decreases and responding with increased arginine uptake [28]. Therefore, the arginine-uptake control appears to be an important limiting factor to parasite survival inside macrophages [17,29].

*Leishmania* has a polycistronic transcription, and the control of gene expression is mainly performed through protein levels and mRNA stability [30]. In this study, we evaluated the importance of arginine transporter mRNA levels on the physiology of arginine uptake in *L. (L.) amazonensis*. Our data indicated that these organisms control the arginine transporter expression by regulating the transporter-coding mRNA stability. We also
showed that the level of arginine transporter mRNA varies in promastigote development, and, using arginase-deficient mutants, we showed that possible changes in the internal arginine pool could be responsible for altering the transporter-coding mRNA levels.

Results
Characterization of the two L. (L.) amazonensis arginine transporter-coding DNA sequences
A DNA probe based on the AAP3 ORF sequence of L. donovani [22] was used to screen a L. (L.) amazonensis genomic-cosmid DNA library [31]. The partial DNA sequence of the selected cosmid revealed the presence of two copies in tandem from a putative homologous gene. The ORF regions of the two copies showed 93% similarity to the AAP3 ORF in L. donovani (not shown). A northern blot analysis showed the presence of two distinct mRNAs for the gene (5.1 kb and 4.7 kb) (not shown). We named these transcripts 4.7 AAP3 mRNA and 5.1 AAP3 mRNA. The mRNA identities were confirmed by sequencing RT-PCR products that were obtained using oligo-dT reverse transcription and primers based on the cosmid sequence. These sequences were deposited in GenBank with accession numbers of HQ912026 (5.1 AAP3) and HQ912027 (4.7 AAP3).

The ORFs of each copy, that are 98% identical were cloned into pYES2 plasmid and the recombinant plasmids were used to transform a Saccharomyces cerevisiae mutant lacking amino-acid transporter coding region: GAP1/ YHR039W (Material and Methods S1). The transformed yeast recovered the growth capacity in medium containing L-arginine confirming the transporter character of the protein encoded by those sequences (Figure S1).

The two transcribed copies of the gene presented different 5’ untranslated regions (5’UTRs). This allowed for the design of specific primers to differentially quantify each copy by quantitative reverse transcription PCR (qRT-PCR). The alignment of the 5’UTRs from the two copies, their differences and primers positions are shown in Figure S2.

Arginine uptake by L. (L.) amazonensis promastigotes correlates with the arginine transporter transcript abundance
It is known that L. donovani promastigotes are sensitive to arginine starvation, and they respond with an increase in both arginine transporter expression and transport rate [28]. However, it is not completely clear at which level of protein-expression regulation this control occurs. We performed an arginine starvation on mid-log and stationary L. (L.) amazonensis promastigotes for 4 h at 25°C and then evaluated arginine uptake. Initially, we could note that at time 0 that means the physiological condition in each phase the arginine uptake in mid-log phase parasites is lower than the one detected in the stationary phase parasites. When starved, the mid-log phase parasites showed an increase in the arginine uptake compared to the control parasites at time 0 (p<0.05). As this behavior was not detected in starved stationary phase parasites, we can conclude that stationary phase parasites do not respond to starvation (Figure 1A). However, the increase in arginine uptake was not observed when the mid-log phase parasites were incubated for the same time in the presence of arginine (400 μM) (Figure 1A), but the arginine uptake of the stationary phase parasites decrease, in relation to time 0, when incubated in the presence of arginine (Figure 1A). We also evaluated mRNA level at mid-log parasites, and the increase of arginine uptake correlated with an increase in the relative copy-number of the 5.1 AAP3 mRNA (Figure 1B, p<0.05), suggesting the existence of at least one pre-translational mechanism for controlling protein expression. Moreover, only the 5.1 AAP3 mRNA was sensitive to the amino acid starvation. No differences were observed in the 4.7 AAP3 mRNA copy-number or the mRNAs coding for arginase and Metal1, all normalized by the GAPDH mRNA copy-number (Figure 1B).

To evaluate differences in the amino acid-starvation response between mid-log and stationary parasites, we determined the amount of AAP3 mRNA from the 1st to the 10th day of a culture growth curve as described in methods. We compared the mRNA expression amounts of the 5.1 AAP3 mRNA and the 4.7 AAP3 mRNA normalized to GAPDH mRNA. The arginine transporter mRNA was increased more than 10 times in the stationary parasites compared to the log-phase parasites (Figure 2). Interestingly, both copies increased the mRNA level in the stationary phase, although the 5.1 AAP3 mRNA was at least 30 fold more abundant than the 4.7 AAP3 mRNA at the log-phase, reaching 100 fold in the stationary phase.

Arginine-transporter-coding transcripts stability are affected in axenic promastigotes experiencing arginine deprivation
Treatment with actinomycin and sinefungin causes an inhibition of transcription and splicing mRNA-maturation processes in the parasites [32,33]. RNA obtained from a time-course treatment of mid-log phase promastigotes with actinomycin D and sinefungin, preceded or not by 4 hours arginine starvation was used in qRT-PCR experiments, as described in Material and Methods. The 5.1 AAP3 mRNA showed no decay after 180 min of treatment in cells submitted to arginine starvation but the presence of the amino acid induces a degradation (Figure 3A), in contrast to the observed for both 4.7 AAP3 mRNA [half lives of 45.7 ± 5.4 min (+arg) / 27.7 ± 5.4 [-arg]] and GAPDH [half lives of 40.6 ± 2.1 min (+arg) / 30.0 ± 5.4 [-arg]] (Figure 3B and 3C). The qRT-PCR data were normalized by SSU rRNA copy-number, a RNA that is not sensitive to the inhibitor drugs (Figure 3D).

Figure 1. Amino acid starvation regulates the arginine transporter rate and 5.1 kb AAP3 mRNA level in L. (L.) amazonensis. A. Arginine uptake of mid-log and stationary phase L. (L.) amazonensis in non-starved parasites (black), 4-h starved parasites (white) and 4-h starved + 400 μM arginine parasites (gray). B. Total RNA of non-starved parasites (black), 4-h starved parasites (white) and 4-h starved + 400 μM arginine parasites (gray). RNA was used to prepare the cDNA, as described in Material and Methods. Equal amounts of cDNA were then used in qRT-PCR to determine the copy-number of both copies of the arginine transporter, arginase and Metal1. All determinations were normalized by GAPDH. Results of a representative experiment. Data are shown as the mean±S.E. (n = 3).
doi:10.1371/journal.pone.0027818.g001
The arginine transporter transcript abundance also assesses the sensing of the arginine internal pool: an output relying on L. amazonensis genetic manipulation targeting the arginase-coding gene.

To evaluate the influence of an internal pool of arginine on its uptake, we used a L. (L.) amazonensis arginase-null mutant (arg\(^-\)), which does not use arginine to produce ornithine and present higher amounts of arginine in their cytoplasm, requiring polyamines supplementation (Laranjeira da Silva, submitted). The mutant and WT parasites (5\(\times\)10\(^7\)/mL), at initial stationary phase, were submitted to amino acid starvation and then arginine uptake was evaluated. Although both parasites responded to amino acid starvation, the WT parasites presented greater arginine uptake than the arg\(^-\) (Figure 4). We performed the same assay using a knockout mutant that is genetically complemented with the arginase ORF (arg\(^-\)/ARG) showing a partial recovery in arginase activity (Laranjeira da Silva, submitted). Interestingly, this mutant also presented a partial recovery in arginine uptake, compared to the WT. On the other hand, the complemented mutant, that contained the arginase ORF without the correct glycosomal compartmentalization signal (arg\(^-\)/arg\(^{SKL}\)) and did not present any arginase activity (Laranjeira da Silva, submitted), showed a arginine uptake similar to the arg\(^-\) mutant (Figure 4).

**Discussion**

The data presented in this study show that L. (L.) amazonensis can control arginine uptake when promastigotes parasites are amino acid starved. These observations are similar to those made in L. donovani [28]. Adding to that data, we showed that the internal pool of the amino acid is also important to regulate the uptake. Furthermore, we showed that the higher concentration of one of the AAP3 transcripts is due to a stabilization process in the mature mRNA and not to an increase in the transcriptional rate or mRNA trans-splicing maturation. Most eukaryotes generally control their gene expression at the transcriptional level; however, Leishmania lacks this control mechanism because its transcription is polycistrionic [34]. Besides, there are no known RNA polymerase II

![Figure 2. mRNA quantification from the parasite culture-growth curve.](image)

![Figure 3. mRNA decay of L. (L.) amazonensis total mRNA.](image)
promoter regions that have binding sites for transcriptional regulatory factors. However, these organisms can control gene expression at the mRNA maturation level (poly-adenylation/trans-splicing coupled processes) or by changing mRNA half-lives in different conditions [30]. This digenic organism experiences different environmental conditions, such as pH, temperature and nutrient availability, when it cycles between invertebrate and mammalian hosts [35]. Our findings suggest a possible mechanism for the parasite to overcome the different requirements due to environmental changes that they will find in insect gut along their development, going from a nutrient rich media after insect blood meal through an deprived ambient until the next feed.

A possible physiological explanation for the presence of two copies of the AAP3 gene is that each copy could be differentially regulated according to the environmental conditions of the parasite’s differentiated stage. It is interesting that one of the arginine transporter mRNAs (4.7 AAP3 mRNA) presented the same typical decay behavior observed for GAPDH mRNA when promastigotes were treated with actinomycin and sinfinugin. The other transcript (5.1 AAP3 mRNA) remains stable even 180 min after blocking of transcription and trans-splicing. This could explain the reason why this copy presents at least 30-fold more copies than the 4.7 AAP3 mRNA. Previous reports show that the mRNA stability in these organisms is altered by amino acid starvation. This is possibly because we only observed changes in arginine uptake and arginase activity, and arginine uptake is crucial to parasite survival inside macrophages. In addition, the lower arginine uptake that occurred in response to starvation in the null mutants indicates a mechanism to increase arginine transporter mRNA. This may occur through detecting possible changes in the internal arginine pool because the disruption of one arginine pathway decreases responses to amino acid starvation, which is also described in L. donovani ornithine decarboxylase or spermidine synthase-null mutants [28].

Stationary phase WT parasites have an increased expression of the arginine transporter, in relation to mid-log phase parasites, but did not respond to amino acid starvation. On the other hand, stationary phase arg- mutant responds to arginine starvation like mid-log phase WT parasites (Figure 1A and Figure 4). A possible explanation is that the mutant uses less arginine present in the culture media, keeping a higher concentration compared to the WT growing media. The data suggests that the maximum expression of the transporter may have been already reached at WT stationary phase. Achieving maximum transporter expression may be a response to decreases in nutrient concentrations in the culture medium over time, and it may represent an adaptation to the low-nutrient availability found inside the fly mid-gut after blood digestion was completed. Another mechanism could be that nutrient depletion drives the modifications in promastigote parasites that induce differentiation in the infective stage [43]. Thus, arginine depletion may represent a signal to metacyclogenesis, although we did not observe changes in Meta1 mRNA due to amino acid starvation. This is possible because we only observed them for 4 hours.

The results presented in this study lead us to conclude that arginine uptake is controlled by transporter-coding mRNA levels in L. (L.) amazonensis. They also suggest a mechanism that senses internal arginine concentrations and controls arginine uptake by increasing arginine transporter expression. This may represent a part of metacyclogenesis for achieving the infective stage.

Materials and Methods

Organisms

Wild-type (WT) promastigotes from the L. (L.) amazonensis strain MHOM/BR/1973/M2269 and three arginase mutants [arg-, arg / ARG and arg / argΔSKL] (Laranjeira da Silva, submitted) were maintained at 25°C by inoculating 5×10⁶ parasites in M199 medium (10 mL) supplemented with 10% fetal calf serum (FGS-Invitrogen - Carlsbad, USA) in 25 cm² tissue culture flasks. The supplemented media was changed every 7 days. Arginine-null mutants were also supplemented with putrescine (50 μM).

The mRNA expression and arginine uptake were evaluated along the growth curve by maintaining the parasites at log phase by sub-culturing them every 24 h with the same initial cell ratio (5×10⁶ parasites/mL), as previously described [44].
AAP3 gene cloning

Based on sequences described by Shaked-Mishan et al. [22], we amplified the *L. (L.) donovani* AAP3 ORF from genomic DNA, purified as described previously [45]. We used this amplicon as a template to construct a radioactive probe (0.1 mCi). This probe was constructed using a 32P-dCTP (10 mCi/mL; 3,000 Ci/mmol; GE Healthcare, UK) and Amersham Megaprime DNA Labeling Systems (GE Healthcare, UK) following manufacturer’s standard protocol. This probe was used to screen a *L. (L.) amazonensis* cosmid library [31] (kindly provided by S.R. Uliana, ICB-USP), and cosmid DNA was isolated by alkaline lysis [45]. Sequencing of the cosmid DNA was performed by the Sanger dideoxy protocol as described previously [45].

RNA purification, cDNA synthesis and qRT-PCR

RNA was extracted with Trizol Reagent (Invitrogen) using the manufacturer’s protocol. Reverse transcription was performed with a random primer protocol (Fermentas, M-MuLV RT) using total RNA (2 µg). The obtained cDNA was diluted in water and used in quantitative Real-Time PCR (qRT-PCR) with primers (Table 1) designed to differentially amplify the 5’ UTR region of the two copies of the *AAP3* gene. The expected products were cloned and sequenced to validate the PCR. A protocol described by Daslyuk et al. [28] was used to starve the promastigotes, with one difference: the parasites were kept for 4 h at 25°C. Controls were performed at time 0 by putting the parasites on ice or incubating the parasites in the presence of arginine (400 µM).

Statistical data analysis

Statistical significance was determined by Student’s t test (p<0.05).

Supporting Information

Material and Methods

Material and Methods S1 Methodological description of genetic complementation of yeast mutant. (DOC)

Figure S1 Genetic complementation of yeast mutant certifies the AAP functional character of *L. amazonensis* amino acid transporter ORFs. pYES2 plasmid DNA carrying 5.1 AAP3 and 4.7 AAP3 ORFs complemented *Saccharomyces cerevisiae* mutant GAP1/YHR039W. The transformed yeast clones, ORF 4.7 and ORF 5.1, obtained as described in Material and Methods S1 were able to grow in the minimal medium supplemented with 1 mg/m of L-arg (A). The mutant transformed with the recipient plasmid pYES2 alone, only grew in a medium containing ammonium as (NH4+) as nitrogen source (B).

Figure S2 Sequence alignment of the 5’UTRs from 5.1 AAP3 mRNA and 4.7 AAP3 mRNA. Cyan-colored box represents the Spliced-Leader RNA sequence. Gray box represents the ORF beginning. Blue boxes show the primers for amplifying the 5.1 AAP3 mRNA, and the green boxes show the primers for amplifying the 4.7 AAP3 mRNA.

Acknowledgments

We thank Dr. Lisvane Silva Paes and Profs. Luis Eduardo Soares Netto, Ariel M. Silber for helping with yeast experiments, and Ricardo de Andrade Zampieri for technical assistance.

AAP3 gene cloning.

Based on sequences described by Shaked-Mishan et al. [22], we amplified the *L. (L.) donovani* AAP3 ORF from genomic DNA, purified as described previously [45]. We used this amplicon as a template to construct a radioactive probe (0.1 mCi). This probe was constructed using a 32P-dCTP (10 mCi/mL; 3,000 Ci/mmol; GE Healthcare, UK) and Amersham Megaprime DNA Labeling Systems (GE Healthcare, UK) following manufacturer’s standard protocol. This probe was used to screen a *L. (L.) amazonensis* cosmid library [31] (kindly provided by S.R. Uliana, ICB-USP), and cosmid DNA was isolated by alkaline lysis [45]. Sequencing of the cosmid DNA was performed by the Sanger dideoxy protocol as described previously [45].

RNA purification, cDNA synthesis and qRT-PCR

RNA was extracted with Trizol Reagent (Invitrogen) using the manufacturer’s protocol. Reverse transcription was performed with a random primer protocol (Fermentas, M-MuLV RT) using total RNA (2 µg). The obtained cDNA was diluted in water and used in quantitative Real-Time PCR (qRT-PCR) with primers (Table 1) designed to differentially amplify the 5’ UTR region of the two copies of the *AAP3* gene. The expected products were cloned and sequenced to validate the PCR. A protocol described by Daslyuk et al. [28] was used to starve the promastigotes, with one difference: the parasites were kept for 4 h at 25°C. Controls were performed at time 0 by putting the parasites on ice or incubating the parasites in the presence of arginine (400 µM).

Statistical data analysis

Statistical significance was determined by Student’s t test (p<0.05).

Supporting Information

Material and Methods S1 Methodological description of genetic complementation of yeast mutant. (DOC)

Figure S1 Genetic complementation of yeast mutant certifies the AAP functional character of *L. amazonensis* amino acid transporter ORFs. pYES2 plasmid DNA carrying 5.1 AAP3 and 4.7 AAP3 ORFs complemented *Saccharomyces cerevisiae* mutant GAP1/YHR039W. The transformed yeast clones, ORF 4.7 and ORF 5.1, obtained as described in Material and Methods S1 were able to grow in the minimal medium supplemented with 1 mg/m of L-arg (A). The mutant transformed with the recipient plasmid pYES2 alone, only grew in a medium containing ammonium as (NH4+) as nitrogen source (B).

Figure S2 Sequence alignment of the 5’UTRs from 5.1 AAP3 mRNA and 4.7 AAP3 mRNA. Cyan-colored box represents the Spliced-Leader RNA sequence. Gray box represents the ORF beginning. Blue boxes show the primers for amplifying the 5.1 AAP3 mRNA, and the green boxes show the primers for amplifying the 4.7 AAP3 mRNA.

Acknowledgments

We thank Dr. Lisvane Silva Paes and Profs. Luis Eduardo Soares Netto, Ariel M. Silber for helping with yeast experiments, and Ricardo de Andrade Zampieri for technical assistance.

Table 1. Primer sequences used in PCR reactions and the amplified products.

| Name         | Sequence (5’-3’)                  | Used to                  |
|--------------|-----------------------------------|--------------------------|
| TArgSU1KF    | GGT CCC CGA TAC ACA CAT TC        | Amplify 5’UTR of 5.1 mRNA |
| TArgSU1KR    | GTC TCC CGT TTT GCA AGA GA        |                          |
| TArgSu500bf  | ACC ATT GTG GGT TAG TTA TAG ATC C  | Amplify 5’UTR of 4.7 mRNA |
| TArgSu500br  | CAA GAT CGC TAG CAG TGG AG         |                          |

Table 1. Primer sequences used in PCR reactions and the amplified products.
Author Contributions
Conceived and designed the experiments: EC-M MLdS MdS LF-W. Performed the experiments: EC-M MLdS MdS SM. Analyzed the data: EC-M MLdS MdS LF-W. Wrote the paper: EC-M MLdS MdS LF-W.

References
1. Dejeux P (2004) Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 27: 305–318.
2. Murray HW (1992) Cell-mediated immune response in experimental visceral leishmaniasis - II: Oxygen-dependent killing of intracellular Leishmania donovani amastigotes. Journal of Immunology 149: 531–537.
3. Murray HW, Cartelle DM (1985) Killing of intracellular Leishmania donovani by human mononuclear phagocytes. Evidence for oxygen-dependent and - independent leishmanicidal activity. J Clin Invest 72: 32–44.
4. Murray HW, Sazoo-Sudel A, Wellner D, Oca MJ, Granger AM, et al. (1989) Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages. Infect Immun 57: 1449.
5. Liew FY, Millott S, Parkinson C, Palmer RM, Moncada S (1990) Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J Immunol 144: 4794–4797.
6. Assreuy J, Conha PQ, Eppielein M, Noerhina-Dutra A, O'Donnell CA, et al. (1994) Production of nitric oxide and oxygen intermediates by activated macrophages and killing of Leishmania major. Eur J Immunol 24: 672–676.
7. Liew FY, Xin D, Chan WL (1999) Immune effector mechanism in parasitic infections. Immunol Lett 65: 101–104.
8. Murray HW, Nathan CF (1999) Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral Leishmania donovani. J Exp Med 189: 741–746.
9. Mubkhopadhyay R, Mathubala R (1995) Leishmania donovani: cellular control of ornithine decarboxylase in promastigotes. Int J Biochem Cell Biol 27: 947–952.
10. Fairlamb AH, Cerrani A (1992) Metabolism and functions of trypanothione in the Kinetoplastida. Annu Rev Microbiol 46: 695–729.
11. Yoshida N, Camargo EP (1978) Ureotelism and ammonotelism in trypanosomatids. J Protozool 25: 622–628.
12. Murray HW, Cartelle DM (1987) Killing of intracellular Leishmania donovani by human mononuclear phagocytes. Evidence for oxygen-dependent and - independent leishmanicidal activity. J Clin Invest 72: 32–44.
13. Roach TL, Kiderlen AF, Blackwell JM (1991) Role of inorganic nitrogen oxides in the development of Leishmania parasites. Immunol Rev 120: 165–187.
14. Iniesta V, Gomez-Nieto LC, Corraliza I (2001) The inhibition of arginase by reactive nitrogen versus oxygen intermediates in the killing of intracellular Leishmania. J Immunol 166: 267–273.
15. Fairlamb AH, Cerrani A (1992) Metabolism and functions of trypanothione in the Kinetoplastida. Annu Rev Microbiol 46: 695–729.
16. Murray HW, Cartelle DM (1987) Killing of intracellular Leishmania donovani by human mononuclear phagocytes. Evidence for oxygen-dependent and - independent leishmanicidal activity. J Clin Invest 72: 32–44.
17. Liew FY, Millott S, Parkinsson C, Palmer RM, Moncada S (1990) Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J Immunol 144: 4794–4797.
18. Assreuy J, Conha PQ, Eppielein M, Noerhina-Dutra A, O'Donnell CA, et al. (1994) Production of nitric oxide and oxygen intermediates by activated macrophages and killing of Leishmania major. Eur J Immunol 24: 672–676.
19. Liew FY, Xin D, Chan WL (1999) Immune effector mechanism in parasitic infections. Immunol Lett 65: 101–104.
20. Murray HW, Nathan CF (1999) Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral Leishmania donovani. J Exp Med 189: 741–746.
21. Mubkhopadhyay R, Mathubala R (1995) Leishmania donovani: cellular control of ornithine decarboxylase in promastigotes. Int J Biochem Cell Biol 27: 947–952.
22. Fairlamb AH, Cerrani A (1992) Metabolism and functions of trypanothione in the Kinetoplastida. Annu Rev Microbiol 46: 695–729.
23. Yoshida N, Camargo EP (1978) Ureotelism and ammonotelism in trypanosomatids. J Protozool 25: 622–628.
24. Murray HW, Cartelle DM (1987) Killing of intracellular Leishmania donovani by human mononuclear phagocytes. Evidence for oxygen-dependent and - independent leishmanicidal activity. J Clin Invest 72: 32–44.
25. Fairlamb AH, Cerrani A (1992) Metabolism and functions of trypanothione in the Kinetoplastida. Annu Rev Microbiol 46: 695–729.
26. Yoshida N, Camargo EP (1978) Ureotelism and ammonotelism in trypanosomatids. J Protozool 25: 622–628.
27. Murray HW, Cartelle DM (1987) Killing of intracellular Leishmania donovani by human mononuclear phagocytes. Evidence for oxygen-dependent and - independent leishmanicidal activity. J Clin Invest 72: 32–44.
28. Liew FY, Millott S, Parkinsson C, Palmer RM, Moncada S (1990) Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J Immunol 144: 4794–4797.
29. Assreuy J, Conha PQ, Eppielein M, Noerhina-Dutra A, O'Donnell CA, et al. (1994) Production of nitric oxide and oxygen intermediates by activated macrophages and killing of Leishmania major. Eur J Immunol 24: 672–676.
30. Liew FY, Xin D, Chan WL (1999) Immune effector mechanism in parasitic infections. Immunol Lett 65: 101–104.
31. Murray HW, Nathan CF (1999) Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral Leishmania donovani. J Exp Med 189: 741–746.
32. Mubkhopadhyay R, Mathubala R (1995) Leishmania donovani: cellular control of ornithine decarboxylase in promastigotes. Int J Biochem Cell Biol 27: 947–952.
33. Fairlamb AH, Cerrani A (1992) Metabolism and functions of trypanothione in the Kinetoplastida. Annu Rev Microbiol 46: 695–729.
34. Yoshida N, Camargo EP (1978) Ureotelism and ammonotelism in trypanosomatids. J Protozool 25: 622–628.
35. Murray HW, Cartelle DM (1987) Killing of intracellular Leishmania donovani by human mononuclear phagocytes. Evidence for oxygen-dependent and - independent leishmanicidal activity. J Clin Invest 72: 32–44.
36. Liew FY, Millott S, Parkinsson C, Palmer RM, Moncada S (1990) Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J Immunol 144: 4794–4797.
37. Assreuy J, Conha PQ, Eppielein M, Noerhina-Dutra A, O'Donnell CA, et al. (1994) Production of nitric oxide and oxygen intermediates by activated macrophages and killing of Leishmania major. Eur J Immunol 24: 672–676.
38. Liew FY, Xin D, Chan WL (1999) Immune effector mechanism in parasitic infections. Immunol Lett 65: 101–104.
39. Murray HW, Nathan CF (1999) Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral Leishmania donovani. J Exp Med 189: 741–746.
40. Mubkhopadhyay R, Mathubala R (1995) Leishmania donovani: cellular control of ornithine decarboxylase in promastigotes. Int J Biochem Cell Biol 27: 947–952.
41. Roach TL, Kiderlen AF, Blackwell JM (1991) Role of inorganic nitrogen oxides and tumor necrosis factor alpha in killing Leishmania donovani amastigotes in gamma interferon- lipopolysaccharide-activated macrophages from Lhs and Lhs congenic mouse strains. Infect Immun 59: 3935–3944.
42. Evans TG, Reed SS, Hibb-Jr JB (1996) Nitric oxide production in murine leishmaniasis: correlation of progressive infection with increasing systemic synthesis of nitric oxide. Am J Trop Med Hyg 54: 486–489.
43. Liew FY, Wei XQ, Proudfout L (1997) Gyskines and nitric oxide as effector molecules against parasitic infections. Philos Trans R Soc Lond B Biol Sci 352: 1311–1315.
44. Iniesta V, Gomez-Nieto LC, Corraliza I (2001) The inhibition of arginase by N(omega)-hydroxy-arginine controls the growth of Leishmania inside macrophages. J Exp Med 193: 777–784.
45. Kropf P, Fuentes JM, Fahurich E, Arpa I, Heralh S, et al. (2005) Arginase and polyamine synthase are key factors in the regulation of experimental leishmaniasis in vivo. FASEB J 19: 1009–1012.
46. Giraldo MV, Silber AM, Pereira CA, Uliana SR (2005) Characterization of a novel motif in amino acid permease genes from Leishmania. Biochem Biophys Res Commun 325: 153–166.