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Review

Role of trypanosomatid's arginase in polyamine biosynthesis and pathogenesis

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ABSTRACT

L-Arginine is one of the precursor amino acids of polyamine biosynthesis in most living organisms including *Leishmania* parasites. L-Arginine is enzymatically hydrolyzed by arginase producing L-ornithine and urea. In *Leishmania* spp. and other trypanosomatids a single gene encoding arginase has been described. The product of this gene is compartmentalized in glycosomes and is the main source of L-ornithine for polyamine synthesis in these parasites. L-Ornithine is substrate of ornithine decarboxylase (ODC) – one of the key enzymes of polyamine biosynthesis and a validated target for therapeutic intervention – producing putrescine, which in turn is converted to spermidine by condensing with an aminopropyl group from decarboxylated S-adenosylmethionine. Unlike trypanosomatids, mammalian hosts have two arginases (arginase I and II), which have close structural and kinetic resemblances, but localize in different subcellular organelles, respond to different stimuli and have different immunological reactivity. Arginase I is a cytosolic enzyme, mostly expressed in the liver as a pivotal component of the urea cycle, providing in addition L-ornithine for polyamine synthesis. In contrast, arginase II localizes inside mitochondria and is metabolically involved in L-proline and L-glutamine biosynthesis. More striking is the role played by L-arginine as substrate for nitric oxide synthase (NOS2) in macrophages, the main route of clearance of many infectious agents including *Leishmania* and *Trypanosoma cruzi*. In infected macrophages L-arginine is catalysed by NOS2 or arginase, contributing to host defense or parasite killing, respectively. A balance between NOS2 and arginase activities is a crucial factor in the progression of the *Leishmania* infection inside macrophages. In response to T-helper type 2 (Th2) cytokines, resident macrophages induce arginase I inhibiting NO production from L-arginine, thereby promoting parasite proliferation. Conversely, the response to T-helper type 1 (Th1) cytokines is linked to NOS2 induction and parasite death. Moreover, induction of any of these enzymes is accompanied by suppression of the other. Specifically, arginase reduces NO synthesis by substrate depletion, and N^{ω}-hydroxy-L-arginine, one of the intermediates of NOS2 catalysis, competitively inhibits arginase activity.

In spite of abundant data concerning arginases in mammals as well their involvement in parasite killing, there are very few papers regarding the actual role of arginase in the parasite itself. This review is an update on the recent progress in research on leishmanial arginase including the role played by this enzyme in the establishment of infection in macrophages and the immune response of the host. A comparative study of arginases from other kinetoplatids is also discussed.

Contents

Abbreviations: ODC, ornithine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; ADC, arginine decarboxylase; NO, nitric oxide; NOS2, inducible nitric oxide synthase; Th1, T-helper type 1 lymphocytes; Th 2, T-helper type 2 lymphocytes; NOHA, Nω-hydroxy-L-arginine; R&D, research and development; NTD, neglected tropical diseases; HIV, human immunodeficiency virus; AIDS, acquire immunodeficiency syndrome; ROS, reactive oxygen species; RNOS, reactive nitrogen oxidized species; $ONOO^-$, peroxynitrite; CAT, cationic amino acid transporter; y^+, basic amino acid transporter; LPG, lipophosphoglycan; gp63, glycoprotein 63; PS, phosphatidyl serine; Trp, trypanothione; fPPG, filamentous proteophosphoglycan.

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1. The dimension of the problem

The aetiological agents of the so-called neglected tropical diseases (NTD) – malaria, cryptosporiodiosis, sleeping sickness, Chagas disease and leishmaniases, among others – are unicellular protozoan parasites the management of which is based on prevention and chemotherapy since no effective vaccines are currently available. At present, antiparasitic chemotherapy is expensive, has multiple adverse effects and, moreover, generates no profits for pharmaceutical companies, which accordingly concentrate their R&D in more profitable markets [1]. Leishmaniases are a complex of NTDs prevalent on four continents and in 88 countries 66 of which are in the Old World. The severity of these diseases has led to be considered as a priority by WHO within the "Special Programme for Research and Training in Tropical Diseases" (http://apps.who.int/tdr/). They are classified according to the organs and tissues damaged, the species involved and their geographic location: (i) cutaneous leishmaniasis is a mild presentation producing skin lesions that heal spontaneously (it is caused by *L. tropica* and *L. major* in countries of the Old World, and by *L. mexicana* and *L. amazonensis* in Central and South America); (ii) mucocutaneous leishmaniasis is a more severe and deforming presentation caused by *L. braziliensis* complex in countries of the New World; and (iii) visceral leishmaniasis is the most dangerous, often fatal, presentation of the disease producing swelling of the spleen, liver, bone marrow and lymph nodes (it is caused by *L. donovani* and *L. infantum* in people living in Old World countries and by *L. chagasi* in America) [2–4]. An estimate of the global prevalence of leishmaniases is of 12 million people affected, with an annual incidence of 1.5–2 million new cases of cutaneous leishmaniasis and 500,000 of visceral leishmaniasis (http://www.who.int/leishmaniasis/burden/en/). However, available official data underestimate this reality due to several limiting factors: (a) many cases are not diagnosed or not reported; (b) most official data are obtained solely from passive detection of cases; and (c) the number of people infected, but asymptomatic, is much greater than the number of overt clinical cases [5].

American trypanosomiasis, Chagas disease, affects about 8 million people (with ca. 50,000 new cases every year), of whom 30–40% either have or will develop severe chronic cardiomyopathy. Unlike leishmaniases, Chagas disease is confined to Central and South America where it has spread to 18 countries [6,7]. However, despite the low risk of zoonotic transmission, the number of Chagas disease patients in European countries has grown rapidly in recent years due to the migratory movements from endemic countries [8]. Humans contract Chagas disease when they are exposed to faeces of triatomine bugs (Order: Hemiptera). During the acute phase, the few days after infection, trypomastigotes of *Trypanosoma cruzi* invade the bloodstream and multiply inside host macrophages; myocarditis [9–11] and meningoencephalitis [12], especially in infants and immunosuppressed patients, are lesions associated with this phase. Chronic disease may appear many years later affecting one third of the patients who survive the acute phase. During this later phase, the parasite disappears from the bloodstream invading non proliferating cells particularly in the heart and smooth muscle [13]. Indeed, the heart is the organ that is most affected and cardiomyopathy frequently develops, congestive heart failure being the most common cause of death [14].

Since the early 1990s, these diseases have ceased to be restricted to poor, underdeveloped or developing countries, and have become a growing source of concern in countries of the so-called "First World". Large cities, surrounded by overcrowded residential areas, lacking adequate sanitary conditions, are optimal for proliferation of the disease [15,16]. In the case of leishmaniases, global warming is playing a role in spreading vectors to geographic areas where they were traditionally absent [17]. In addition, in the case of Chagas disease, despite the vector being absent in Europe and North America, migratory movements are producing a growing incidence of this disease [18]. Furthermore, HIV-*Leishmania* co-infections are being recognized as an emerging disease in Southern European countries, where 25–70% of adults with visceral leishmaniasis are co-infected with HIV [19,20]. Both pathogens seem to have a synergistic and mutually enhancing effect on infection by the other, resulting in greater immunosuppression and a more rapid progression of both diseases. The prevalence of leishmaniasis as opportunistic disease was as high as 1.5–9% of patients with AIDS mostly in Spain, France, Italy and Portugal, before the introduction of anti-retroviral therapy [21].

Nowadays, it is well known how the products of macrophage elicitation act on *Leishmania* and *T. cruzi* amastigotes, but it is less clear whether parasite L-arginine metabolism does or does not promote pathogen development during infection. In this review, we discuss the metabolism of L-arginine in the parasite, its differences with respect to that in the host and their interrelationship, as well as the latest results obtained with arginase null mutants in trypanosomatids and the potential of arginase as a therapeutic target.

2. Arginine metabolism in mammals

L-Arginine is a semi-essential amino acid for adult humans that, despite the existence of its own biosynthetic pathway, should be incorporated into host diet to avoid nutritional diseases [22]. Specifically, L-arginine is involved in several metabolic pathways, including those responsible for the synthesis of nitric oxide (NO), agmatine, glutamate, creatine, and urea. Together with other nitric oxide synthase (NOS) (EC: 1.14.13.39) isoforms (nNOS in neuronal tissue, and eNOS in endothelial cells), inducible NOS (iNOS) is found in various different cell types in the immune system [23–25] and also in cardiomyocytes [26]. NOS is an oxidoreductase responsible for the synthesis of NO from the terminal nitrogen atom of L-arginine in the presence of NADPH and oxygen, producing L-citrulline. The reaction occurs via two successive reactions; a monooxygenase that generates the intermediate N^{ω}-hydroxy-L-arginine (NOHA) and a further hydrolysis producing L-citrulline, NO and the superoxide radical which leads to the formation of peroxynitrite. iNOS generates both NO and superoxide ion (O_2^-) which leads to peroxynitrite ($ONOO^-$) production [27]. NO, $ONOO^-$ and reactive oxygen species (ROS) such as O_2^- and H_2O_2, produced by NOS, are remarkably effective at destroying pathogens, but they also have high autotoxicity and, when present in excess, can cause disease [28].