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The role of metalloproteases in *Leishmania* species infection in the New World: a systematic review

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Abstract

This is a systematic review on the role of metalloproteases in the pathogenicity of the American tegumentary leishmaniasis (ATL) caused by New World Leishmania species. The review followed the PRISMA method, searching for articles in PubMed, EMBASE, LILACS and ISI Web of Science, by employing the following terms: 'leishmaniasis', 'cutaneous leishmaniasis', 'mucocutaneous leishmaniasis', 'diffuse cutaneous leishmaniasis', 'Leishmania' and 'metalloproteases'. GP63 of New World *Leishmania* species is a parasite metalloproteases involved in the degradation and cleavage of many biological molecules as kappa-B nuclear factor, fibronectin, tyrosine phosphatases. GP63 is capable of inhibiting the activity of the complement system and reduces the host's immune functions, allowing the survival of the parasite and its dissemination. High serological/tissue levels of host matrix metalloproteases (MMP)-9 have been associated with tissue damage during the infection, while high transcriptional levels of MMP-2 related with a satisfactory response to treatment. Host MMPs serological and tissue levels have been investigated using Western Blot, zymography, and Real Time polymerase chain reaction. GP63 detection characterizes species and virulence in promastigotes isolated from lesions samples using techniques mentioned previously. The monitoring of host MMPs levels and GP63 in Leishmania isolated from host samples could be used on the laboratory routine to predict the prognostic and treatment efficacy of ATL.

Introduction

Leishmaniasis is a neglected infectious disease considered a public health problem, especially in developing countries such as Argentina, Bolivia, Brazil and other Latin American countries. The disease is caused by protozoa of the genus *Leishmania* and can develop as an infection with cutaneous, mucosal, and visceral lesions. These clinical forms have their development according to the pathogenicity of infecting species and host's immunity. The World Health Organization estimates that, in the last 5 years, about 1.3 million cases of cutaneous leishmaniasis and 300 000 cases of visceral leishmaniasis (WHO, 2010) have been reported, with around 20 000 deaths per year (Brazil, Ministry of Health, 2017). The importance of American tegumentary leishmaniasis (ATL) lies in its high incidence and wide geographic distribution, as well as in the capacity of assuming forms that can determine destructive, disfiguring and disabling lesions, with great repercussion in the individual's psychosocial approach field as self-stigma and social stigma, until suicidal ideations (Gontijo *et al.* 2003; Bennis *et al.* 2017).

In the vector, the parasite survives in its salivary glands and the metacyclic promastigotes forms (flagellates) are inoculated into the individual through the bite on host's skin during the blood meal (WHO, 2010). Once in the skin, the parasites are phagocytosed by cells of the phagocytic mononuclear system and polymorphonuclear cells, transforming into amastigote form (not flagellated), which uses escape mechanisms to resist death, multiplying until cell lysis to infect new cells (WHO, 2010; Oliveira *et al.* 2014). Also, there is evidence that mononuclear cells play an essential role in the spread of infection to other tissues, and that enzymes from metalloprotease complexes in the host and parasite are involved in this process (Chang and Werb, 2001).

In general, metalloproteases are a group of enzymes necessary for cell proliferation, differentiation, extracellular remodeling, vascularization and cell migration in physiological and pathological processes (Chang and Werb, 2001). These are divided into two groups: matrix metalloproteases (MMPs), such as MMP-9 and MMP-2 produced mainly by host activated macrophages (Birkedal-Hansen *et al.* 1993); and disintegrin metalloproteases (ADAMS), such as glycoprotein 63 (GP63), parasite metalloprotease present in the membrane of the

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Leishmania genus. This GP has been increasingly studied for the understanding of progression and clinical forms of leishmaniasis (Chang and Werb, 2001; Cuervo *et al.* 2006; Gomez *et al.* 2009*a*; Shio and Olivier, 2010; Isnard *et al.* 2012; de Oliveira *et al.* 2013). Infection with *Leishmania* sp. stimulates host MMPs production by infected macrophages, leading to the breakdown of cell matrix, which promotes the dissemination of parasite to another site (Liarte *et al.* 2001; Carvalhal *et al.* 2004). The role of host MMP has been recently studied and it is related to the clinical development of visceral leishmaniasis in humans and dogs (Machado *et al.* 2010; Marangoni *et al.* 2011; Melo *et al.* 2011; de Oliveira *et al.* 2013; Gadisa *et al.* 2017). Host MMPs serological or tissue levels have been considered as new and simple biomarkers for monitoring the success of therapy and predicting favourable clinical outcome of human visceral leishmaniasis (de Oliveira *et al.* 2013; Gadisa *et al.* 2017).

The host and parasite metalloproteases represent an important role in the understanding of host immune response to infection, parasite pathogenicity, progression and cure of the disease. There are no recent systematic reviews on the role of parasite and host metalloproteases in tegumentary leishmaniasis, specifically those caused by the New World *Leishmania*. Therefore, this paper is a systematic literature review about the function of the enzymatic complex of metalloproteases in infection by New World *Leishmania* species causing ATL.

Methodology

Research strategy

The PRISMA (Beller *et al.* 2013) was used for making this systematic literature review. The strategy for research in the PubMed (US National Library of Medicine) consisted of the use of MeSH database tool, by employing the descriptors 'leishmaniasis', 'cutaneous leishmaniasis', 'mucocutaneous leishmaniasis', 'diffuse cutaneous leishmaniasis', '*Leishmania*' and 'metalloproteases'. The same terms were also used for the research in EMBASE databases, LILACS (Latin American and Caribbean Center on Information in Health Sciences) and ISI Web of Science.

Inclusion and exclusion criteria

Articles published from 01/01/2006 to 12/31/2016 have been included in this review. As inclusion criteria, only the articles that studied the role of metalloproteases in New World *Leishmania* species causative of ATL, following the WHO clasification (2011), were included. Thus, papers which studied metalloproteases in *Leishmania braziliensis, Leishmania amazonensis, Leishmania mexicana, Leishmania guyanensis, Leishmania panamensis, Leishmania lainsoni, Leishmania naiffi* and *Leishmania peruviana* were selected. Also, we included *Leishmania infantum*, responsible for ATL or atyphical CL. Therefore, studies that investigated other *Leishmania* species or species that do not cause human infection and visceral leishmaniasis were excluded from the review. Only articles that were published in Spanish, Portuguese or English and had available abstract were selected. Case reports, commentary, editorials, errata publications, interviews and guidelines were also excluded from this review.

Selection of articles

Independently, six researchers belonging to Group I (IGD, LSM, TFPM, BMC, QALN and JVPS) searched the databases for relevant abstracts to be included in the study. The selected articles were randomly distributed into Group I, which judged the articles based on the inclusion and exclusion criteria. The first step of article selection was the research for potential publications through the reading of the titles and abstracts, excluding this way the articles that did not attend to selection criteria. After the initial selection, the articles were chosen by pairs of judges to establish a consensus on articles to be selected. Publications in which four or more researchers agreed to include the article for the systematic review were included as definitive articles. If the consensus was found between three or two researchers, the articles were considered as indeterminate. Following the same consensus, those articles selected by one researcher were excluded from the review.

In a second step, three reviewers from Group II (JVPS, LSM and IGD) independently evaluated the full articles selected in the first stage. After consensus eligibility assessment of Group II researchers, the articles that were finally included in this systematic review were chosen.

Methodological quality and risk of bias evaluation

At the second moment of selection, the three researchers from Group II independently assessed the quality and risk of biases in each included study. The researchers also evaluated the selected articles according to data analysis, sample selection, animal species or cell type and methods for detection of metalloproteases. Regarding the biases, the articles were evaluated for their limitations pointed out by the researchers themselves and for those detected by the reviewers.

Extraction of data and analysis of results

The data extraction was carried out by structuring the relevant information of the articles in the form of tables. From the selected articles, the researchers in Group I extracted and tabulated the data. After the synthesis of the data in the form of tables, the judges of Group II made their corrections, checking the data extracted in the tables in two stages. The first stage consisted of the random distribution of publications among the judges of Group I, who promoted the first corrections. In the second stage, the judges of Group II did the correction in pairs and randomly.

Results and discussion

In this systematic review, 17 original articles were included (Fig. 1) after applying the inclusion and exclusion criteria and consensus among the researchers. Thirteen articles were related to the GP63 of New World *Leishmania* sp. species (Cuervo *et al.* 2006, 2008; Thiakaki *et al.* 2006; Mauricio *et al.* 2007; Gregory *et al.* 2008; Kulkarni *et al.* 2008, 2009; Tupperwar *et al.* 2008; Gomez *et al.* 2009*a*, *b*; Lima *et al.* 2009; Contreras *et al.* 2010; Petropolis *et al.* 2014) and four articles were related with host's MMP-9 (Maretti-Mira *et al.* 2011*a*, *b*; Fraga *et al.* 2012; Campos *et al.* 2014).

Leishmania sp. Glycoprotein 63

GP63 is a multifunctional enzyme related to complement components inactivation (Isnard *et al.* 2012), matrix extracellular degradation, contributing to cellular migration (Yao, 2010) and inhibition of natural killer cells activation, which contributes to infection persistence and disease progression (Shio and Olivier, 2010; Shio *et al.* 2012). In addition, the invasive and immunomodulatory role of GP63 and other proteases differ between the clinical form of the disease and the virulence of strains in patients from the same region (Cuervo *et al.* 2006).

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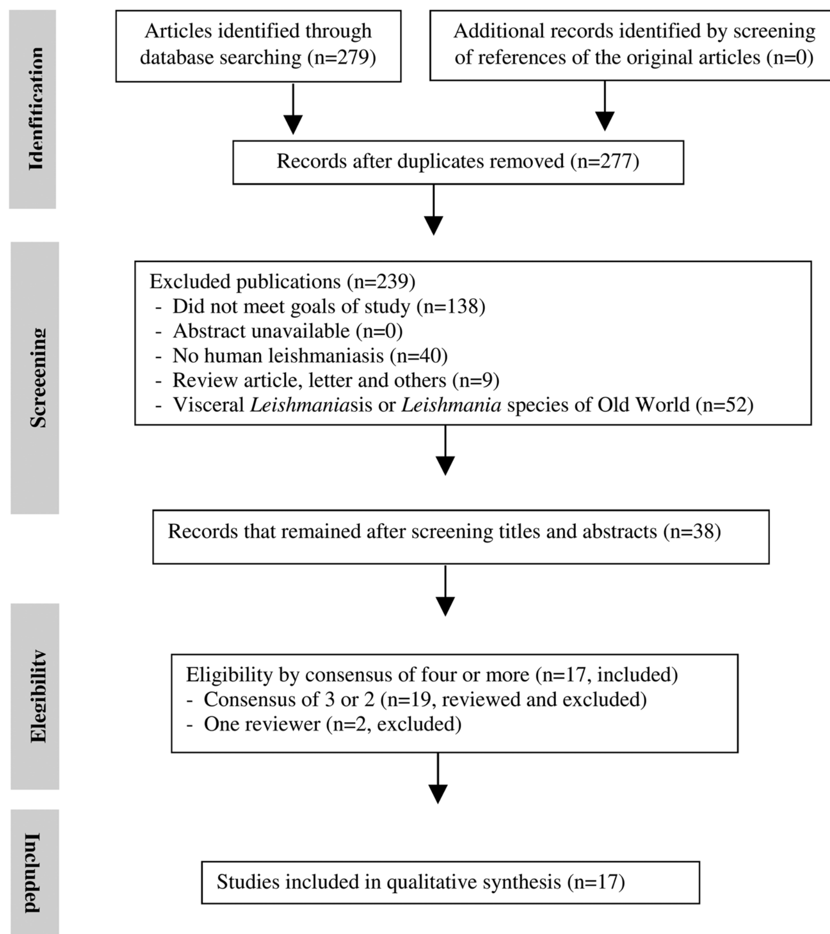

Fig. 1. PRISMA flowchart with selection criteria of the articles included in the systematic review.

GP63 studies characteristics

From the articles on GP63 ($n = 13$), four were conducted in Canada (Gregory *et al.* 2008; Gomez *et al.* 2009*a*, *b*; Contreras *et al.* 2010), four in Brazil (Cuervo *et al.* 2006, 2008; Lima *et al.* 2009; Petropolis *et al.* 2014), two in the USA (Kulkarni *et al.* 2008, 2009), one in the UK (Mauricio *et al.* 2007), one in India (Tupperwar *et al.* 2008), and one in Greece (Thiakaki *et al.* 2006). In 13 studies, 11 performed *in vitro* studies (Cuervo *et al.* 2006, 2008; Mauricio *et al.* 2007; Tupperwar *et al.* 2008; Gregory *et al.* 2008; Kulkarni *et al.* 2008, 2009; Gomez *et al.* 2009*a*, *b*; Lima *et al.* 2009; Contreras *et al.* 2010; Petropolis *et al.* 2014;), one was retrospective (Tupperwar *et al.* 2008) and one *in vivo* (Thiakaki *et al.* 2006). Six studies performed statistical analysis of their results (Thiakaki *et al.* 2006; Mauricio *et al.* 2007; Kulkarni *et al.* 2008; Gomez *et al.* 2009*b*; Lima *et al.* 2009; Petropolis *et al.* 2014), while five did not report the statistics (Cuervo *et al.* 2008; Gregory *et al.* 2008; Gomez *et al.* 2009*a*; Kulkarni *et al.* 2009; Contreras *et al.* 2010) and one did not perform the analysis (Cuervo *et al.* 2006; Tupperwar *et al.* 2008).

Different leishmaniasis clinical forms were investigated in the studies, with three studies addressing cutaneous form (Thiakaki *et al.* 2006; Lima *et al.* 2009; Petropolis *et al.* 2014), one studied cutaneous and mucosal forms (Cuervo *et al.* 2008), two studied cutaneous and visceral forms (Gomez *et al.* 2009*b*; Contreras *et al.* 2010), five studied different forms of disease manifestation (Cuervo *et al.* 2006; Mauricio *et al.* 2007; Gregory *et al.* 2008; Kulkarni *et al.* 2008; Tupperwar *et al.* 2008; Gomez *et al.* 2009*a*) and one investigated the diffuse cutaneous and cutaneous form (Kulkarni *et al.* 2009). The New World *Leishmania* studied were *L. amazonensis*, *L. mexicana*, *L. guyanensis*, *L. panamensis*, *L. braziliensis* and *Leishmania venezuelensis* (Table 1).

About the objectives of the selected articles, the role/mechanism and structure of GP63 in promastigotes (Cuervo *et al.* 2006, 2008; Gregory *et al.* 2008; Kulkarni *et al.* 2008; Lima *et al.* 2009), amastigotes (Kulkarni *et al.* 2008), animal model (BALB/c mice) (Thiakaki *et al.* 2006) and in 3D type I collagen matrices (Petropolis *et al.* 2014) were studied. Another study (Tupperwar *et al.* 2008) evaluated a new method to detect *Leishmania* sp. Metalloproteases, and two other studies investigated cellular (Cuervo *et al.* 2008) and gene localization (Kulkarni *et al.* 2009) of metalloproteases (Table 1).

Most of the studies used cell culture as the sample, while others used cell (Kulkarni *et al.* 2009) and nuclear (Gregory *et al.* 2008) extracts from promastigotes. Besides these, three articles used murine macrophages (Gregory *et al.* 2008; Kulkarni *et al.* 2008), one used embryonic fibroblasts (Gomez *et al.* 2009*b*) and others used recombinants proteins (Gomez *et al.* 2009*a*) and genes (Kulkarni *et al.* 2009). Many methods have been used to detect GP63: most of the studies used Western Blotting (Thiakaki *et al.* 2006; Cuervo *et al.* 2008; Kulkarni *et al.* 2008, 2009; Gomez *et al.* 2009*a*, *b*; Lima *et al.* 2009; Contreras *et al.* 2010) and the other methodologies employed were gelatin SDS-PAGE (Cuervo *et al.* 2006; Lima *et al.* 2009; Petropolis *et al.* 2014), complement lysis assays (Thiakaki *et al.* 2006), DNA sequencing (Mauricio *et al.* 2007), zymography (Cuervo *et al.* 2008; Kulkarni *et al.* 2008, 2009; Petropolis *et al.* 2014), indirect fluorescence microscopy (Gomez *et al.* 2009*a*; Kulkarni *et al.* 2009; Lima *et al.* 2009), immunoprecipitation (Gomez *et al.* 2009*a*; Contreras *et al.* 2010), evaluation of protein complex by Bradford method (Gomez *et al.* 2009*a*), electrophoretic mobility shift assay (EMSA) (Gregory *et al.* 2008), confocal

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Table 1. Characteristics of the selected articles on the role of GP63 in New World *Leishmania*sis

Author/ country	Design	Clinical manifestation	New world *Leishmania* species	Statistics	Objetive
Thiakaki *et al.* (2006)/Greece	Experimental *in vivo*	CL	*L. amazonensis*	Y	To demonstrate the role of GP63 in *L. amazonensis* infectivity *in vivo* by using the BALB/c mouse model.
Mauricio *et al.* (2007)/UK	*In vitro* assays	Different forms	*L. mexicana, L. amazonensis, L. guyanensis, L. panamensis, L. braziliensis, L. venezuelensis*	Y	To exploit gp63EXT gene to investigate the genetic structure and evolution of the *L. donovani* complex and other OW *Leishmania*.
Cuervo *et al.* (2006)/Brazil	*In vitro* assays	CL and ML	*L. (V.) braziliensis*	NR	The protease activities of various *L. (V.) braziliensis* strains, from Brazilian and Colombian patients presenting diverse clinical manifestations, were characterized and compared using whole promastigote extracts and extracellular secretions, through zymographic assays.
Cuervo *et al.* (2008)/Brazil	*In vitro* assays	CL and ML	*L. (V.) braziliensis*	NR	To investigate the cellular localization of these MMPs in promastigotes of *L. (V.) braziliensis* and whether these enzymes are related to the major surface protease of *Leishmania* sp.
Gregory *et al.* (2008)/Canada	*In vitro* assays	Different forms	*L. mexicana, L. braziliensis*	NR	To evaluate the effect of infection with promastigotes on macrophage NF-kB, and its role in chemokine regulation.
Kulkarni *et al.* (2008)/USA	*In vitro* assays	Different forms	*L. amazonensis*	Y	To show that a distinct parasite cell surface protein facilitates binding to FN and that both promastigotes and amastigotes of *Leishmania* spp degrade fibronectin.
Tupperwar *et al.* (2008)/ India	Retrospective	Different forms	*L. mexicana*, *L. braziliensis*	N	To develop a highly accurate and sensitive real-time PCR assay to detect and quantify *Leishmania* parasites.
Gomez et al. (2009*a*, *b*)/ Canada	*In vitro* assays	CL and VL	*L. mexicana*	Y	To determine the mechanism underlying protein tyrosine phosphatases modulation in *Leishmania* infection.
Hallé *et al.* (2009)/Canada	*In vitro* assays	Different forms	*L. mexicana*, *L. braziliensis*	NR	To describe distinctive effects of *Leishmania* infection on cell signaling in fibroblasts, which happens in part by altering the tyrosine phosphorylation state of several proteins and rearrangement of the actin cytoskeleton.
Kulkarni *et al.* (2009)/USA	*In vitro* assays	CL and DCL	*L. amazonensis*	NR	To study TcGP63 expression and localization.
Lima *et al.* (2009)/Brazil	*In vitro* assays	CL	*L. braziliensis*	Y	To report the production of metalloproteinases and cysteine peptidases in virulent and avirulent promastigote forms of *L. braziliensis*.
Contreras *et al.* (2010)/ Canada	*In vitro* assays	CL and VL	*L. mexicana*	NR	To investigate how GP63 contributes to the inactivation of AP-1 and the degradation of its subunits.
Petropolis *et al.* (2014)/ Brazil	*In vitro* assays	LC	*L. amazonensis*	Y	To characterize *L. amazonensis* invasion, migration, and matrix remodeling in 3D COL I matrices, focusing on the functions of *Leishmania* proteases.

CL, cutaneous *Leishmania*sis; ML, mucosal *Leishmania*sis; VL, visceral *Leishmania*sis; MMPs, metalloproteases; FN, fibronectin; NR, not related; gp63^{EXT}, class of GP63 genes; OW, Old World; NF-kB, nuclear factor kappa beta; PCR, polymerase chain reaction; TcGP63, recombinant GP63 of *T. cruzi*; AP-1, activated protein-1; SNAREs, soluble N-ethylmaleimide-sensitive factor attachment receptor; 3D COL I, 3D Collagen I.

microscopy (Gomez *et al.* 2009*b*; Contreras *et al.* 2010), confocal fluorescence microscopy (Cuervo *et al.* 2008), flow cytometry (Cuervo *et al.* 2008; Kulkarni *et al.* 2008; Lima *et al.* 2009) and polymerase chain reaction (PCR) (Tupperwar *et al.* 2008) (Table 2).

Role of new world Leishmania sp. (GP63)

Lima *et al.* showed that there are alterations in the GP63 expression according to the *Leishmania* strain and its ability to interact with macrophages, which confirms that the presence of peptidases is essential to genus virulence in nutrition, cell invasion, intracellular survival, differentiation and proliferation. The authors observed that the virulent strain of *L. braziliensis* presented a distinct and complex proteolytic profile, composed of metal and cysteine peptidases, whose hydrolytic activities were modulated by pH conditions. During analysis of the SDS-PAGE gel, the virulent strain of *L. braziliensis* showed a more complex peptidase pattern than the non-virulent, presenting four proteolytic bands at

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Table 2. Samples, methods of detection and conclusion of articles included on GP63 of New World *Leishmania* sp.

Reference	Sample	GP63 detection	Conclusion
Thiakaki *et al.* (2006)	Cell culture	Complement lysis assay; WB	Down-regulation of GP63 increases extracellular lysis of the mutants by complement, in the *in vivo* environment, and reduces their infection of MØs, resulting in a type 1 immune response seen at the site of inoculation and DLNs.
Mauricio *et al.* (2007)	Cell culture, genomic DNA	DNA sequencing	GP63EXT gene is an excellent marker for phylogenetics and genotyping of OW *Leishmania*. GP63 in using the host immune system, rather than evasion through antigenic variability. The gene conversion, between different alleles of the same GP63 gene class and between different members of this gene family, is a determinant factor in generating genetic diversity, but also in maintaining homozygosity and homogeneity between genes in the GP63 family.
Cuervo *et al.* (2006)/Brazil	Promastigote extracts and extracellular secretions	Zymography	Distinct profiles of MMPs were observed among the studied *L. (V.) braziliensis* strains. Differences among the microorganisms might be related to the geographical origin of the strains and/or to the clinical presentation.
Cuervo *et al.* (2008)	Cell culture	WB; zymographic and inhibition assays; CFM; FC.	Differential zymographic profiles of MMP exhibited by *L. (V.) braziliensis* isolates remain unaltered during prolonged *in vitro* culture, suggesting that the proteolytic activity pattern is a stable phenotypic characteristic of *Leishmania*.
Gregory *et al.* (2008)	MØs, nuclear extracts, *Leishmania* and synthetic oligonucleotides	EMSA; supershift	The infection with *Leishmania* promastigotes produces a modified form of NF-kB that is involved in chemokine induction; the pathogen can subvert a MØs regulatory pathways to alter NF-kB activity.
Kulkarni *et al.* (2008)	Mϕ and extracts, promastigotes, amastigotes, ECM proteins	FC; WB; Zymography	Both parasite stages of *Leishmania* sp. bind to and proteolytically degrade FN at the parasite surface and distantly through secreted proteases; the degraded forms of FN can influence the activation state of parasite-infected MØ.
Tupperwar *et al.* (2008)	Cells or tissue	Real-time PCR; conventional PCR;	The assay is sensitive enough to quantify parasite load in the absence of overt lesions and reveals a systemic distribution of *Leishmania*, which has implications for our understanding of the disease.
Gomez *et al.* (2009*a*, *b*)	Cell culture, BMMΦ; Knockout strains of *L. major*	WB; CM	GP63 is likely to be the protease that cleaves PTPs in *Leishmania*-infected MØs. To SHP-1, TCPTP and PTP1B are activated upon *Leishmania* infection by GP63-dependent cleavage, and we identify PTP1B as necessary for the initial stages of disease development. GP63 functions as a key *Leishmania* virulence factor, allowing its successful development as an obligate intracellular parasite.
Hallé *et al.* (2009)	Cell culture, extracts and recombinant proteins.	IFM IP; PCA, WB	Results establish that GP63 plays a central role in a number of host cell molecular events that likely contribute to the infectivity of *Leishmania*.
Kulkarni *et al.* (2009)	Cell culture, recombinant TcGP63, cell extracts	WB; IFM; zymography	The 61-kDa TcGP63 protein is likely to be a transmembrane protein and this may help mediate complement resistance, in conjunction with other factors, at some stages of *Trypanosoma cruzi*, which might protect this stage from complement-mediated lysis, as is the case for GP63 of *Leishmania*.
Lima *et al.* (2009)	Cell culture	SDS-PAGE; WB; IFM and FC	There are significant alterations in the peptidase expression in avirulent and virulent strains of *L. braziliensis* as well as their distinct susceptibility to proteolytic inhibitors and interaction capability with MØs, reaffirming the participation of peptidases in vital processes and virulence of *Leishmania* parasites.
Contreras *et al.* (2010)	Cell culture	EMSA; supershift assays; WB; IP; CM	Alterations in AP-1 activity can dramatically contribute to the down-regulation of innate immune functions observed during the early stages of *Leishmania* infection. This novel mechanism of evasion by *Leishmania* further demonstrates the complex negative regulatory mechanisms developed by the parasite, which has permitted its adaptation to the harsh intracellular environment leading to its survival and propagation within its mammalian host.
Petropolis *et al.* (2014)	Cell culture	SDS-PAGE; zymography	*Leishmania amazonensis* promastigotes release proteases and actively remodel their 3D environment, facilitating their migration.

WB, Western Blotting; DLN: IFM, immunofluorescence microscopy; IP, immunoprecipitation; PCA, protein complex analysis; EMSA, electrophoresis mobility shift assay; CM, confocal microscopy; CFM, confocal fluorescence microscopy; FC, flow cytometry; MMP, metalloproteases; PTP, protein tyrosine phosphatase; PCR, polymerase chain reaction; FN, fibronectin; BMMΦ, bone marrow–derived MØ; SDS-PAGE; DLNs, draining lymph nodes; $GP63^{EXT}$, class of GP63 genes; OW, Old World; MMP, metalloprotease; NF-kB, nuclear factor kappa-beta; FN, fibronectin; SHP-1, type of tyrosine phosphatase; TCPTP, T cell phosphatase; PTP1B, type of tyrosine phosphatase; TcGP63, recombinant GP63 of *T. cruzi*; AP-1, activated protein-1; VAMP8, SNAREs present on phagosomes; SNAREs, soluble N-ethylmaleimide-sensitive factor attachment receptor.

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acid and alkaline pH, while the non-virulent strain showed only one band, representing GP63 metalloprotease. All differences found in the production of proteases are possibly associated with the mechanism of infection and virulence of *L. braziliensis*. The comparison of proteolytic activity in strains of different virulence patterns may contribute to the study of the participation of these enzymes during infection in the host (Lima *et al.* 2009).

Cuervo *et al.* (2006) has studied the proteases activity of several strains of *L. braziliensis* using promastigotes and SDS-page. The authors tested different pHs and conditions and obtained distinct profiles of metalloproteases. They observed distinct zymographic patterns in *L. (V). braziliensis* strains isolated from cutaneous, mucosal and disseminated leishmaniasis from subjects coming from Brazil and Colombia. Brazilian isolates exhibited the same protease profile as the reference isolate, while isolates from Colombian patients showed a different pattern. While Brazilian isolates remained infective, the other did not show infectivity after the cryopreservation process. Differences among the microorganisms can be related to the geographical origin of the strains and/or to the clinical manifestations (Cuervo *et al.* 2006).

Real Time PCR technique has been shown as a sensitive assay for GP63 detection and quantification of the parasite load in the absence of lesions caused by *Leishmania* infection, and still allows verification of the systemic parasite distribution (Tupperwar *et al.* 2008). Also, in the methods used for GP63 identification, the $GP63^{EXT}$ gene (GP63 gene) may be an excellent marker for *Leishmania* genotyping using polymorphism in the recombinant domain of this gene. The authors also pointed out that from this genotyping it is possible to distinguish two species of the Old World, *L. infantum* and *Leishmania archibaldi*. The gene conversion between various alleles of the same GP63 gene class and between different members of this gene family is not only a determining factor in the generation of genetic diversity but also in the maintenance of homozygosis and heterozygosis among genes of the GP63 family (Mauricio *et al.* 2007).

GP63 may be related to host complement proteins resistance, protecting the parasite from complete lysis mediated by this system (Kulkarni *et al.* 2009). This pattern of proteolytic activity of GP63 is a stable phenotypic characteristic of *Leishmania* sp. (Cuervo *et al.* 2008). Gregory *et al.* demonstrated *in vitro* that promastigote forms of pathogenic species, including *L. mexicana* and *L. braziliensis*, promote the cleavage of the D-subunit of nuclear factor kappa-B (NF-κBp65RelA) in the cytoplasm of infected murine macrophages, in a process depending on GP63 protease activity (Gregory *et al.* 2008). NF-κB plays an important role in the onset of the innate response after the pathogen invasion in the host, regulating the expression of chemokines, cytokines, adhesion molecules and enzymes that produce secondary inflammatory mediators, which reduces the inflammatory response capacity (Calegari-Silva *et al.* 2009).

In both parasitic stages (promastigote and amastigote), GP63 from *L. amazonensis* binds and degrades host fibronectin, influencing the activation of macrophages infected by the parasite. Fibronectin is a multifunctional protein that has domains for interaction with matrix extracellular (ECM) components such as heparin, collagen and fibrin, exerting structural function in ECM and decreasing the activation capacity of macrophages (Kulkarni *et al.* 2008). Petropolis *et al.* studied the interaction between promastigotes and the extracellular matrices of the host, using a three-dimensional collagen matrix composed of type I collagen. It was observed that *L. amazonensis* promastigotes adhered strongly to the fibres of the matrix of collagen, altering the organization of these fibers, remodeling the entire matrix. Also, the parasite degrades collagen, and this degradation is reduced only in the presence of protease inhibitors. Incubation with anti-GP63 showed a small but significant reduction in the degradation of collagen fibres, suggesting that this metalloprotease is involved in this process. The group showed that the metalloproteases played an important role in the migration of 3D matrices so that fibre remodeling contributes to the invasion of the parasite (Petropolis *et al.* 2014).

GP63 is also responsible for cleaving tyrosine phosphatases (PTPs) in murine macrophages infected with *Leishmania*, such as SHP-1 (protein tyrosine phosphatase), TCPTP (T cell phosphatase) and PTP1B (protein tyrosine phosphatase). These enzymes are activated in the infection by a GP63-dependent cleavage process, and PTP1B is required for the development of *Leishmania*sis in its early stages. Therefore, in addition to the GP63 cleavage of kinases and transcriptional factors, it further modulates tyrosine phosphatases that regulate cell signaling pathways and may subvert macrophage signaling and its functions (Isnard *et al.* 2012). Thus, GP63 acts as an important virulence factor in the development of the intracellular parasite and establishment of the infection (Gomez *et al.* 2009*b*).

The *in vivo* reduction in the amount of GP63 from *Leishmania* sp. seems to be related to the increase of extracellular lysis by complement, decreasing infection in macrophages. Thiakaki *et al.* observed that single GP63 mutants exposed to cellular and humoral attacks *in vivo* were more susceptible and had greater difficulty in establishing intracellular parasitism. The reduction of GP63 also induced a Th1-like cellular immune response (T helper 1), and it is crucial in host defense against *Leishmania* infection (Thiakaki *et al.* 2006). The Th1 immune response is essential due to its inducting of the production of interferon-gamma (IFN-γ) and interleukin 12 (IL-12), which are responsible for the activation of macrophages to produce leishmanicidal substances, leading to the death of the parasite. The induction of Th1 cell subpopulations is known to inhibit the proliferation and differentiation of Th2 cells, which are involved in the production of cytokines that inhibit the activation of macrophages and promote inflammation. Thus, these cells promote parasite persistence and evolution of the infection (Espir *et al.* 2014). Thus, decreasing of GP63 would be necessary for the development of Th1 type immune response and to favor parasite death (Fig. 2).

In *Leishmania tarentolae* infection (not included for this review), GP63 was mentioned as responsible for the cleavage of vesicle-associated membrane proteins (VAMP8) and vesicular surface proteins (SNARE, Soluble NSF Attachment Receptor) present in phagocytic phagosomes. GP63 cleaves a subgroup of SNAREs, including VAMP8, and this phenomenon prevents the binding of the NADPH oxidase complex to phagosomes by altering the pH and degradation properties of phagosomes. Therefore, the presentation of the exogenous antigens of *Leishmania* by MHC molecules (Major Histocompatibility Complex) class I does not occur, so that there is reduced activation of T lymphocytes, persisting infection and significantly reducing parasitic death (Matheoud *et al.* 2013).

GP63 also contributes to the reduction of immune functions in the early stages of infection through changes in the activity of protein 1 (AP-1), a transcription factor that controls gene regulation in response to physiological and pathological stimuli, such as cytokines, growth factors, stress signals, apoptosis, bacterial and viral infections and oncogenic responses. Changes in AP-1 caused by GP63 during infection by *Leishmania* sp. allow an adaptation of the parasite to the harmful intracellular environment, leading to its survival and spreading of the infection through the organism (Contreras *et al.* 2010).

From these GP63 articles, three studies reported biases, such as lack of information on clinical data and injury time (Cuervo *et al.* 2008; Kulkarni *et al.* 2008; Lima *et al.* 2009). Petropolis *et al.* (2014) used the stationary phase promastigotes and not enriched

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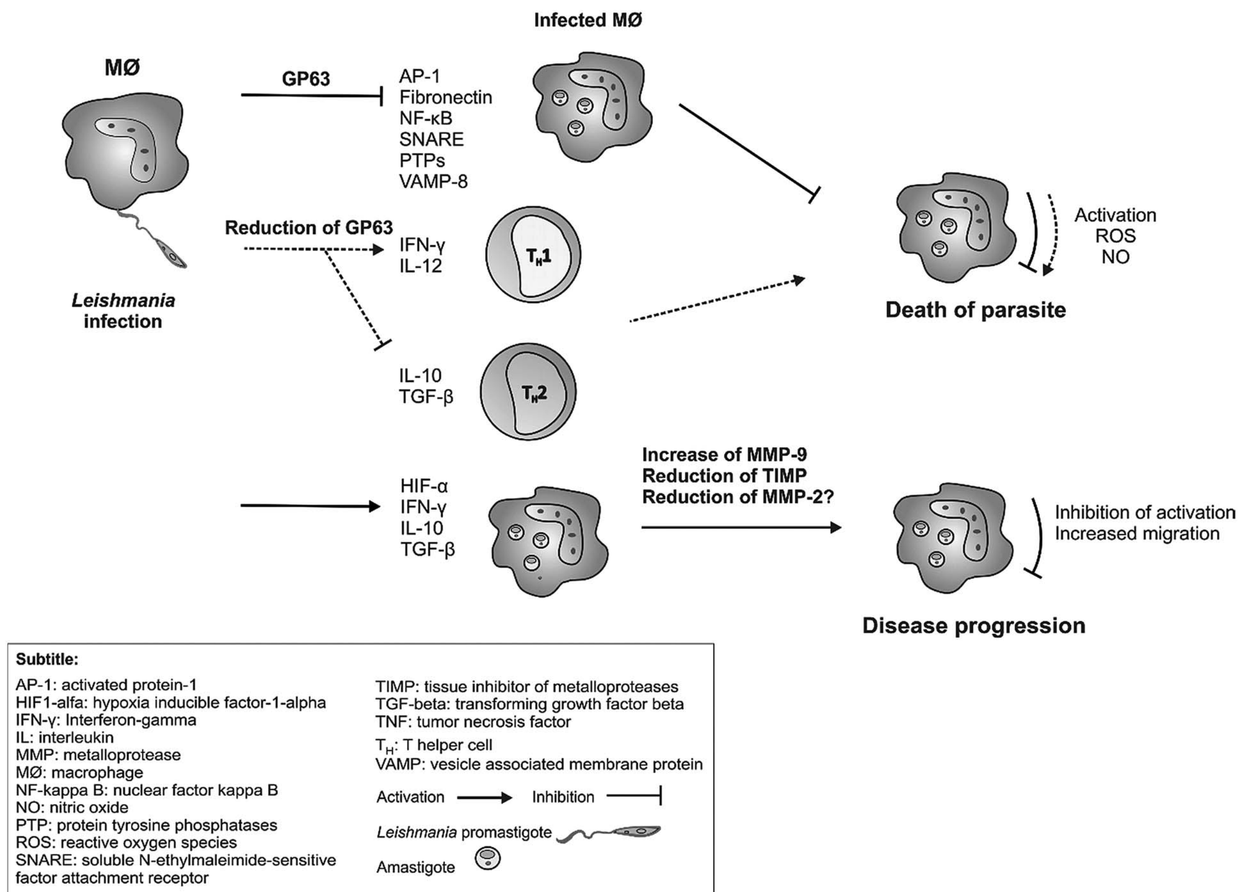

Fig. 2. Role of GP63 and MMP-9 metalloproteases in the induction of immune response during New World *Leishmania* sp. infection (hypothetical scheme). GP63 of *Leishmania* sp. promotes the cleavage of several molecules involved in the activation of macrophages, such as activated protein-1 (AP-1), fibronectin, nuclear factor kappa B (NF-kappa B), soluble N-ethylmaleimide-sensitive factor attachment receptor (SNAREs), protein tyrosine phosphatases (PTPs) and vesicle associated membrane protein 8 (VAMP-8). This inhibition of the macrophage does not lead to the production of microbicidal substances, such as oxygen reactive species (ROS) and nitric oxide (NO) and, consequently, does not lead to the death of the parasite. Some studies show that the reduction of GP63 is able to induce the production of cytokines interferon-gamma (IFN-γ) and interleukin (IL) 12 by Th1 cells and macrophages, which in turn activate the macrophages to produce the microbicidal substances with consequent death of the parasite. Furthermore, this reduction of GP63 is able to inhibit the production of IL-10 and to transform growth factor beta (TGF-β) by reducing the Th2-type response. During the infection, cells from the phagocytic mononuclear system produce IFN- γ, IL-10, TGF-β and hypoxia-induced factor 1-alpha (HIF1-α), which stimulate increased production of metalloprotease 9 (MMP-9) and (TIMP) inhibitor, all of which promote macrophage inhibition and cell migration to other tissues, persistence of infection and disease progression.

or metacyclic forms. The research group has not accessed the metacyclic/procyclic ratio of the promastigote culture so that it is possible that these forms behavior in 3D cultures. Also, other classes of proteases could not be detected by zymography under the conditions tested, which may limit the use of this method for *Leishmania* proteases. Tupperwar *et al.* (2008) suggest that the qRT-PCR performed using varied labeled fluorescent probes become limited for the detection of unique DNA sequences that could be involved in the detection of GP63.

Host metalloproteases

Cells of the immune response produce MMP and other proteases involved in the pathogenesis of the disease. In this review, the selected articles reported that immune cells, mainly activated macrophages, produce high levels of MMP-9 during infection by New World *Leishmania* species. This enzyme has been considered one of the factors responsible for digesting extracellular matrix components and regulating microvascular permeability in the primary inflammatory response, in addition to being involved in leukocyte recruitment (Reichel *et al.* 2008). Although the macrophages are the main route of infection elimination, the parasite possesses escape mechanisms of immune response allowing its survival and dissemination, which alters the production of cytokines and other mediators of immune response (Oliveira *et al.* 2014).

MMP-related studies were conducted in Brazil (Maretti-Mira *et al.* 2011*a*, *b*; Fraga *et al.* 2012; Campos *et al.* 2014). From the five articles included in this review, two performed *in vitro* studies (Maretti-Mira *et al.* 2011*a*; Campos *et al.* 2014), one conducted a retrospective study (Fraga *et al.* 2012), one a prospective study (Maretti-Mira *et al.* 2011*a*) and another one clinical essays (Maretti-Mira *et al.* 2011*a*). Four studies have reported a statistical analysis of their results (Maretti-Mira *et al.* 2011*a*, *b*; Fraga *et al.* 2012; Campos *et al.* 2014). The cutaneous (Maretti-Mira *et al.* 2011*b*; Campos *et al.* 2014), and mucosal (Fraga *et al.* 2012) clinical forms were studied. The species of *Leishmania* related were *L. braziliensis* (Maretti-Mira *et al.* 2011*a*, *b*) and one did not report the specie (Table 3). Regarding the objectives of the selected articles, three studies have investigated the role of MMP-9 in the pathogenesis and development of infection in human macrophages (Maretti-Mira *et al.* 2011*a*), cells from human skin fragments (Maretti-Mira *et al.* 2011*b*), peripheral blood cells and biopsy of lesions (Campos *et al.* 2014). Also, Maretti-Mira *et al.* (2011*b*) also investigated the role of MMP-2 in chronic cutaneous form and skin re-epithelialization.

The studies used blood, (Maretti-Mira *et al.* 2011*a*; Campos *et al.* 2014), human macrophages (Maretti-Mira *et al.* 2011*a*),

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Table 3. Characteristics of selected articles on the role of host metalloproteases in New World *Leishmania*sis

Author/ country	Design/period	Number of patients and age range (years)	Clinical manifestation	*Leishmania* species	Statistics	Objetive
Maretti-mira *et al.* (2011*a*)/ Brazil	Clinical trial and *in vitro* assays/ NR	21; 18–70 (42 ± 18)	LM	*L. braziliensis*	Y	To test if *L. braziliensis* infection would induce MMP9 activity in human macrophages and would correlate to enhanced macrophage dissemination, characterized by the development of mucosal *Leishmania*sis.
Maretti-mira *et al.* (2011*b*)/ Brazil	Prospective; NR	39; 37	ACL	*L. braziliensis*	Y	Investigate the participation of gelatinases in the resolution of human CL lesions and determine some of the factors that influence gelatinase activity in these lesions and therefore interfere in the resolution process.
Fraga *et al.* (2012)/Brazil	Retrospective/ NR	54	ATL	NR	Y	To verify whether the HIF-1 α protein expression may be associated with VEGF-A, VEGFR2 and MMP9 in *Leishmania*l lesions.
Campos *et al.* (2014)/Brazil	*In vitro* assays/ 2014	133 (19 early CL *Leishmania*sis; 85 CL ; 29 healthy subjects; NR	LC	*L. braziliensis*	Y	Participation of MMP9 in the pathogenesis of *L. braziliensis* infection and evaluation of the frequency of MMP9 in monocyte subsets and its mechanism of production.

NR, not related; CL, cutaneous *Leishmania*sis; ML, mucosal *Leishmania*sis; ATL, American tegumentary *Leishmania*sis; ACL, American cutaneous *Leishmania*sis; Y, yes; HIF-1a, hypoxia inducible factor-1-alpha; VEGF-A, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor; MMP, metalloprotease.

skin fragments (Maretti-Mira *et al.* 2011*b*), skin lesions (Fraga *et al.* 2012) and culture of promastigotes and amastigotes of *L. braziliensis* (Maretti-Mira *et al.* 2011*a*). The methods for detection of MMP, which were used in the selected studies, were zymography (Maretti-Mira *et al.* 2011*a*, b), immunoprecipitation (Maretti-Mira *et al.* 2011*a*), Western blotting (Maretti-Mira *et al.* 2011*a*), RNA Real Time PCR (Maretti-Mira *et al.* 2011*a*, *b*), immunohistochemistry (Maretti-Mira *et al.* 2011*b*; Fraga *et al.* 2012), flow cytometry (Campos *et al.* 2014) and ELISA (enzyme-linked immunosorbent assay) (Campos *et al.* 2014) (Table 4).

Whereas some studies associate MMP-9 as prognostic biomarker for visceral leishmaniasis (de Oliveira *et al.* 2013; Gadisa *et al.* 2017), one study related that MMP-9 may represent a biomarker to prognosticate an increased risk for parasite dissemination and development of mucosal leishmaniasis (Maretti-Mira *et al.* 2011*a*). The high levels of this enzyme indicate an increased risk of parasitic spread and development of the mucosal form of leishmaniasis. Macrophage cultures infected by *L. braziliensis* increased MMP-9 activity levels, so the authors suggest that the infection stimulates macrophages to increase MMP-9 production, which is related to increased cell migratory activity. This shows that the metalloprotease plays an important role in host defense against the progression of leishmaniasis. Furthermore, macrophages from patients with different forms of ATL showed several patterns in the production and activity of MMP-9. Some studies obtained human monocyte-derived macrophages from peripheral blood mononuclear cells (PBMC) and isolated from patients with the mucosal leishmaniasis. In these cells increased levels of MMP-9 were observed before and after infection when compared with samples from patients with localized cutaneous form. The superior gelatinolytic activity of macrophages from individuals that have evolved into the mucosal form may be related to the migration of these cells to other tissues. The matrix degradation acts facilitating this migration process to tissues such as the mucosa, leading the individual to develop the mucosal form of the disease. Conversely, macrophages from individuals with reduced MMP-9 activity may have a *L. braziliensis* infection controlled at the entry site, so that these individuals develop only the localized cutaneous form of the disease (Maretti-Mira *et al.* 2011*a*).

Thus, the therapeutic modulation of MMP-9 could be a useful approach to improve the prognosis of patients infected with *L. braziliensis* (Maretti-Mira *et al.* 2011*a*). The patients in the pre-ulcerative stage of the disease produce lower levels of MMP-9 than patients in the ulcerative phase, suggesting that progression of the disease to the ulcerative phase may be associated with increased MMP-9 levels, as well as by an imbalance between MMP-9 and its inhibitor, TIMP-1. These results corroborate with the studies cited above, which suggest that the monitoring of MMP-9 early in the disease to establish the likelihood of clinical progression and therapeutic cure. The inflammatory cytokine TNF (tumor necrosis factor), besides playing a role in cellular apoptosis and other biological processes, also participates in the induction of metalloproteases. In cutaneous leishmaniasis, TNF works in the regulation of MMP-9, since the addition of exogenous TNF increases the production of MMP-9 in healthy patients, whereas the neutralization of this cytokine negatively regulates the synthesis of MMP-9 in patients with the cutaneous form of the disease. The excessive production of TNF by monocytes from patients with the cutaneous form increases the production of MMP-9 leading to the imbalance of the enzyme and its inhibitor, degradation of the basal membrane, migration of inflammatory cells and development of the lesion. These results propose that TNF cytokine is the primary regulator of MMP-9 production during *L. braziliensis* infection (Campos *et al.* 2014).

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Table 4. Samples, methods of detection and completion of articles included on host metalloproteases in New World *Leishmania* sp.

Author and country	Sample	MMP detection methods	Results	Conclusion
Maretti-mira *et al.* (2011*a*)/ Brazil	Blood, MØs cells, promastigotes and amastigotes	IP; WB; Zymography; qPCR	*In vitro* infection of human MØs with *L. braziliensis* increased the secretion and activation of MMP9. MØs from healthy cured individuals with previous history of ML had increased MMP9 activity compared with LCL cured individuals.	The intrinsic capacity of MMP9 activation of each individual might influence the intensity of MØ efflux and dissemination of *L. braziliensis* infection to different anatomic areas. MMP9 may represent a biomarker to prognosticate an increased risk for parasite dissemination and development of ML.
Maretti-mira *et al.* (2011*b*)/ Brazil	Skin tissue fragments	RNA Real Time PCR; *In situ* zymography; IHC	An association between gelatinase activity and increased numbers of cells making IFN-γ, IL-10 and TGF-β in lesions from poor responders. High levels of MMP2 mRNA and enhanced MMP2: TIMP2 ratios were associated with a satisfactory response to antimonials treatment. A high gelatinolytic activity was found in the wound beds, necrotic areas in the dermis and within some granulomatous infiltrates	These results indicate the importance of gelatinase activity in the skin lesions caused by CL. The immune response profile may be responsible for the gelatinase activity pattern and may ultimately influence the persistence or cure of CL lesions.
Fraga *et al.* (2012)/Brazil	Paraffin blocks from skin lesions	IHC	There was an increase of VEGFR2 and MMP9 protein expressions in HIF-1 α higher group of epithelial cells. In epithelial cells there was correlation between VEGF-A and MMP9, VEGFR2 and MMP9 protein expression.	Considering leukocyte cells, VEGFR2 was negatively correlated to MMP9 protein levels. This pathway possibly prepares the cells for a higher activity in a hypoxic or an angiogenic microenvironment.
Campos *et al.* (2014)/Brazil	Blood and cutaneous lesion	FC; ELISA	The excessive production of TNF by monocytes observed in CL increases the production of MMP9 leading to an imbalance between production of this enzyme and its inhibitor (TIMP1). Consequently, there is an excessive degradation of the basal membrane, migration of inflammatory cells to the site of infection and ulcer development.	Monocytes and MMP9 may play key roles in tissue destruction. Thus, therapeutic modulation of MMP9 may be a useful approach for improving disease outcome in *L. braziliensis* patients.

kDa, kilodalton; WB, Western Blotting; FC, flow cytometry; MMPs, metalloproteases; IHC, Immunohistochemistry; IP, immunoprecipitation; qPCR, quantitative polymerase chain reaction; ELISA, enzyme-linked immunesorbent assay; ML, mucosal *Leishmania*sis; LCL, localized cutaneous *Leishmania*sis; IFN, interferon gamma; IL, interleukin; TGF, *transforming growth factor;* VEGFR2, vascular endothelial growth factor; HIF-1, hypoxia inducible factor-1-alpha; VEGF-A, vascular endothelial growth factor A; MIP1a, macrophage inhibitory protein 1a.

In addition to TNF and TIMP-1, other factors are involved in the increased production of MMP-9 by immune cells, such as hypoxia-inducible factor-1 (HIF1-α). This factor, when associated with its beta subunit, acts as a transcription factor of VEGF-A (vascular endothelial growth factor A) in the lesions of patients infected with *Leishmania* sp. These factors are involved in essential body processes, such as embryogenesis, angiogenesis, cell proliferation, metastasis and also play an important role in response to hypoxia. The HIF1-α protein is maintained in response to hypoxia. HIF1-α is expressed mainly in parasitophorous vacuoles and cytoplasm of infected macrophages. In leishmaniasis lesions, a microcirculation deficiency, leukocyte metabolic demand, parasite proliferation and secondary bacterial infection are observed, with HIF1- α protein representing a hypoxic event in the lesion (Fraga *et al.* 2012). There is evidence that HIF1-α accumulates in the inflamed tissue of *Leishmania*sis lesion due to the presence of macrophages (Arrais-Silva *et al.* 2005). In the study by Fraga *et al.* (2012), the researchers observed an increase in MMP-9 in the group of individuals who had high levels of HIF1-α. Also, levels of VEGF-A and VEGFR2 (vascular endothelial growth factor receptor 2) are also positively correlated with those of MMP-9. Since HIF1-α factor increases the expression of VEGFR2 and MMP-9, inflammatory cellular response and angiogenesis in leishmanial lesion sites are promoted (Fraga *et al.* 2012). The increase of MMP-9 occurs along with HIF1-α and VEGF (Fraga *et al.* 2012), the expression of HIF1-α may induce the elements necessary to provide the establishment of angiogenesis in the inflammatory response, aiding these specific sites of lesions caused by *Leishmania* (Zarember and Malech, 2005).

In the present review, just one study related MMP-2 to ATL (Maretti-Mira *et al.* 2011*b*), while concerning VL, there are some studies describing its role in the pathogenesis of disease in human and dogs (Machado *et al.* 2010; Marangoni *et al.* 2011; Melo *et al.* 2011; Melo *et al.* 2012). In ATL, Maretti-Mira *et al.* (2011*b*) reported low levels of MMP-2 and high levels of MMP-9 gelatinolytic activity in lesions of patients with low response to antimony treatment. This enzymatic profile may be related to the increased number of cells producing IFN-γ, TGF-β (growth-transforming factor) and IL-10. The increase of MMP-2 is also associated with the process of re-epithelialization of the lesions and therapeutic success. In cutaneous leishmaniasis caused by *L. braziliensis*, various cytokine profiles can be found in the lesions. The TGF-β and IL-10 cytokines characterize a Th2-type cellular immune response profile, being related to persistent infections and chronic lesions, whereas IFN-γ is important in the resolution of cutaneous form. Lesions from individuals with low response to treatment had cells producing IFN-γ and high levels of MMP-9 gelatinase activity; this shows that the production of these cytokines in recent lesions suggests that the first few months are the most important to establish an effective immune response that can result in the success or failure of treatment. The high levels of proinflammatory cytokines found in

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lesions of patients with low response also suggest that excessive IFN-γ may have the opposite effect and impair resolution of lesions, whereas, in individuals with a good immune response, the predominance of these cytokines such as IFN-γ may be responsible for the low level of gelatinolytic activity of MMP-9 in the lesions (Maretti-Mira *et al.* 2011*b*).

IL-10 appeared to be the only cytokine capable of suppressing the production and activity of metalloproteases, thus playing a necessary role in protecting the matrix during infection. These results show the importance of gelatinases activity in skin lesions of leishmaniasis. The modulation of IL-10, IFN-γ and TGF-β may be responsible for the activity pattern of the metalloproteinases early in the infection, and may influence the persistence or cure of lesions (Maretti-Mira *et al.* 2011*b*; de Oliveira *et al.* 2013; Oliveira *et al.* 2014) (Fig. 2).

Regarding biases, only one study reported them. The limitation of the study was the lack of information on the clinical data of lesions and their time of evolution. Therefore, to confirm its results, *in vitro* and *in vivo* studies could clarify the mechanism underlying the induction and activity of the factors involved in cutaneous leishmaniasis (Fraga *et al.* 2012).

Conclusions

In this systematic review, we found sufficient literature about parasite metalloproteases related pathogenicity, virulence and specie detection, but some species have not been studied and their role remains unclear, such as *Leishmania lainsoni, L. naiffi, L. peruviana* and *L. infantum* (causing atypical CL and ATL). It was observed that the GP63 complex has different enzymatic profiles according to the *Leishmania* species that causes ATL in the New World. GP63 is significantly related to the development and establishment of the infection. It can inhibit complement system activity, stimulate the degradation of fibronectin and promote cleavages of transcriptional molecules, such as NF-κB, membrane proteins and tyrosine phosphatases. In addition, GP63 has been shown as an essential virulence factor in the establishment of infection, contributing to the reduction of immune functions in the early stages of the disease, which provides the adaptation of the parasite to the intracellular environment and allows the survival and propagation of the protozoan to other tissues of the body.

Based on the results of this review, there are few studies on host metalloprotease involved in ATL, but there is a consensus that these studies have high methodology quality and scientific rigor, and, due to it, we considered relevant to compile the results about host metalloprotease. Thus, it becomes evident that new clinical and experimental studies are needed to demonstrate the role of these enzymes and the mechanisms that are involved in the progression of cutaneous and mucocutaneous leishmaniasis caused by New World *Leishmania*.

Among the host matrix metalloproteases, MMP-9 can be considered a biomarker of bad prognostic that indicates a high risk of parasite dissemination and development to the mucosal form of leishmaniasis, especially if detected at the beginning of the cutaneous lesion. Also, its modulation may improve prognosis in *L. braziliensis* infections and may be considered a therapeutic and laboratory follow-up target. Since MMP-2 has also been considered an important marker to diagnosis and prognosis of VL in human and dogs, MMP-2 activity appears to be involved with therapeutic success in ATL when present at elevated levels and with the re-epithelialization process, but other studies must be conducted to affirm the potential of MMP-2 for the ATL development. From this systematic review of the literature on the role of metalloproteases in ATL, it is suggested that the serological and tissue levels of MMP-9 should be monitored early in the onset of the cutaneous lesion and used as a marker of disease progression to the mucosal form. The monitoring of MMP-9 levels could be performed using Western Blot, zymography and Real Time PCR in serological and tissue samples. The inclusion of one or more techniques in the laboratory routine can improve the diagnosis and monitoring of cases of cutaneous leishmaniasis, since, if it is detected in the first months of infection, it can predict the prognosis of the clinical form and resolution of leishmaniasis after treatment.

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References

Arrais-Silva WW, Paffaro VA, Yamada AT and Giorgio S (2005) Expression of hypoxia-inducible factor-1-alpha in the cutaneous lesions of BALB/c mice infected with *Leishmania amazonensis. Experimental and molecular pathology* **78**, 49–54.

Beller EM, Glasziou PP, Altman DG, Hopewell S, Bastian H, Chalmers I, Gøtzsche PC, Lasserson TTovey D and Group, for the P. for A. (2013). PRISMA for abstracts: reporting systematic reviews in journal and conference abstracts. *PLoS Medicine* **10**, e1001419.

Bennis I, Thys S, Filali H, De Brouwere V, Sahibi H and Boelaert M (2017) Psychosocial impact of scars due to cutaneous leishmaniasis on high school students in Errachidia province, Morocco. *Infectious Diseases of Poverty* **6**, 46.

Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A and Engler JA (1993) Matrix metalloproteinases: a review. *Critical Reviews in Oral Biology and Medicine: An Official Publication of the American Association of Oral Biologists* **4**, 197–250.

Brazil, Ministry of Health (2017) *Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Manual de vigilância da leishmaniose tegumentar [recurso eletrônico]/Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância das Doenças Transmissíveis.*–Brasília: Ministério da Saúde, p. 189.

Calegari-Silva TC, Pereira RMS, Dione L, De-melo B, Saraiva EM, Soares DC, Bellio M and Lopes UG (2009) NF-kB-mediated repression of iNOS expression in *Leishmania amazonensis* macrophage infection. *Immunology Letters* **127**, 19–26.

Campos TM, Passos ST, Novais FO, Beiting DP, Costa RS, Queiroz A, Mosser D, Scott P, Carvalho EM and Carvalho LP (2014) Matrix metalloproteinase 9 production by monocytes is enhanced by TNF and participates in the pathology of human cutaneous *Leishmania*sis. *PLoS Neglected Tropical Diseases* **8**, e3282.

Carvalhal DGF, Barbosa AJ, D'El-Rei Hermida M, Clarencio J, Pinheiro NFJ, Veras PST and dos-Santos WLC (2004) The modelling of mononuclear phagocyte-connective tissue adhesion *in vitro*: application to disclose a specific inhibitory effect of *Leishmania* infection. *Experimental Parasitology* **107**, 189–199.

Chang C and Werb Z (2001) The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. *Trends in Cell Biology* **11**, S37–S43.

Contreras I, Gómez MA, Nguyen O, Shio MT, McMaster RW and Olivier M (2010) *Leishmania*-induced inactivation of the macrophage transcription factor AP-1 Is mediated by the parasite metalloprotease GP63. *Plos Pathogens* **6**, e1001148.

Cuervo P, Saboia-Vahia L, Costa Silva-Filho F, Fernandes O, Cupolillo E and De Jesus JB (2006) A zymographic study of metalloprotease activities in extracts and extracellular secretions of *Leishmania* (*Viannia*) *braziliensis* strains. *Parasitology* **132**, 177–185.

Cuervo P, Santos AL, Alves CR, Menezes GC, Silva BA, Britto C, Fernandes O, Cupolillo E, de Jesus BJ (2008) Cellular localization and

expression of gp63 homologous metalloproteases in Leishmania (Viannia) braziliensis strains. *Acta Tropica* **106**, 143–148.

de Oliveira FA, Vanessa Oliveira Silva C, Damascena NP, Passos RO, Duthie MS, Guderian Ja, Bhatia A, de Moura TR, Reed SG, de Almeida RP and de Jesus AR (2013) High levels of soluble CD40 ligand and matrix metalloproteinase-9 in serum are associated with favorable clinical evolution in human visceral *Leishmania*sis. *BMC Infectious Diseases* **13**, 331.

Espir TT, Figueira LDP, Naiff MDF, Guimarães A, Ramalho-ortigão M, Malheiro A, Maria A and Franco R (2014) The role of inflammatory, anti-inflammatory, and regulatory cytokines in patients infected with cutaneous *Leishmania*sis in Amazonas State, Brazil. *Journal of Immunology Research* **2014**, 1–10.

Fraga AC, Oliveira MV, Alves LR, Viana AG, Sousa AA, Carvalho SF, De Paula AM, Botelho AC and Guimarães AL (2012) Immunohistochemical profile of HIF-1 α, VEGF-A, VEGFR2 and MMP9 proteins in tegumentary *Leishmania*sis. *Anais Brasileiros de Demartologia* **87**, 709–713.

Gadisa E, Tasew G, Abera A, Gelaye W, Chanyalew M, Abebe M, Laskay T and Aseffa A (2017) Serological signatures of clinical cure following successful treatment with sodium stibogluconate in Ethiopian visceral leishmaniasis. *Cytokine* **91**, 6–9.

Gomez MA, Contreras I, Halle M, Tremblay ML, McMaster RW and Olivier M (2009a) *Leishmania* GP63 alters host signaling through cleavage-activated protein tyrosine phosphatases. *Science Signaling* **2**, ra58.

Gomez MA, Stuible M, Shimizu H, Mcmaster WR, Olivier M and Tremblay ML (2009b) The Leishmania surface protease GP63 cleaves multiple intracellular proteins and actively participates in p38 mitogen-activated protein kinase inactivation. **284**, 6893–6908.

Gontijo B, De M and De Carvalho LR (2003) Leishmaniose tegumentar Americana. *American Cutaneous leishmaniasis. Medicina Tropical* **36**, 71–80.

Gregory DJ, Godbout M, Contreras I, Forget G and Olivier M (2008) A novel form of NF-kB is induced by *Leishmania* infection: involvement in macrophage gene expression 1. *European Journal of Immunology* **38**, 1071–1081.

Hallé M, Gomez MA, Stuible M, Shimizu H, McMaster WR, Olivier M and Tremblay ML (2009) The Leishmania surface protease GP63 cleaves multiple intracellular proteins and actively participates in p38 mitogen-activated protein kinase inactivation. *The Journal of Biological Chemistry* **284**, 6893–6908.

Isnard A, Shio MT and Olivier M (2012) Impact of *Leishmania* metalloprotease GP63 on macrophage signaling. *Frontiers in Cellular and Infection Microbiology* **2**, 72.

Kulkarni MM, Jones EA, Mcmaster WR, Mcgwire BS, Kulkarni MM, Jones EA, Mcmaster WR and Mcgwire BS (2008) Fibronectin binding and proteolytic degradation by *Leishmania* and effects on macrophage activation fibronectin binding and proteolytic degradation by *Leishmania* and effects on macrophage activation. *Infection and Immunity* **76**, 1738–1747.

Kulkarni MM, Olson CL, Engman DM and Mcgwire BS (2009) *Trypanosoma cruzi* GP63 proteins undergo stage-specific differential posttranslational modification and are important for host cell infection. *Infection and Immunity* **77**, 2193–2200.

Liarte DB, Mendonca IL, Luz FC, Abreu EA, Mello GW, Farias TJ, Ferreira AF, Millington MA and Costa CH (2001) QBC for the diagnosis of human and canine American visceral *Leishmania*sis: preliminary data. *Revista da Sociedade Brasileira de Medicina Tropical* **34**, 577–581.

Lima AKC, Elias CGR, Souza JEO, Santos ALS and Dutra PML (2009) Dissimilar peptidase production by avirulent and virulent promastigotes of *Leishmania braziliensis*: inference on the parasite proliferation and interaction with macrophages. *Parasitology* **136**, 1179–1191.

Machado GF, Melo GD, Moraes OC, Souza MS, Marcondes M, Perri SHV and Vasconcelos RO (2010) Differential alterations in the activity of matrix metalloproteinases within the nervous tissue of dogs in distinct manifestations of visceral leishmaniasis. *Veterinary Immunology and Immunopathology* **136**, 340–345.

Maretti-Mira AC, de Pinho Rodrigues KM, de Oliveira-Neto MP, Pirmez C and Craft N (2011*a*) Acta Tropica MMP-9 activity is induced by *Leishmania braziliensis* infection and correlates with mucosal *Leishmania*sis. *Acta Tropica* **119**, 160–164.

Maretti-Mira AC, De Oliveira-Neto MP, Da-Cruz AM, De Oliveira MP, Craft N and Pirmez C (2011*b*) Therapeutic failure in American cutaneous *Leishmania*sis is associated with gelatinase activity and cytokine expression. *Clinical and Experimental Immunology* **163**, 207–214.

Marangoni NR, Melo GD, Moraes OC, Souza MS, Perri SHV and Machado GF (2011) Levels of matrix metalloproteinase-2 and metalloproteinase-9 in the cerebrospinal fluid of dogs with visceral leishmaniasis. *Parasite Immunology* **33**, 330–334.

Matheoud D, Moradin N, Bellemare-Pelletier A, Shio MT, Hong WJ, Olivier M, Gagnon É, Desjardins M and Descoteaux A (2013) *Leishmania* evades host immunity by inhibiting antigen cross-presentation through direct cleavage of the SNARE VAMP8. *Cell Host and Microbe* **14**, 15–25.

Mauricio IL, Gaunt MW, Stothard JR and Miles MA (2007) Glycoprotein 63 (gp63) genes show gene conversion and reveal the evolution of Old World *Leishmania*. *International Journal for Parasitology* **37**, 565–576.

Melo GD, Marangoni NR, Marcondes M, Lima VMF and Machado GF (2011) High levels of serum matrix metalloproteinases in dogs with natural visceral leishmaniosis: a preliminary report. *Veterinary Journal (London, England : 1997)* **188**, 243–245.

Melo GD, Marcondes M and Machado GF (2012) Canine cerebral leishmaniasis: potential role of matrix metalloproteinase-2 in the development of neurological disease. *Veterinary Immunology and Immunopathology* **148**, 260–266.

Oliveira WN, Ribeiro LE, Schrieffer A, Machado P, Carvalho EM and Bacellar O (2014) The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of human tegumentary *Leishmania*sis. *Cytokine* **66**, 127–132.

Petropolis DB, Rodrigues JCF, Viana NB, Pontes B, Pereira CFa and Silva-Filho FC (2014) *Leishmania amazonensis* promastigotes in 3D Collagen I culture: an *in vitro* physiological environment for the study of extracellular matrix and host cell interactions. *PeerJ* **2**, e317.

Reichel CA, Rehberg M, Bihari P, Moser CM, Linder S, Khandoga A and Krombach F (2008) Gelatinases mediate neutrophil recruitment *in vivo*: evidence for stimulus specificity and a critical role in collagen IV remodeling. *Journal of Leukocyte Biology* **83**, 864–874.

Shio MT and Olivier M (2010) Editorial: *Leishmania* survival mechanisms: the role of host phosphatases. *Journal of Leukocyte Biology* **88**, 1–3.

Shio MT, Hassani K, Isnard A, Ralph B, Contreras I, Gomez MA, Abu-Dayyeh I and Olivier M (2012) Host cell signalling and *Leishmania* mechanisms of evasion. *Journal of Tropical Medicine* **2012**, 819512.

Thiakaki M, Kolli B, Chang K and Soteriadou K (2006) Down-regulation of gp63 level in *Leishmania amazonensis* promastigotes reduces their infectivity in BALB / c mice. *Microbes and Infection* **8**, 1455–1463.

Tupperwar N, Vineeth V, Rath S and Vaidya T (2008) Development of a real-time polymerase chain reaction assay for the quantification of *Leishmania* species and the monitoring of systemic distribution of the pathogen. *Diagnostic Microbiology and Infectious Disease* **61**, 23–30.

WHO, World Health Organization (2010) Control of the Leishmaniasis: Report of a Meeting of the WHO. Expert Committee on the Control of Leishmaniasis. “WHO technical report series”, WHO: Geneva, Switzerland, no. 949, pp. 201.

Yao C (2010) Major surface protease of *Trypanosomatids*: one size fits all. *Infection and Immunity* **78**, 22–31.

Zarember KA and Malech HL (2005) HIF-1 α: a master regulator of innate host defenses. *The Journal of Clinical Investigation* **115**, 2003–2005.

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