Review Article

Biochemical Properties Of Parasite Virulence Factor: Lesson Learned From Leishmania

ABSTRACT

Leishmania, a parasitic protozoan, a single-celled organism of the genus trypanosomes that are responsible for the disease leishmaniasis. Transmission occured by sandflies of the genus Phlebotomus in the Old World, and of the genus Lutzomyia in the New World. Globally, at least 93 sandfly species are proven or probable vectors. Their primary hosts are vertebrates; Leishmania commonly infects hyraxes, canids, rodents, and humans. Leishmaniasis encompasses diverse clinical syndromes, including cutaneous, mucosal, and potentially life-threatening visceral forms. Three widely known virulence factors belongs to the genus Leishmania include the active compound named proteophosphoglycan (PPG), GP63 metalloprotease and lipophosphoglycan (LPG). these substance established on the surface of the parasite. The aim of this review article is to make an insight of the biochemical characteristics of Leishmania spp virulence factors, the armamentarium that predispose their pathogenesis, its invasion and virulence to the mammalian host.

Keywords: protozoan, Trypanosomes, proteophosphoglycan, GP63 metalloprotease and lipophosphoglycan, leishmaniasis

1. INTRODUCTION

Parasites are organisms that live on or within their hosts. As intelligent organisms, parasitic agents have the ability to evade the host's immune system [1,2]. Their goal is to ensure its existence is permanently sustainable in the host's body. Although at the same time, parasitic organisms must obtain optimal nutrition from their host in order to stay alive [3]. Its continual sensing accomodation and adapt to environmental shift is condemnatory for all organisms to carry on homeostasis and eventually its for survival [4].

Every parasites actually experience sophisticated life cycles; this process consist of a broad array of cellular distinction stages in probably different host compartments [5]. The potency of transmission might also occurs across multiple hosts [6]. As any parasites primarily depend on its host assets, it is crystal clear they have evolved the most efficient mechanisms to sense alterations and modify itself to any resources which is available; in a wide range of conditions in their environments. Virulence strategies also modified and adjusted by parasites to invade its host and it must be suitable for different kind and type of tissue. Parasite also must be able to enhance its clonal replication and escalate, as well as other action for immunomodulation or immunoevasion of their host immune responses.

Here we provide an insight of the biochemical properties of parasite virulence factor with focus on *Leishmania* spp.; properties that facilitate their disease formation including their virulence and invasion to the mammalian host.

2. LEISHMANIA SPP., LEISHMANIASIS AND ITS GLOBAL EPIDEMIOLOGY

Leishmania (/liːˈʃmeɪniə/) is a genus of parasitic organisme belongs to the *Trypanosomes.* This organism causing leishmaniasis, a parasitic disease that is commonly found in parts of the tropics, subtropics, and southern Europe. Based on the occasion or time of occurence, the vector divide into two: the sandflies from the genus *Phlebotomus* in the Old World, and on the other hand, of the genus *Lutzomyia* in the New World. So far, not less than 93 species of sandfly are Entomologically evince or have the status as potential or probable vectors, globally. This protozoan parasite actually has a vertebrate organism as its primary host. *Leishmania* repeatedly found to infect rodents, canids, hyraxes, and even humans [7,8]. Leishmaniasis encompasses diverse clinical syndromes, including cutaneous, mucosal, and potentially life-threatening visceral forms [9,10].

Leishmaniasis is endemic in the vast area across the globe from the tropics, subtropics, and southern Europe [9-11]. It is estimated more than one billion individuals are at threat of leishmaniasis with an annual incidence of more than two million cases throughout tropical and subtropical region (in number might reach to 100 countries) [11]. Recent literature revealed significant increase elevation regarding imported leishmaniasis cases in developed, non-endemic countries, *e.g*., Italy, and this took place in conjunction with improvement in mass and rapid transportation intercontinentally, massive international tourism, asylum seekers/immigrants from endemic countries and even multinational based military operations in endemic areas [13,14]

Area where Leishmaniasis acquired is already suspected; South America is the main source area of cutaneous leishmaniasis, and escapade tourists on long-term vacation in highly-endemic forested areas are at certain peril1[5,16]. On contrary, international tourists are in danger while they travel to certain Mediterranean or middle east destinations where there is emerging risk of unfortunate acquisition of visceral leishmaniasis [17,18].

Leishmaniasis should be appraised in vulnerable individuals suffer from well-matched clinical syndrome along with a recent history of travelling to and staying in an endemic area, even if this occurred several months or years ago; this become important key factor in making correct diagnosis [11,14,16] Appropriate counseling should be provided to adventure travelers, military personnel, researchers, and other groups of travelers likely to be exposed to sandflies in endemic areas [20].

Overall, leishmaniasis in humans is created by approximately 20 genus that belongs to the Leishmania spp. classified in the sub-genera *Leishmania* and *Viannia* [20-22]. Epidemiologically, it is possible that in certain condition there might be more than one species of *Leishmania* spp. found in the same geographic area [20]. The effort of making correct identification of the species often has clinical relevance, such as implications regarding whether and which initial medication is urgently indicated and whether and how to closely asses for the consequence of potential sequelae regarding the infection (*e.g*., the condition of mucosal leishmaniasis, which is ordinarily created by the New World species belongs to the group of the *Viannia* subgenus, particularly, but must kept in mind that it is not barely, by the genus *Leishmania* [*Viannia*] *braziliensis* in certain restricted terresterial areas) [21,22].

Approximately, 350 million individual globally are at hazard of infecting leishmaniasis and an estimated 1.6 million new cases actually occur, annually [7,22]. The disease primarily infects impoverished individuals lining in low socio-economy level of countries in Africa, Latin America and Asia, and this condition is often linked with underlying condition such as malnutrition, refugees that made fast migration across borders, countries and even continents, unfortunate poverty-stricken housing conditions, limited assets due to the inability of the authorities and frail personal immune system [23].

The ability of the immune system to fight infectious diseases must also be related to the virulence factors of the pathogenic agent. The following section will discuss some of the virulence factors of the Leishmania; especially the biochemical aspect.

3. VIRULENCE FACTORS

Virulence is described as an internal properties of an organism that enabled them to infect their host, a substance pinned internally and can cause a disease in vulnerable host. Virulence factors are the molecules that assist the organism colonize its host at the cellular level. These factors are either secretory, membrane associated or cytosolic in nature. In terms of bacteria, the cytosolic factors facilitate the bacterium to undergo quick adaptive-metabolic, physiological and morphological shifts [24].

Three widely known virulence factors belongs to the genus Leishmania include the active compound named proteophosphoglycan (PPG), GP63 metalloprotease and lipophosphoglycan (LPG). These substance established on the surface of the parasite [25-27]

Leishmania spp. Actually induce autophagy in a variety of cell types, eventhough that published results regarding the effects of autophagic modulation on Leishmania survival inside their host's cella are contradictory. Upon infecting the innate immune cells, namely the macrophage, Leishmania parasite soon launch into an organelle named parasitophorous vacuole. It soon begins to control and 'hijack' the cell, with the inner vacuole actually acting as a safeguard against the host cell's immunity [28] *Leishmania* then take over the macrophage's membrane fusion machinery, didacting them to work according to its will, to export their important virulence factors out of the vacuole [29]. The protozoan parasite Leishmania, is particularly adept at shifting the macrophage to become a suitable and hospitable host cell for their existence inside their host, so that the host's cellular immune system failed to recognize them [30]

As the parasite transfers its virulence factors to the other side of the vacuole's membrane, it was necessary to learn the compartment used to contain these factors [31]. The virulence factors actually were found in a cell organelle called the endoplasmic reticulum (ER) and this step was pivotal in the lay out of virulence factors within the inhabited cell.

3.1. BIOCHEMICAL PROPERTIES AND CHALLENGES ON THEIR GENOMES DATA

The biochemistry and cell biology of *Leishmania* spp. is similar to that of other kinetoplastids. They share the same main morphological features, including a single flagellum which has an invagination, the flagellar pocket, at its base, a kinetoplast, which is found in the single mitochondrion, and a subpelicular array of microtubules, which make up the main part of the cytoskeleton.

The result of genomes sequencing regarding three major species of *Leishmania* spp (*L. braziliensis, L. infantum, L. major*) has succesfully apportioned the initial diagrams of the metabolism pathway belongs to these protozoans [32]. Another systems approach was used to initiate another metabolism network for the *L. major Friedlin* strain and in continuation with that is to make forecasts in conjunction with possible essential genes and pathway usefulness. However, > 65% of the protein-encoding sequences in the parasite *Leishmania* genome cannot yet be allocated any single functi on based on homology searches, and therefore it is likely that *in silico* models must be continously upgraded and improved as recent metabolic pathways are recognized, just like the approach conducted by Bora and Jha that developed an *in silico* metabolic pathway analysis identifying target against Leishmaniasis – a kind of kinetic modeling approach which can be a breakthrough in problem alleviation approaches [33,34]

Leishmania genomic database in majority is available in the GeneDB genome resource, the effort first confirmed by scientists from the Sanger Institute, and then soon made available via the Eukaryotic Pathogens Database Resource (EuPathDB) [35,36]. GeneDB was in the beginning aimed to keep all genomic data regarding *T. brucei, L. major*, and *S. pombe*, and was later broaden to comprise carefully collected curated data regarding a vast number of different organisms, including fungi, bacteria, and protozoa [37]. GeneDB authorizes the act of gene searching-finding, protein feature predictions, and any other form of searches against tailored and or protein domain/families databases [37,38]. It provides several functional instruments for inquiring genomic features needed, including (1) BLAST searches, (2) plain text searches, (3) regular expressions enabled motif searches, and (4) AmiGO browsing of genes [39]. Unfortunately, although GeneDB is a crucial assets for the Leishmania investigation group, this genome resource does not incorporate all recent globally available genomic data into biochemical networks, or in other words it is not automatically connected.

Other famous database that can be mentioned is the Kyoto Encyclopedia of Genes and Genomes (KEGG) that combines three kind of data: (1) chemical, (2) genomic, and also (3) functionality information for a wide array of species [40] Eventhough this top-down method easily help the incorporation of all accessible data/information and only need visual exploration of pathways regarding dissimilar organisms, but unfortunately the lack of organism-species specialization frequently means that, for more doubtful organisms, that the specific information need is not easily approachable, and in some conditions, not even incorporated.

An interesting dissimilar accesion provided by the BioCyc project, which method is constructed regarding the ontology evolved in order to express certain biological tasks based on the combination of cellular and molecular grade [41] On contrary to the incorporated accesion provided by the KEGG database, the BioCyc databases are highly dispersed. The BioCyc comprises of MetaCyc (an extensive reference database regarding metabolism pathways) and a set of organism-specific databases which delineate starting from genes to gene products to metabolites and continued to their relationships and the incorporation into metabolism pathways. MetaCyc accomodates preliminary elucidated metabolism pathways from a wide diversity of species. Actually, many organism-specific BioCyc databases are still under continously agile buildout and continous curation [41].

With the advancement of biomolecular science, there is almost no scientific limit in studying and studying something. getting deeper and more detailed, each comes with advantages and disadvantages. Time has always been the catalyst for many of these advances; scientists from far apart places can continue to contribute so that scientific progress can continue to be accelerated.

Next, we will discuss the biochemical aspects of several parasite-related compounds that are considered to be virulence factors. In case of *Leishmania* spp., the list of its virulence factors are as follow: (1) lipophosphoglycan (LPG), (2) glicoinositolphospholipids (GIPLs), (3) proteophosphoglycan (PPG) and (4) the 11 kDa kinetoplastid membrane protein (KMP-11). Eventhough the precise impact of these *Leishmania* biologic properties on the clinical manifestations observed in mammalian hosts is not yet revealed clearly, and there is confirmation that these components able to facilitate and even modify the Leishmania-host immune cells relationship.

3.2. LIPOPHOSPHOGLYCAN (LPG)

Leishmania parasite owns a LPG, a class of molecules that made up of two parts; a lipid part and a (also called glycan) part, that surround over the outer part of the cell wall [25,42]. Immunologically, *Leishmania*'s LPG have the ability to TLR-2, a specific signalling receptor elaborated in precipitating an initial activation of the immune response, *e.g*, the innate immune cells, in mammals [43].

The exact formation and composition of LPG content actually very dynamic and oscillates over time, depending on two things namely (1) the species involved and (2) its lifecycle phase [42,44]. Regarding its content, the amount and composition of the polysaccharide glycan in the LPG is exceptionally fluctuating and contrasting. The amount and variants of lLPG are actually exploitable in terms making them as a biochemical marker. Distinct lifecycle stages of the parasite *Leishmania* might produce different LPG. Furthermore, Lectins, a set of proteins which attach to several different categories of glycans, are repeatedly used to perceive and sense these LPG variants, *e.g*., peanut agglutinin specifically attachs to a particular LPG located on the facet of the infective form of *L. major* [46].

Lipophosphoglycan is actually empowered strongly by the parasite primarily to maintain its survival inside their host [46]. The exact techniques that used by the parasite apply is not clearly revealed; but this property being the midpoint around modifying the immune response of their primary host. Considering this is very critical to the disease formation, due to the fact that (1) the *Leishmania* parasites live inside the host's cellular innate immune cell named macrophages and (2) it really need to avert the inhabited macrophages from processing them further and ends with killing them.47 Lipophosphoglycan also has a duty in (1) facilitating resistance and preventing activation of the complement system armamentarium, (2) inhibiting host's oxidative burst response, and also (3) initiating an adequate inflammation and (4) preventing the natural killer T cells realizing that the host's macrophage is already infected with the Leishmania parasite [25,48]. There may be an association between the immune cell's response to Leishmania and the exact cell stage/subset being evaluated, with differentiated macrophages being more permissive to infection in vitro than the monocytes.

In order to keep away from destruction and killing by the immune cells and also to facilitate its thrive, the *Leishmania* actually 'disguise' itself inside its host's immune cells [46,48]. This safe location actually facilitates them to circumvent the work of the humoral immune feedback because in this situation, the pathogen is keep safe inside an intact cell that belongs to their host's and actually not in blood vessels where open blood flow is likely to increase its contact with the immune cells. Furthermore, it may avert the immune cells from destroying the host's own tissue through the mechanism of non-danger surface signals which unfortunately for the host dettered the process of apoptosis [49] The primary cell types that the parasite Leishmania actually attack and then infiltrates are subset of phagocytotic

cells, *e.g*., neutrophils and macrophages, and this is what determines the fate of the chronicity of this infection [50].

Regularly, a phagocytotic cell, *e.g*., macrophage, will internalize and further kill a pathogen covered within an enclosed endosome and in order to do so, they then pervade this endosome with certain enzymes which will digest the pathogen [25,46-48]. However, in the case of *Leishmania*, these enzymes of macrophage have no effect to the parasite. This allowing internalized *Leishmania* even to undergo multiplication, fastly and enormously [51]. This almost unstopable growth of parasites eventually submerges the host's macrophage and other type of the host's immune cell available, and even making the infected host's cell to die [51,52].

The protozoan parasites of *L. major* may change the regular pattern of the first immune defense from eating-inflammation-killing and turn it upside down to eating-with no inflammation production- further no killing; and all of this took place inside of their host phagocyte. Unfortunately, this smart parasite corrupt its defence properties for their own welfare [27,28,52]. They use the mechanism of immune evasion by using phagocytosing cell named the polymorphonuclear neutrophil granulocytes (PMNs) carefully as their hidden vehicle, where they proliferate silently and undetected from the immune system and then enter the long-lived macrophages, unnoticed by the immune armamentarium to create a "*dormant*" infection [47,50].

According to van Zandbergen *et al* [52] that cited Sunderkotter *et al* which experimentally infecting mice with 1–2 × 10^6 *Leishmania*, The first phagocytic cells that infiltrate the site of experimental infection are the bunch of neutrophilic granulocytes (polymorphonuclear neutrophil granulocytes (PMN), and immediately act in accordance with the coming of a stream of macrophages (MF) in approximately in the following 48 hours. The PMN cells have the 'built-in' ability to internalize *Leishmania* promastigotes [28,51]. Unfortunately, within the PMN, these parasite can manipulate its actual primary function, make them 'toothless' and hijack the PMN antiparasite properties for their own survival [5,53]. Eventhough, during this intracellular 'staycation' the parasites failed to multiplicate, an interesting phenomena whose answers are still hidden and need to be explored further. Perhaps as far as we know, these cells might solely available as the parasite temporary shelter within the first hours or even days after infection established [54].

The PMN cell actually only have a very short life span and soon will undergo spontaneous apoptosis within the duration of 6–12 hours. According to van Zandbergen *et al*, [52] that infection with Leishmania actually slows down what supposed to be happen soon, named the apoptotic cell death program of PMN; this retardation can even delay it until up to 40+ hour and, therefore, promotes longevity of the parasite. However, after 42 hours, even most of infected PMN soon encounter apoptosis. An interesting phenomena that need further exploration is the fact that the time point at which infected PMN undergo apoptotic process, it coincides with the peak migration of the parasite into the infected tissue. Thus, *in situ*, the parasites would encounter apoptotic PMN harboring intracellular parasites rather than free extracellular Leishmania promastigotes [46,52]

A key factor in elongating infection is by way of the reticence of the adaptive immune cells [48-50]. This took place primarily during the intracellular inhabition phases, when amastigotes search for newly prone uninfected macrophages and then infecting them [44,51,52]. By underwent this process, the parasite actually are less prone to immune reactions. Almost all types of phagocytes are attacked [46]. For example, mincle has been described to be selected by the parasite *L. major*. Interaction between mincle and a protein

liberated by the infecting parasite results in actual weakened immune response in dendritic cells.

Lipophosphoglycan, biochemically, is a macrophage ligand which function immediately elaborated in the early steps of the occuring infection [55]. An interesting assays conducted with a mutant type of *L. major* which lacking in the gene lpg1 (lpg1-) actually revealed that this type of mutant organism are lessened for virulence when ongoing infection of murine macrophages, eventhough phenotypically there is no considerable changes [56]. These parasites actually do not harbor any LPG, but still accommodated normal levels of related GPI-anchored proteins and also glycoconjugates enzyme [57].

The lpg1- promastigotes are extremely prone to the activated complement system and also to the oxidative end-products of the host cells [25,57]. In addition to that condition, they failed to prevent phagolysosome fusion [42]. It has also been reported that L. major LPG2 null mutants (lpg2-) cannot live inside sandflies or in mammalian host cells. This type of organisms were even more revised than the lpg1- mutants strain and be short of all type of phosphoglycans enzyme, including LPG and proteophosphoglycans. Leishmania LPG has been shown to diminish the nuclear translocation of NF-κB in monocytes, bring about a subsequent decline in the assembly of IL-12. It can also affect the host's early immune reaction by modifying dendritic cells via the inhibition of antigen presentation and boosting an early response of IL-4 [56].

3.3. GLICOINOSITOLPHOSPHOLIPIDS (GIPLs)

Glicoinositolphospholipids (GIPLs) facilitates the survival of *L.major* inside macrophages by way of suppressing the enzyme nitric oxide synthase and also protein kinase C. Schneider *et al*.,[58] revealed the relation between the rate of macrophage infection by *L. braziliensis* and the GILP-containing detergent-resistant membrane domains of this parasite [58].

In both parasite developmental stages, the amount of the enzyme glycoinositol phospholipids (GIPLs) actually expressed at near-constant amount [59]. The construction of the enzyme GIPLs from amastigotes obtained from the tissue have been determined by hplc analysis of the deaminated and reduced glyc an head classes, and also by profiling the chemical and enzymic sequencing. The deduced structures appear to form a complete biosynthetic series, ranging from Man alpha 1-4GlcN-phosphatidylinositol (PI) to Gal alpha 1-3Galf beta 1-3Man alpha 1-3Man alpha 1-4GlcN-PI (GIPL-2). A small proportion of GIPL-2 was further extended by addition of a Gal residue in either alpha 1-6 or beta 1-3 linkage. From gc-ms analysis and mild base treatment, all the GIPLs were shown to contain either alkylacylglycerol or lyso-alkylglycerol lipid moieties, where the alkyl chains were predominantly C18:0, with lower levels of C20:0, C22:0 and C24:0. The parasite *L. major* amastigotes also contained at least two PI-specific phospholipase C-resistant glycolipids which are absent from promastigotes [60].

These neutral glycolipids were defiant to both mild acid and or mild base hydrolysis, contained terminal beta-Gal residues and were restrained during immense purification of amastigotes from cell membranes of the host. It is likely that these glycolipids actually are glycosphingolipids earn from the mammalian host. There have been studies comparing the GIPL profile of *L. major* amastigotes, *L. major* promastigotes and *L. donovani* amastigotes [58].

3.4. PROTEOPHOSPHOGLYCANS (PPG)

Other biochemical substance that also behave as the parasite's virulence factors is called Proteophosphoglycans.61 It is a highly glycosylated polypeptides with O-glycosylations; a structure indistinguishable to those found in the LPG and also in acid phosphatase [62]. Proteophosphoglycans are a growing family of highly glycosylated proteins belongs to *Leishmania* with many atypical and some idiosyncratic architectural features [61-63]. The obscure protein-glycan linkage in proteophosphoglycans - phosphoglycosylation of Ser by lipophosphoglycan-like structures– actually appear as a prime configuration of protein glycosylation in this parasite organism [62].

The main role of membrane PPGs actually is only partially revealed, but some experts postulated that its long chain configuration that enclosess the surface of parasite's plasma membrane might take part partially in its binding to the macrophage receptors [25]. The emmision of modified PPG by parasites when they colonized the macrophages seems to contribute to the maintenance of the parasitophorous vacuole [31]. Furthermore, the PPG is also have the ability to trigger the complement via the route of mannose-binding protein.

During the course of infection, Leishmania parasites are transmitted to its vertebrate hosts by the aid of female sand flies from the genus of Phlebotomine as they obtain blood from its host by puncturing deep into the dermis's upper capillaries with their spiked mouthparts [7-9]. In the sand fly midgut, secreted specific proteophosphoglycans from Leishmania actually form a biological plug known as the promastigote secretory gel (PSG), which blocks the gut and facilitates the regurgitation of infective parasites [64]. In a study using animal model, PSG injected to BALB/c mouse skin lead to the differential expression of 7900+ copy of transcripts and those transcript transiently up-regulated during the initial six hours post-wound and become more augmented for potently exacerbated cutaneous infection, and in turn will improved the probability of developing a patent cutaneous lesion, parasite growth and the evolution of the lesion [65].

3.5. 11 KDA KINETOPLASTID MEMBRANE PROTEIN (KMP-11)

KMP-11 is a hydrophobic protein that has been described to be associated to LPG which show strong immunoregulatory properties [66]. Kinetoplastid Membrane Protein -11 is present in both promastigotes and also amastigotes. The protein KMP-11 was associated with the membrane composition, which to some amount available at the cellular facet, flagellar pocket and also in the intracellular vesicles. The amount of its surface expression is actually higher in amastigotes than in promastigotes and the concnetration escalates during the stage of metacyclogenesis [67].

The rising expression of the protein KMP-11 in metacyclic promastigotes, and especially in the stage amastigotes, designates a role for this molecule in the close interaction of the parasite with its mammalian host. The presence of this molecule in amastigotes is consistent with the previously demonstrated immunoprotective capacity of vaccine prototypes based on the KMP-11-coding gene and the presence of humoral and cellular immune responses to KMP-11 in Leishmania-infected humans and animals [67,68].

This protein already recognized through its immunoregulatory properties and ahve the ability to induce the expression of IL-10 in cells from patients suffer from cutaneous and mucocutaneous leishmaniasis; unfrotunately, the mechanism through which this effect occurs remains unrevealed [66-68].

3.6. PROTEINASES

Proteinases also a crucial virulence properties that belongs to Leishmania. It can be grouped according to their catalytic domains, as serine-, threonine-, aspartyl-, metallo- and cysteine-proteinases . Among these, only the aspartyl-, metallo- and cysteine-proteinase classes have been extensively studied in *Leishmania*.[56].

Proteinases also considered as a crucial virulence factor of *Leishmania*, because as enzymes and through direct contact, it has the ability to hydrolyze any peptide bonds. This enzyme have the potency to destroy any proteins and peptides that might engage in a wide scale of biological purposes, including the making and establishing an infection [69]. The enzyme Proteinases actually occur pervasively in all living biological systems [70]. It is rich in functions, e.g., in human, varying from the digestion of proteins in order to achieve nutritive motives to the magnificent control of general protein role, *e.g.,* by hydrolyzing a extremely particular peptide bond in a certain protein surfactant [69,70].

Parasite proteinases widely known being elaborated in the (1) Pathogenesis, (2) Invasion-migration of the parasite through host tissues, (3) Degradation of immune related proteins, (4) Immune evasion and (5) Activation of inflammation [71,72]. Among protozoan parasites, the enzyme proteinases play crucial part in several acitivites, including (1) Transition of the parasite's life cycle, (2) Invasion of hosts, (3) Migration through tissue barriers, (4) Degradation of hemoglobin and other blood proteins, (5) Immune evasion, and (6) Activation of inflammation in the mammalian host [71-73].

Analysis of the genom carried out with different species of Leishmania that have been sequenced revealed that the amount of proteinase genes is maintained constantly among the various species [73]. Nonetheless, its heterogeneity is very diverse, *e.g*., the result of genomic survey on multiple databanks unveil that *L. braziliensis* alone has at least forty-four cysteine proteinases, twenty-three serine proteinases and ninety-seven metalloproteinase [74] Therefore, due to the wide range of action of *Leishmania* proteinases while the parasite is inside the mammalian host, it is equitable to seek for the relation between proteinase enzymatic activity and the clinical manifestation of leishmaniasis.

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