Review Article

Biochemical Properties Of Parasite Virulence Factor: Lesson Learned From Leishmania

ABSTRACT

Leishmania, a parasitic protozoan, is a single-celled organism of the genus trypanosomes that are responsible for the disease leishmaniasis. Transmission ~~occured~~ occurred 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~~ belong to the genus *Leishmania* ~~include~~ including the active compound named proteophosphoglycan (PPG), GP63 metalloprotease, and lipophosphoglycan (LPG). ~~these substance~~This substance established on the surface of the parasite. The aim of this review article is to make an insight ~~of~~ into the biochemical characteristics of *Leishmania* spp. virulence factors, the armamentarium that ~~predispose~~ predisposes their pathogenesis, its invasion, and virulence to the mammalian host.

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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~~can 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~~ accommodation and adapt to environmental ~~shift~~ shifts is condemnatory for all organisms to carry on homeostasis and eventually ~~its~~ it's for survival [4].

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

other ~~action~~ actions for immunomodulation or ~~immuno~~immune-evasion of their host immune responses.

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

2. *LEISHMANIA* SPP., LEISHMANIASIS AND ITS GLOBAL EPIDEMIOLOGY

Leishmania (/liːʃˈmeɪniə/) is a genus of parasitic ~~organisme~~ organism ~~belongs~~ belonging to the *Trypanosomes.* This organism ~~causing~~ causes 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~~occurrence, the vector ~~divide~~ divided 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~~evinced or have the status as potential or probable vectors, globally. This protozoan parasite ~~actually has~~has a vertebrate organism as its primary host. *Leishmania* is repeatedly found to infect rodents, canids, hyraxes, and ~~even~~ 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~~ a 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 the tropical and subtropical region (in number might reach to 100 countries) [11]. Recent literature revealed a significant increase elevation ~~regarding~~ in 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~~intercontinental, massive international tourism, asylum seekers/immigrants from endemic countries, and even multinational based military operations in endemic areas [13,14]

Area ~~where Leishmaniasis acquired~~, where Leishmaniasis acquired, is already suspected; South America is the main source area of cutaneous leishmaniasis, and escapade tourists on long-term ~~vacation~~ vacations in highly-endemic forested areas are at certain peril1[5,16]. On contrary, international tourists are in danger ~~while~~ when they travel to certain the Mediterranean or middle east destinations where there is an 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~~ traveling to and staying in an endemic area, even if this occurred several months or years ago; this become an important key factor in making a 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~~ conditions 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

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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~~ individuals 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~~ living in a low socio-economy level of countries in Africa, Latin America and Asia, and this condition is often linked with underlying ~~condition~~ conditions 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.

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3. VIRULENCE FACTORS

Virulence is described as an internal ~~properties~~ property of an organism that enabled them to infect their host, a substance pinned internally and can cause ~~a~~ disease in a 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~~membrane-associated or cytosolic in nature. In terms of bacteria, the cytosolic factors facilitate the bacterium to undergo quick adaptive-metabolic, ~~physiological~~physiological, and morphological shifts [24].

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

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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~~acting as a safeguard against the host cell's immunity [28] *Leishmania* then take over the macrophage's membrane fusion machinery, ~~didacting~~ didactic 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]

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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~~layout of virulence factors within the inhabited cell.

3.1. BIOCHEMICAL PROPERTIES AND CHALLENGES ~~ON~~ TO THEIR GENOMES DATA

The biochemistry and cell biology of *Leishmania* spp. ~~is~~ are similar to that of other kinetoplastids. They share the same main morphological features, including a single flagellum ~~which~~ that has an invagination, the flagellar pocket, at its base, a kinetoplast, which is found in the single mitochondrion, and a ~~subpelicular~~subpellicular 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~~ successfully apportioned the initial diagrams of the metabolism pathway ~~belongs~~ belonging 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~~function based on homology searches, and therefore it is likely that *in silico* models must be ~~continously~~ continuously 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 the 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~~ broadened 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~~family's databases [37,38]. It provides several functional instruments for inquiring about 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~~ asset 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~~ Another famous database that can be mentioned is the Kyoto Encyclopedia of Genes and Genomes (KEGG) ~~that~~ which combines three ~~kind~~ kinds of data: (1) chemical, (2) genomic, and also (3) functionality information for a wide array of species [40] ~~Eventhough~~ Even though 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~~ accession 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~~ accession 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~~ accommodates preliminary elucidated metabolism pathways from a wide diversity of species. Actually, many

organism-specific BioCyc databases are still under ~~continously~~ continuously agile buildout and ~~continous~~ continuous 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~~are virulence factors. In the case of *Leishmania* spp., the list of its virulence factors ~~are~~ is as follow~~:~~ (1) lipophosphoglycan (LPG), (2) glicoinositolphospholipids (GIPLs), (3) proteophosphoglycan (PPG) and (4) the 11 kDa kinetoplastid membrane protein (KMP-11). ~~Eventhough~~ Even though 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 are able to facilitate and even modify the Leishmania-host immune cells relationship.

3.2. LIPOPHOSPHOGLYCAN (LPG)

Leishmania parasite owns ~~a~~ an LPG, a class of molecules that is 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~~ has the ability to TLR-2, a specific ~~signalling~~ signaling 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 are ~~actually very~~very dynamic and ~~oscillates~~ oscillate 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~~the polysaccharide glycan in the LPG is exceptionally fluctuating and contrasting. The amount and variants of lLPG are ~~actually exploitable~~exploitable in terms of making them as a biochemical marker. Distinct lifecycle stages of the parasite *Leishmania* might produce different LPG. Furthermore, Lectins, a set of proteins ~~which~~ that attach to several different categories of glycans, are repeatedly used to perceive and sense these LPG variants, *e.g*., peanut agglutinin specifically ~~attachs~~ attaches to a particular LPG located on the facet of the infective form of *L. major* [46].

Lipophosphoglycan is ~~actually empowered~~empowered strongly by the parasite primarily to maintain its survival inside ~~their~~ its host [46]. The exact techniques ~~that~~ used by the parasite apply is not clearly revealed~~;~~ , but this property ~~being~~ is 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~~ needs 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~~ 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' ~~'disguises' 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~~is kept safe inside an intact cell that belongs to ~~their ~~its ~~host's ~~hosts 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 ~~deterred the process of apoptosis [49] The primary cell types that the parasite Leishmania actually ~~attack ~~attacks and then infiltrates are a subset of phagocytotic cells, *e.g*., neutrophils and macrophages, ~~and ~~and this is what determines the fate of the chronicity of this infection [50].

Regularly, a phagocytotic cell, *e.g*.~~, macrophage~~, 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 ~~macrophages have no effect ~~to ~~on the parasite. This ~~allowing ~~allows internalized *Leishmania* even to undergo multiplication, fastly and enormously [51]. This almost ~~unstopable~~unstoppable growth of parasites eventually submerges the host's macrophage and ~~other type~~another type of the host's immune cell available, and even ~~making ~~makes 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~~, and all of this took place inside of their host phagocyte. Unfortunately, this smart parasite ~~corrupt ~~corrupts its ~~defence ~~defense properties for ~~their ~~its own welfare [27,28,52]. They use the mechanism of immune evasion by using phagocytosing ~~cell ~~cells 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~~*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~~this parasite can manipulate its actual primary function, make them 'toothless' and hijack the PMN antiparasite properties for their own survival [5,53]. ~~Eventhough~~Even though, during this intracellular 'staycation' the parasites failed to multiplicate, ~~an interesting phenomena~~interesting phenomenon whose answers are still hidden and need to be explored further. Perhaps as far as we know, these cells might solely be available as the ~~parasite ~~parasite's temporary shelter within the first hours or even days after infection is established [54].

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The PMN cell ~~actually only~~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~~happen soon, named the apoptotic cell death program of PMN; this retardation can even delay it until up to 40+ ~~hour ~~hours and, therefore, promotes longevity of the parasite. However, after 42 hours, even most ~~of ~~infected PMN soon encounter apoptosis. ~~An interesting~~Interesting phenomenon~~a~~ that need further exploration is the fact that the time point at which infected PMN ~~undergo ~~undergoes 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~~ inhibition phases, when amastigotes search for newly prone uninfected macrophages and then infecting them [44,51,52]. By ~~underwent~~ undergoing 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.

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Lipophosphoglycan, biochemically, is a macrophage ligand ~~which~~ that function immediately elaborated in the early steps of the ~~occuring~~ occurring infection [55]. ~~An interesting assays~~Interesting assays conducted with a mutant type of *L. major* which lacking in the gene lpg1 (lpg1-) ~~actually revealed~~revealed that this type ~~of mutant~~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~~do not harbor any LPG, but still accommodated normal levels of related GPI-anchored proteins ~~and also~~and glycoconjugates ~~enzyme~~ enzymes [57].

The lpg1- promastigotes are extremely prone to the activated complement system ~~and also~~and 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~~ organism were even more revised than the lpg1- mutants strain and ~~be~~ was 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~~ bringing 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~~ facilitate the survival of *L.major* inside macrophages by way of suppressing the enzyme nitric oxide ~~synthase~~ synthase and also protein kinase C. ~~Schneider~~ 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].

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In both parasite developmental stages, the amount of the enzyme glycoinositol phospholipids (GIPLs) is actually expressed at a near-constant amount [59]. The construction of the enzyme GIPLs from amastigotes obtained from the tissue ~~have~~ has been determined by ~~hplc~~ 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.*

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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~~ earned 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~~ Another biochemical substance that also ~~behave~~ behaves as the parasite's virulence factors is called Proteophosphoglycans.61 It is a highly glycosylated ~~polypeptides~~ polypeptide with O-glycosylations; a structure indistinguishable ~~to~~ from those found in the LPG and also in acid phosphatase [62]. Proteophosphoglycans are a growing family of highly glycosylated proteins ~~belongs~~ belonging 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~~ appears 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~~long-chain configuration that ~~enclosess~~ encloses the surface of the parasite's plasma membrane might take part partially in its binding to the macrophage receptors [25]. The ~~emmision~~ emission 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~~also has ~~has~~ has the ability to trigger the complement via the ~~route~~ route of mannose-binding protein.

During the course of infection, Leishmania parasites are transmitted to ~~its~~ their 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 an animal model, PSG injected to BALB/c mouse skin lead to the differential expression of 7900+ copy of transcripts, and ~~those transcript~~those transcripts transiently up-regulated during the initial six hours post-wound and become more augmented for potently exacerbated cutaneous infection, and in turn will ~~improved~~ improve 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~~ with LPG which ~~show~~ shows strong immunoregulatory properties [66]. Kinetoplastid Membrane Protein -11 is present in both promastigotes and ~~also~~ 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~~ concentration 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~~ 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 is already recognized through its immunoregulatory properties and ~~ahve~~ has the ability to induce the expression of IL-10 in cells ~~from~~ of patients to suffer from cutaneous and mucocutaneous leishmaniasis; ~~unfrotunately~~unfortunately, the mechanism through which this effect occurs remains unrevealed [66-68].

3.6. PROTEINASES

Proteinases are also ~~a~~ crucial virulence properties that ~~belongs~~ belong 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 are 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~~ has the potency to destroy any proteins and peptides that might engage in a wide ~~scale~~ 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~~humans, 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~~ an extremely particular peptide bond in a certain protein surfactant [69,70].

Parasite proteinases ~~widely known~~are widely known being elaborated in the (1) Pathogenesis, (2) Invasion- migration of the parasite through host tissues, (3) Degradation of ~~immune related~~immune-related proteins, (4) Immune evasion, and (5) Activation of inflammation [71,72]. Among protozoan parasites, the enzyme proteinases play a crucial part in several ~~acitivites~~activities, 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~~ genome 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 the 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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