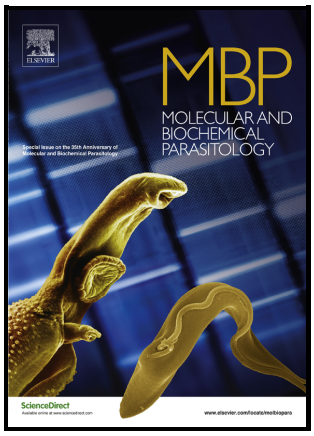
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Surface Molecules of *Leishmania*: From Virulence Determinants to Therapeutic and Vaccine Targets

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Keywords: *Leishmania*; surface virulence factors; therapeutic targets; vaccine development; metacyclogenesis; host-parasite interaction.

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Abstract:

Leishmaniasis is a group of neglected tropical diseases (NTDs) caused by protozoa of the genus *Leishmania* that affect vulnerable populations in tropical and subtropical regions. The disease manifests in cutaneous, mucocutaneous, and visceral clinical forms. This major public health disease presents high morbidity, and despite the global impact of leishmaniasis, there are few therapeutic options available and no currently licensed human vaccines. Besides, the available therapeutic agents are associated with high toxicity and treatment failure. These limitations highlight the importance of identifying new therapeutic targets, which will contribute to the development of more effective, safer and shorter treatment options. In this context, surface molecules of *Leishmania* emerge as attractive therapeutic targets due to their roles in host cell adhesion, immune evasion, and intracellular survival. In addition to their translational potential for drug discovery and vaccine development, these surface molecules are key virulence factors that play central roles in parasite biology and disease pathogenesis. Understanding their structure and function is essential not only for elucidating mechanisms of host–parasite interaction, but also for identifying novel therapeutic and prophylactic strategies. Importantly, molecules such as GP63 (a major surface metalloprotease), LPG (lipophosphoglycan), and KMP-11 (kinetoplastid membrane protein 11) combine essential biological functions with demonstrated immunogenic properties, making them promise as targets for both chemotherapeutic and prophylactic interventions. This review aims to explore the structural and functional characteristics of major surface virulence factors in *Leishmania*, highlighting their roles in the parasite–host interaction and discussing their translational potential for therapeutic and vaccine development.

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1. INTRODUCTION

Leishmaniasis is a vector-borne disease caused by protozoa of the genus *Leishmania* (Euglenozoa: Trypanosomatidae), obligate intracellular parasites that primarily infect macrophages of the mononuclear phagocyte system (1). Transmission occurs through the bite of infected female phlebotomine sand flies, which introduce the parasite into the mammalian host during a blood meal (2) Of the ~30 known *Leishmania* species, approximately 21 are pathogenic to humans, with infections predominantly occurring in tropical and subtropical regions of the Americas, Asia, Africa, and the Mediterranean (3).

Despite its widespread impact, leishmaniasis remains neglected in terms of therapeutic innovation. Current treatment options are limited by high toxicity, prolonged regimens, and the emergence of drug-resistant strains. No vaccine is licensed for human use, and immunoprophylactic development is hindered by the parasite's antigenic variability and capacity to modulate host immune responses (4–6).

The disease's pathogenesis is tightly linked to the parasite's digenetic life cycle. Within the sand fly vector, *Leishmania* undergoes metacyclogenesis, a differentiation process in which non-infective procyclic promastigotes become infective metacyclic forms. This transition is accompanied by metabolic reprogramming and upregulation of key surface virulence factors, which are essential for host cell invasion and immune evasion (7,8). Following transmission to the vertebrate host, metacyclic promastigotes are phagocytosed by macrophages, differentiate into amastigotes, and replicate within the phagolysosome. Their survival in this hostile environment depends on surface molecules that protect against microbicidal mechanisms, manipulate host signaling pathways, and interfere with immune detection (9,10)

These surface molecules, including LPG, GP63, KMP-11, proteophosphoglycans (PPGs), and glycoinositolphospholipids (GIPLs), form a dynamic and multifunctional glycocalyx critical to *Leishmania* pathobiology. They mediate adhesion to host cells, modulate immune signaling through Toll-like receptors, assist in nutrient acquisition, and influence disease outcome by shaping tissue tropism and immunopathology (5,11). Given their central role in infection and immune modulation, surface virulence factors have gained attention as promising targets for novel therapeutics and vaccines. Our research group has contributed to this field by investigating a diverse array of natural and synthetic compounds with antileishmanial activity, including guanidine derivatives, marine algal metabolites, and bioactive peptides. Although the precise molecular targets of these compounds are not yet fully elucidated, accumulating evidence suggests that part of their leishmanicidal mechanisms may involve interactions with, or disruption of, the parasite's surface architecture. This includes interference with the integrity, biosynthesis, or function of surface molecules essential for parasite survival, host cell adhesion, and immune evasion. Such findings reinforce the surface of *Leishmania* as a critical interface for therapeutic intervention, even when specific surface receptors or enzymatic targets remain to be characterized (12–16).

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This review provides a comprehensive analysis of *Leishmania* surface molecules with established or potential roles in virulence and immune evasion. We explore their structural features, biological functions, and translational potential, while identifying knowledge gaps and outlining future directions for therapeutic and vaccine development.

2. SURFACE VIRULENCE FACTORS

Surface molecules of *Leishmania* (Figure 1) play a central role in parasite virulence by mediating adhesion, immune evasion, and intracellular survival, whilst also representing promising targets for drug discovery and vaccine development. Their involvement in host-pathogen interactions, particularly through engagement with Toll-like receptors (TLRs) and complement receptors, emphasizes their potential as candidates for therapeutic intervention (17–20). Molecules such as lipophosphoglycan (LPG), glycoinositolphospholipid (GIPL), and glycoproteins (GP63) have been shown to modulate key immune pathways and disease progression (21–23). To guide the identification of promising therapeutic and vaccine targets, the following section explores the structural diversity and functional relevance of *Leishmania* surface molecules, emphasizing their roles in host–pathogen interactions. These molecules are organized into five categories based on membrane association and biological function: (i) surface glycocalyx components (e.g., LPG, PPG, and GIPLs); (ii) GPI-anchored proteins (such as GP63 and GP46); (iii) type I integral membrane enzymes (including acid phosphatases and nucleotidases); (iv) polytopic membrane transporters essential for nutrient acquisition and drug resistance; and (v) peripheral and lipid-associated elements (such as HASPs, amastins, glycosphingolipids, and host-interacting glycoproteins). This structure provides a comprehensive understanding of how these surface structures contribute to *Leishmania* pathogenesis and highlights their potential as targets for therapeutic and prophylactic strategies. For this, to establish the context for the discussion, we provide some aspects regarding the biological context in which *Leishmania* surface molecules are upregulated and functionally activated. In this regard, metacyclogenesis plays a pivotal role as it marks the differentiation stage where key virulence factors become prominent and functionally relevant. This transitional stage is marked by surface remodeling and selective enrichment of key virulence factors, setting the molecular basis for host invasion.

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2.1 Metacyclogenesis and surface remodeling

Metacyclogenesis is a critical differentiation process in the *Leishmania* life cycle, during which non-infective procyclic promastigotes differentiate into infective metacyclic forms within the gut of the phlebotomine vector. Traditionally defined by morphometric criteria, such as cell body slendering and flagellar elongation, this process is now increasingly recognized as a complex molecular remodeling event, involving extensive changes in gene expression, surface composition, and metabolic activity (24–26).

One of the hallmark features of metacyclogenesis is the restructuring of the parasite surface, particularly the upregulation and modification of virulence-associated glycoconjugates, such as LPG and the zinc-dependent metalloprotease GP63. These molecules are substantially enriched in metacyclic forms, contributing to increased resistance to complement-mediated lysis and enhanced infectivity both *in vitro* and *in vivo* (27–29). Surface remodeling can be evaluated by biochemical and immunological parameters, including decreased lectin or monoclonal antibody binding, increased complement resistance, and elevated capacity to infect human macrophages (30).

Proteomic analyses have revealed that metacyclic promastigotes exhibit a proteome biased toward motility and host invasion, with a higher abundance of proteins such as paraflagellar rod protein 1D, α-tubulin, and β-tubulin. In contrast, components involved in protein biosynthesis tend to be downregulated at this stage, consistent with the absence of cell division in metacyclic forms (26). Furthermore, transcriptome analyses revealed that metacyclic promastigotes display an amastigote-like profile, marked by enhanced expression of surface proteins and metabolism-related genes to amastigote forms; these similarities suggest a preadaptation to the mammalian host intracellular environment. Amastin, an amastigote-related protein, appears to have its mRNAs downregulated in procyclic promastigotes, but upregulated in metacyclic promastigotes at levels comparable to those observed in amastigotes. Both LPG and GP63 show increased mRNA levels in metacyclic promastigotes, whereas LP63 appears to be downregulated, but strongly upregulated in procyclic promastigotes. Amino acid transporters and autophagy related genes have also increased levels in metacyclic forms (228). Mitochondrial enzymes, including the β-subunit of succinyl-CoA synthetase and cytochrome c oxidase subunit IV, also show stage-specific expression patterns,

suggesting metabolic adjustments that support energy demands associated with infectivity.

Complementing the proteomic data, NMR-based metabolomic profiling has identified distinct metabolite signatures between logarithmic and stationary-phase promastigotes, with alterations in metabolites such as citraconic acid, isopropyl acid, L-leucine, ornithine, caprylic acid, capric acid, and acetic acid (31). These findings highlight biochemical shifts that may underlie or facilitate the differentiation process and point to potential metabolic vulnerabilities exploitable for therapeutic intervention. Although not all surface molecules discussed in this review are directly modulated during metacyclogenesis, this stage constitutes a biological bottleneck in which specific virulence factors are selectively upregulated, enabling the parasite to survive host defenses and initiate infection.

2.2 Surface glycocalyx components

2.2.1 Lipophosphoglycan (LPG)

LPG is the main cell surface glycoconjugate in *Leishmania*, abundantly found in metacyclic promastigote. This heterogeneous molecule has a molecular weight of nine kDa and is composed of phosphorylated units of di-, tri-, and tetra saccharides, containing mainly arabinose (Ara), mannose (Man), and galactose (Gal), which are anchored to the cell surface via 1-O-alkyl-2-lyso-phosphatidylinositol moieties. In *Leishmania*, these glycolipids are inserted into a plasma membrane distinct from that of mammalian cells, where ergosterol replaces cholesterol as the main sterol and inositol phosphoceramide (IPC) is the predominant sphingolipid (32). Structurally, there are generally 16 sugar units arranged linearly, connected by α-glycosidic bonds in LPG (33). During *Leishmania* metacyclogenesis, structural modifications in the expression of this molecule occur. In metacyclic promastigotes, LPG presents a more complex structure (Figure 2), as well as changes in the number of phosphoglycan (PG) repeats (7,34).

Pioneering studies by Beverley and Turco (35) were fundamental in establishing the central role of LPG in *Leishmania* virulence. Their work with LPG-deficient mutants

demonstrated that this surface glycoconjugate is essential for parasite survival both in the sand fly vector and within mammalian host macrophages. LPG-deficient promastigotes exhibited markedly reduced persistence in phagolysosomal compartments and increased susceptibility to host-derived nitric oxide (NO), highlighting the molecule's protective function against oxidative stress and the hostile phagolysosomal environment. These seminal findings laid the foundation for subsequent mechanistic studies that revealed how metacyclic promastigotes of *Leishmania donovani* exploit LPG to inhibit phagolysosome biogenesis by promoting the accumulation of peripheral F-actin around the phagosome, thereby forming a physical barrier that impedes its fusion with early endosomes and late lysosomes (36). This strategy allows the parasite to hijack the host cell cytoskeleton at the onset of infection, facilitating the formation of a permissive parasitophorous vacuole. In parallel, LPG has also been implicated in impairing the recruitment of lysosome-associated membrane protein 1 (LAMP-1) during phagosome maturation, a phenomenon observed in *Leishmania braziliensis* (37). Collectively, these studies underscore LPG as a critical factor in immune evasion and intracellular survival, validating its long-standing recognition as a promising target for therapeutic intervention (38). However, Thomas Ilg (229) reported that *L. mexicana* mutants lacking PG repeats, and thus deficient in LPG, retained the ability to infect mice and macrophages, demonstrating that PG repeat synthesis is probably not required for the virulence of *L. mexicana*. Polymorphism was detected among various strains of *L. braziliensis* from distinct clinical isolates, especially regarding the type and the size of sugars linked to the repeat unit motif of LPG (230). In addition, *L. infantum* strains also exhibited different types of LPG, which has a substantial impact in host cell activation through Toll-like receptors (TLRs), a mechanism that will be further investigated below (231,232). The PG domain of *L. major, L. mexicana, L. infantum and L. tropica* exhibits comparable glycosylation patterns (position C3 of the galactose); *L. aethiopica* commonly displays a different glycosylation motif at another position (C2 of the mannose) (231). Nevertheless, *L. donovani* possesses a linear LPG as this species lacks side-chain substitutions in the PG repeats (233). Because LPG structures vary among *Leishmania* species and even among strains from the same species, and also procyclic and metacyclic LPG have different structures, the complexity and heterogeneity can be a hindrance to vaccine development based on LPG (231).

Beyond its role in promoting parasite survival within host phagocytes, LPG also engages directly with the host immune system, acting as a potent modulator of innate and adaptive

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responses. Indeed, peripheral blood mononuclear cells from cured Kala-Azar patients were found to proliferate and produce IFN-γ *in vitro* in response to LPG isolated from *L. major* (39). Surface glycolipids play an important role in parasite-phagocyte interactions, stimulating T cell-mediated immune responses. Immunization assays further revealed that T cell activation is associated with the core structure of LPG, rather than with its lipid or repetitive phosphoglycan moieties, suggesting that the conserved glycan core is central to its immunogenicity (40). One of the key immunological interfaces of LPG is with TLRs, especially TLR2, which function as pattern recognition receptors (PRRs) central to the initiation of innate immune responses. The LPG–TLR2 interaction activates downstream pathways involving kinases such as tumor progression locus 2 (TPL2), modulating inflammatory responses (41). Experimental models have shown that inoculation of *L. mexicana* or purified LPG into the dermis of BALB/c mice triggers T cell proliferation and TLR2-dependent immune activation (18). These findings position LPG not only as a virulence factor but also as an immunomodulatory molecule with clear translational relevance for vaccine development.

Recent experimental data have demonstrated with increasing clarity that LPG also activates another receptor, TLR4 (37,42). Promastigotes stimulate TLR4 to facilitate parasite phagocytosis. To confirm TLR4's role in internalization, a recent study showed that silencing this receptor in macrophages reduced the infection rate, highlighting the crucial role of the LPG-TLR interaction in internalization (17). LPG from *L. amazonensis* and *L. braziliensis*, as well as whole parasites, are capable of inducing proinflammatory interleukins (ILs) such as IL-32, IL-1β, and IL-6 in human PBMCs, and this regulation depends on TLR4 and NOD2 receptors (23). Beyond macrophages, LPG is a key component in interactions with neutrophils, as LPG-knockout parasites fail to induce autophagy of infective forms in these cells (43). Neutrophils are known to release neutrophil extracellular traps (NETs) to capture promastigotes. Recently, extracellular vesicles (EVs) from *L. amazonensis* containing LPG were shown to induce NET formation in a TLR-dependent manner, as the addition of TLR2 and TLR4 inhibitors significantly reduced NET induction by EVs (44). Interestingly, *L. mexicana* LPG indirectly activates natural killer T (NKT) cells by initially stimulating TLR2 on dendritic cells (DCs), which leads to increased expression of TLR2, major histocompatibility complex class II (MHC II), and cluster of differentiation 86 (CD86), as well as IL-12 production, ultimately promoting IFN-γ synthesis by NKT cells during the innate phase

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of infection (45). Both soluble and membrane-associated LPG can induce the production of reactive oxygen species (ROS) in PBMCs. Interestingly, ROS production is blocked by anti-TLR2 antibodies, but not by anti-TLR4 (46). Structurally, TLRs contain leucine-rich repeats (LRRs), and mutations impair the LPG-TLR4 interaction, suggesting that LPG-TLR binding may be specific and restricted to LRR domains. Therefore, disrupting the LRR-LPG association may be an important factor in reducing the internalization of infective forms, and identifying the LPG domains responsible for this interaction may contribute to future control strategies (47,48). Although the LPG–TLR4 interaction leads to pro-inflammatory responses that are desirable for vaccine purposes, the effects of this activation are not always beneficial. This interaction may also trigger modulation via TLR2 and potentially TLR4, activating PI3K/Akt pathways that promote Th2-like responses, which can reduce microbicidal activity. At the same time, these same ligands can stimulate TLR4 to induce increased expression of iNOS, TNF, and IFN-γ, favoring a more protective Th1 response (49).

CR1 and CR3 receptors are also LPG targets (20). CR3 was shown to bind LPG from *L. mexicana* (50). Supporting these findings, a mutant strain of *L. major* lacking surface LPG, and exhibiting attenuated virulence, is highly susceptible to the human complement system and unable to inhibit phagosomal fusion, making it vulnerable to oxidative stress. In contrast, *L. major* expressing LPG binds complement C3b, resists low pH and phagosomal proteases, and persists intracellularly by inhibiting host signaling pathways (51).

The aforementioned studies establish LPG as a promising candidate for both drug development and vaccine strategies. The inhibition of key enzymes in the LPG biosynthetic pathway could impair parasite viability and disrupt essential host–parasite interactions, providing a dual benefit of reducing infectivity and improving host immune clearance. Besides, there is a potential of using LPG-derived fragments in the formulation of conjugated or subunit vaccines to elicit protective immunity, particularly by enhancing antigen presentation and T-cell responses (5).

2.2.2 Proteophosphoglycan (PPG)

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PPG can be found in two forms: a secreted, water-soluble form, and a structural form anchored to the cell membrane via glycosylphosphatidylinositol (GPI) anchors (52). This phosphoglycan is present on the surface of both amastigotes and promastigotes, as well as in the flagellar pocket, endosomes, and lysosomes (53) PPG is secreted in large quantities by amastigotes through the flagellar pocket into the parasitophorous vacuoles of host cells (54). Unlike LPG, this molecule contains a protein backbone composed of different amino acid residues. In *L. major*, the structure is rich in serine, alanine, and proline residues, with serine residues glycosylated through phosphoester linkages. The sugars present in this molecule are like those found in LPG. Moreover, the structure of PPG is highly like mucins found in mammalian cells (54,55). Analysis of this phosphoglycan, recovered through extraction and purification processes, reveals a composition of 28% protein, 5% phosphate, and 67% carbohydrate. Additionally, PPG's high molecular weight and extensive glycosylation render it resistant to protease degradation (55,56). Its anchoring to the plasma membrane is mediated by GPI structures, which in *Leishmania* share a conserved core but are synthesized via pathways divergent from those of mammalian hosts, offering potential selectivity for therapeutic targeting (57,58).

Metacyclic promastigotes produce in the midgut of the sand fly vector a viscous gel-like matrix, the Promastigote Secretory Gel (PSG), which is primarily composed of filamentous mucin-like proteophosphoglycan (fPPG), that enhance the transmission capacity of the metacyclic forms by blocking the anterior midgut lumen and stomodeal valve of the invertebrate vector. As a result, regurgitation of infectious forms is increased, leading to a higher infection rate. This represents a critical strategy employed by the parasite to ensure transmission (59,60). PPG also contributes to *Leishmania*-host cell interaction and modulates macrophage biology during infection (61). This molecule induces vacuolization in peritoneal macrophages *in vitro*. The secretion of this glycoconjugate by intracellular parasites contributes to the expansion of phagolysosomal compartments in infected cells (62). Under normal circumstances, activated macrophages produce IL-12, which stimulates the differentiation and proliferation of Th1 cells. These, in turn, secrete interferon-gamma (IFN-γ), enhancing the macrophage's microbicidal activity through the production of Reactive Oxygen Species (ROS) and NO. However, PPG suppresses the production of IL-12 and IFN-γ in a dose-dependent manner, thereby

downregulating ROS and NO synthesis. This immunosuppressive effect ultimately facilitates parasite persistence within host cells (54,55).

Interestingly, a DNA vaccine using the N-terminal domain of *L. donovani* PPG demonstrated efficacy by increasing levels of NO, IFN-γ, tumor necrosis factor-alpha (TNF-α), and IL-12 while reducing levels of transforming growth factor-beta (TGF-β), IL-4, and IL-10, key determinants of Th2 activation. This suggests that depending on the antigenic region used for vaccine development, it is possible to elicit a favorable response for the elimination of intracellular forms (63).

The intracellular PPG secreted by amastigotes interacts with the complement system to activate it via the lectin pathway, thereby contributing to the development of parasitic infection. Complement activation results in the generation of C3a, C4a, and C5a factors, leading to the recruitment of monocytes susceptible to infection. Hosts deficient in these factors exhibit delayed or absent lesion development during leishmaniasis (62). Interestingly, mice chronically infected with *L. mexicana* typically do not produce antibodies or T cells specific for intracellular PPG. Antibody titers are very low in mice vaccinated with this molecule, and $CD4^+$ T cells are undetectable. It is known that intracellular PPG behaves immunologically like a carbohydrate due to the lack of $CD4^+$ T cell induction. In contrast, recombinant peptides derived from intracellular PPG can stimulate cellular immune responses, as evidenced by the high titers of specific antibodies detected in the sera of vaccinated mice. However, native intracellular PPG exhibits a high resistance to proteolytic degradation and appears to be non-degradable by macrophages. As a result, $CD4^+$ T cell activation is not triggered, since the molecule escapes antigen processing due to its dense and complex glycosylation. These elaborate glycan chains likely represent an evolutionary adaptation to evade recognition by the host immune system (64).

Recent studies have demonstrated the promising potential of the N-terminal domain of PPG as a candidate for DNA vaccine development, such as a vaccine based on this domain from *Leishmania donovani*, which was shown to enhance NO, IFN-γ, TNF-α, and IL-12, whilst downregulate Th2-associated cytokines leading to a protective Th1-type immune response. Additionally, genetic approaches could be used to reduce glycan complexity or introduce protease-sensitive linkers, thus enhancing the molecule's immunogenicity and enabling its inclusion in future DNA-based vaccine platforms. A recombinant plasmid encoding the N-terminal domain capable of secreting soluble

intracellular PPG into the parasitophorous vacuole reduced lesion size and parasite burden in BALB/c mice, while also shifting the IgG2a/IgG1 ratio to greater than (65).

These findings underscore the dual role of PPGs as both structural and immunomodulatory molecules, contributing not only to vector-mediated transmission and intracellular persistence but also offering opportunities for translational applications such as vaccine development. While PPGs exert their effects predominantly through physical shielding and immune suppression, other surface glycoconjugates adopt a more subtle, yet equally strategic role in immune modulation.

2.2.3 Glycoinositolphospholipid (GIPL)

As with many surface macromolecules, including major glycoproteins and the LPG complex, GIPL is anchored to the plasma membrane via glycosylphosphatidylinositol (GPI) moieties. Furthermore, this molecule can be present in the membrane in a free form, not linked to glycoproteins or phosphoglycans (66,67). The composition and structure of GIPL may vary depending on the species. In *L. tropica*, the glycan chain terminates in α-mannose, which is structurally related to the GPI anchors of both surface proteins and LPG, whereas in *L. major* it terminates in α-galactose, being related only to LPG anchors (68). Regarding the complexity of this molecule, in *L. major*, analysis of native and dephosphorylated glycolipids has revealed that the glycosidic portions consist of 4 to 10 sugar residues, typically composed of mannose, galactose, and non-acetylated glucosamine. These GIPLs, like other surface glycolipids, are built upon a phosphatidylinositol core predominantly containing C16:0 and C18:0 fatty acids, and may feature structural variations such as alkylacyl or lyso-alkyl lipid tails, depending on the parasite life stage (66,67). Additionally, some GIPLs present glucose, monophosphorylated hexose residues, and a phosphatidylinositol moiety indicated by a single myo-inositol monophosphate residue (69). GIPL is expressed at nearly constant levels in both promastigote and amastigote forms; however, the GIPL profile in amastigotes is enriched with shorter glycan chains and a lyso-alkyl-PI lipid moiety instead of an alkylacyl-PI portion (70).

Regarding its function, the glycocalyx formed by GIPL may play a critical role in shielding and protecting parasite plasma membrane proteins (such as transporters, glycoproteins, enzymes, etc.), which are important for cellular maintenance and various

biological processes (70). Furthermore, the glycocalyx formed by GIPL in amastigotes protects the internalized parasite against lysosomal hydrolases and may also act as a ligand for host cell interaction, as previously described (71). As documented, in resistant mice, a Th1 response develops after infection with *L. major*, triggering IFN-γ production, which activates macrophages to synthesize NO. However, in susceptible strains, GIPL stimulates IL-4 production, inhibiting NO synthesis in macrophages, promoting a Th2 response and intracellular survival. Most of this inhibitory activity of GIPL has been attributed to its alkylacylglycerol moiety (72). It has been shown that GIPL from *L. infantum* and *L. braziliensis* induces TNF-α and NO production in C57BL/6 mice, mainly via TLR-4 (73). Additionally, binding of anti-GIPL IgG antibodies during the release of amastigote forms induces IL-10 expression and suppresses IL-12 production. This reduces IFN-γ expression in T cells and, consequently, NO production, contributing to the survival of intracellular forms (74). Although Th1 and Th2 responses play opposing roles, maintaining a balance between them is essential for the design of effective and safe vaccine formulations. While a Th1 response characterized by elevated IFN-γ and TNF-α levels is critical for parasite clearance, an uncontrolled or excessively robust Th1 response can lead to excessive inflammation and tissue damage, ultimately harming the host (75). In addition to the induction of a Th1 response, the regulation of IL-10 production by regulatory T cells is crucial for the success of vaccines against *Leishmania*. The presence of high IL-10 levels can suppress the protective immune response even in the presence of IFN-γ. Therefore, achieving an appropriate balance between pro-inflammatory and regulatory cytokines is essential for vaccine efficacy (76). Interestingly, despite the promising data for this molecule, no new *in vivo* vaccine data have emerged for GIPLs since 2018.

In *Leishmania*, a wide range of glycoconjugates contain mannose, including LPG, PPG, and GIPLs themselves, all of which are essential for parasite virulence. The GIPL biosynthetic pathway in *Leishmania* involves enzymes with structural and functional properties distinct from those found in higher eukaryotes, enabling the development of selective inhibitors targeting the parasite. For instance, GDP-mannose pyrophosphorylase (GDP-MP) is an attractive target for the design of specific inhibitors, as it is essential for *Leishmania* survival and displays significant differences from its mammalian counterpart. This enzyme catalyzes the conversion of mannose into its activated form, GDP-mannose, within the glycoconjugate biosynthesis pathway. Notably, rational drug design efforts

targeting this enzyme have led to the development of effective *in vitro* inhibitors, which exhibit activity against intracellular amastigotes with a selectivity index (SI) greater than 10 for *L. donovani* and *L. mexicana* (77). Furthermore, gene knockout of GDP-MP in *L. mexicana* results in an inability to develop within macrophages *in vitro* and a lack of parasite persistence *in vivo* (78,79).

2.2.4 Kinetoplast membrane protein 11 (KMP-11)

KMP-11 is an abundant and highly conserved 11 kDa surface protein among trypanosomatids, present in both promastigote and amastigote forms (80). It localizes to membrane-associated regions, including the cell surface, flagellar pocket, and intracellular vesicles, with increased expression in amastigotes and during metacyclogenesis. In promastigotes, KMP-11 exhibits a defined distribution along the flagellum and flagellar pocket (81). Beyond its structural role, KMP-11 has been linked to host–parasite interaction and immunoprotection, as evidenced by its ability to induce humoral immune responses (82).

The immunogenic potential of KMP-11 has been demonstrated *in vivo* using experimentally infected dogs, where it stimulated the proliferation of peripheral blood mononuclear cells (PBMCs). Its conservation across *Leishmania* species and recognition of B and T cell epitopes in different *L. infantum* hosts support its candidacy as a vaccine antigen against visceral leishmaniasis. Although stimulation with *L. infantum* KMP-11 induced a modest increase in IFN-γ gene expression, no significant changes were observed in TNF-α or IL-4 expression, indicating a limited Th1-like immune activation. These findings suggest that KMP-11 alone may not elicit a sufficiently robust protective response and should be evaluated as part of a multicomponent vaccine formulation incorporating additional antigens or adjuvants to enhance Th1 polarization (83).

However, *in vitro* studies revealed that adding KMP-11 during infection of BALB/c peritoneal macrophages with *L. amazonensis* increased IL-10 secretion, enhanced arginase activity, and suppressed nitric oxide production, ultimately raising the intracellular amastigote load. These results underscore the dual role of KMP-11 in immune modulation and the need for selective epitope mapping to exclude sequences that promote IL-10–mediated immunosuppression, thereby ensuring the safety and efficacy of future vaccines (84,85).

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Recent vaccine design efforts have incorporated KMP-11 into a recombinant chimeric multi-epitope protein (MEV-Fc) alongside other virulence factors, such as cysteine protease. Molecular docking studies indicated potential interactions with TLR-2 and TLR-4 receptors. Immunoinformatic simulations predicted increases in IgM and IgG titers following immunization, as well as activation of B cells, helper T cells, and cytotoxic T lymphocytes (86).

Additionally, IgG antibodies against KMP-11 are present in the sera of patients with visceral, mucocutaneous, and cutaneous leishmaniasis, confirming its immunogenicity. However, due to antigenic cross-reactivity with other trypanosomatids, KMP-11 is not suitable for serological diagnosis in regions endemic for both leishmaniasis and trypanosomiases (82,83,87). Although KMP-11 is frequently studied for its antigenic properties, its role in immune modulation remains unresolved. While *in vitro* experiments suggest it promotes macrophage activation and proinflammatory cytokine production, *in vivo* data often indicate immunosuppressive effects that support parasite persistence. This apparent contradiction reflects the complexity of host–parasite interactions and suggests that the immunological outcome of KMP-11 may depend on factors such as host immune status, parasite stage, and co-expression with other surface molecules. Further studies are warranted to clarify whether KMP-11 primarily functions in immune evasion or activation, and to determine its feasibility as a vaccine candidate.

Despite the structural simplicity and conserved nature of KMP-11, its immunological role remains ambiguous, marked by a delicate balance between activation and suppression of host responses.

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2.3 GPI-anchored proteins

2.3.1 Glycoprotein 63 (GP63)

GP63 is the major surface metalloprotease in *Leishmania* and is abundantly expressed on metacyclic promastigotes. Although present in both parasite stages, distinct isoforms exhibit differential expression and localization. In promastigotes, GP63 is N-glycosylated and predominantly located on the cell surface, whereas in amastigotes it lacks N-glycosylation and localizes to the flagellar pocket, cytosol, and intracellular vesicles (88–

90). Proteomic analyses indicate that GP63 is among the most abundant surface proteins in amastigotes (91). Its membrane anchoring is mediated via a glycosylphosphatidylinositol (GPI) anchor, a structure widely conserved among eukaryotes but remarkably amplified in protozoa, where GPI copies can reach up to 20 million per cell (57).

Functionally, GP63 participates in multiple processes essential for Leishmania pathogenesis, including immune evasion, modulation of host cell signaling, and intracellular survival. It contributes to complement resistance by cleaving C3b and C3bi, thereby preventing their conversion into active opsonins and avoiding complement-mediated lysis (92–94). Simultaneously, it promotes parasite internalization by binding to complement receptor CR3, particularly via its Arg-Gly-Asp motif, and by interacting with host fibronectin receptors such as α4β1 integrin. GP63-expressing parasites are more efficiently phagocytosed than GP63-deficient mutants, and inhibition of β1 integrins reduces this uptake. Notably, although GP63-deficient promastigotes develop normally in the sand fly vector, they are more susceptible to complement-mediated lysis and cause delayed lesion formation in BALB/c mice (93).

In addition to facilitating parasite entry, GP63 plays a central role in modulating innate immune responses. It cleaves and activates host protein tyrosine phosphatases (PTPs), which in turn dephosphorylate key signaling molecules such as JAK2 and IRAK-1, leading to the suppression of iNOS expression and inhibition of macrophage activation (95–97). GP63 also downregulates inflammasome activity by interfering with NLRP3 expression, resulting in decreased IL-1β and ROS production (98). These immunosuppressive effects are enhanced by the enrichment of GP63 in exosomes secreted by metacyclic promastigotes, which act distally to modulate host immune cells (99).

Another important function of GP63 is its ability to disrupt phagosome maturation and vesicle trafficking. It cleaves vesicular transport proteins such as VAMP8, synaptotagmin XI, and syntaxin-5, impairing the formation of a functional NADPH oxidase complex, altering phagosomal pH, and inhibiting antigen presentation and T cell activation (89,100,101). Beyond vesicular processes, GP63 contributes to nutrient acquisition by targeting host iron metabolism. It cleaves the poly(rC)-binding proteins PCBP1 and PCBP2, which are responsible for delivering iron to ferritin, thereby increasing intracellular iron availability and supporting parasite proliferation (21). Additionally,

GP63 modulates adaptive immunity by cleaving chemokines such as CXCL10, thus interfering with T cell chemotaxis via CXCR3, and by stimulating prostaglandin E2 production, which promotes a Th2-skewed immune response favorable to parasite survival (102,103).

Given its multifunctional role in host–parasite interactions and its proven immunogenicity, GP63 has emerged as a promising target for both vaccine and drug development. A baculovirus-based vaccine expressing GP63 elicited robust humoral and cellular immune responses in mice, leading to the generation of immune memory and effective parasite clearance (104). Multi-epitope constructs incorporating GP63 have demonstrated strong predicted antigenicity and IFN-γ induction (85,105). Moreover, pharmacological inhibition of GP63's proteolytic activity has been proposed as a strategy to restore host immune defenses. Its secretion via extracellular vesicles during early infection further reinforces its relevance for therapeutic and prophylactic applications. To consolidate these findings, Table 1 summarizes GP63's key roles in parasite–host interaction and immune modulation.

2.3.2 Promastigote surface antigen-2 (GP46/PSA2)

In contrast to the broad proteolytic activity and immune regulatory functions attributed to GP63, the promastigote surface antigen 2 (PSA2), also known as GP46, represents a structurally distinct class of membrane-associated proteins with pronounced immunogenic potential. GP46 is markedly more abundant in metacyclic promastigotes, presenting approximately 30-fold higher expression levels than in procyclic forms, and its structure is defined by an N-terminal region rich in leucine-rich repeats (LRRs), which mediate protein–protein interactions, and a C-terminal region that includes threonine and serine residues susceptible to post-translational modifications (106–108).

Functionally, the LRR motifs of GP46 facilitate binding to CR3 on host macrophages, a critical step for parasite adhesion and entry. Enhanced GP46 expression in metacyclic promastigotes correlates with increased resistance to complement-mediated lysis, reinforcing its role in parasite survival during vector-to-host transition (108,109). Importantly, immunization studies have shown that GP46 elicits protective responses against *L. amazonensis* infection in mice (110,111).

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Building on its immunogenic profile, *in silico* vaccine strategies incorporating GP46-derived epitopes have demonstrated strong interactions with Toll-like receptor 4 (TLR4) and the ability to stimulate IFN-γ production, a hallmark of protective Th1 responses (105,112). These data highlight GP46 as a promising candidate for multi-epitope vaccines, especially when combined with antigens like GP63 to enhance coverage and immune stimulation.

2.4 Type 1 integral membrane protein

2.4.1 Membrane Acid Phosphatase

Membrane-bound acid phosphatases in *Leishmania* are structurally and functionally adapted to promote parasite survival within hydrolytic environments, such as phagolysosomes. In *L. mexicana*, this enzyme is a type I transmembrane protein, heterogeneously N-glycosylated, and localized predominantly in vesicles near the flagellar pocket of both promastigotes and amastigotes (113). It is uniformly distributed on the parasite surface and detected on the outer plasma membrane, as confirmed by electron microscopy, where the subpellicular microtubules attached to the inner membrane face served as structural markers to distinguish the cytoplasmic side from the external surface (114). The enzyme has a molecular mass of ~70 kDa, and carries about seven N-linked glycans, and is expressed at ~16,000 copies per cell (115).

The activity of membrane-bound acid phosphatases is associated with *Leishmania* virulence. In *L. donovani*, enzymatic activity is 2.4-fold higher in virulent strains compared to avirulent ones (116). In *L. amazonensis*, it is found not only on the plasma membrane but also in the endoplasmic reticulum, Golgi, and vesicles near the flagellar pocket, suggesting roles in secretion and host interaction (117). Functional studies confirm its involvement in immune evasion, since pre-incubation of neutrophils with purified enzyme significantly reduced O_2 consumption, O_2^- and H_2O_2 production, key components of the respiratory burst, by 15–25% (118). Similarly, *L. donovani* overexpressing membrane-bound acid phosphatase exhibited increased infectivity and virulence in murine models. Moreover, this phosphatase is also being explored as a vaccine candidate. Immunization of mice with a plasmid encoding *L. mexicana*

membrane-bound acid phosphatase reduced lesion size and parasite burden, while enhancing lymphocyte proliferation, suggesting its potential as a DNA vaccine against cutaneous leishmaniasis (65).

Importantly, membrane-bound acid phosphatases are absent or structurally divergent in mammalian cells, making them attractive targets for selective drug development. Their dual role in suppressing host oxidative responses and enhancing parasite survival demonstrates their translational relevance both as virulence factors and as candidates for immunoprophylaxis. While membrane-bound acid phosphatases facilitate parasite survival through enzymatic modulation of host immune responses, the following class of enzymes contributes to *Leishmania* virulence by mediating purine acquisition and immune signaling interference.

2.4.2 3’ and 5’ nucleotidase

3′- and 5′-nucleotidases are surface-associated enzymes identified on *L. donovani* promastigotes, with activity concentrated at the plasma membrane (119,120). In *L. amazonensis*, the 3′-nucleotidase/nuclease (3′NT/NU) performs a key step in the parasite’s purine salvage pathway by hydrolyzes extracellular 3′-monophosphorylated nucleosides and nucleic acids to generate adenosine (121). These nucleoside products are imported via specific transporters and routed into the purine biosynthesis pathway, where *Leishmania* compensates for its inability to synthesize purines *de novo* (122).

The activity of 3′NT/NU is modulated by environmental conditions. It increases in nutrient-rich media during early growth and is further elevated in purine- or phosphate-depleted media, suggesting regulatory control in response to nutrient stress. For instance, *L. chagasi* grown under low phosphate conditions showed a 45% increase in macrophage interaction, correlating with enhanced 3′NT/NU activity (123). In *L. major*, however, low purine conditions do not upregulate this enzyme (124).

Importantly, 3′NT/NU supports parasite survival under oxidative stress and contributes to evasion of neutrophil extracellular traps (NETs). In *L. amazonensis*, this enzyme shows 60-fold greater activity than 5′-nucleotidase and is more active in virulent strains (125). Its resistance to ROS and ability to increase parasite survival by ~50% in the presence of NETs supports its classification as a virulence factor (126,127). Given its surface

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localization, ROS resistance, and role in purine acquisition and immune evasion, 3′NT/NU is a promising therapeutic target. While this enzyme has not yet been explored in vaccine development, its structural domains hold potential for both immunological screening or selective inhibition strategies.

2.4.3 Receptor adenylate cyclase

Receptor adenylate cyclase is a membrane-associated protein found in *Leishmania* and other trypanosomatids, playing a pivotal role in signal transduction and parasite differentiation (128–130). During the parasite's migration from the midgut of the sand fly to the mammalian phagolysosome, promastigotes are exposed to environmental changes that demand rapid adaptation (131). To cope with oxidative stress encountered within macrophages, *Leishmania* activates adenylate cyclase, triggering intracellular production of cyclic AMP (cAMP) a stress-sensing molecule that promotes the differentiation of metacyclic promastigotes into amastigotes, enhancing resistance to phagolysosomal lysis and facilitating infection establishment (132). In *L. donovani*, five genes clustered within *rac-A* and *rac-B* loci encode putative adenylate cyclase isoforms, whose catalytic domains share high sequence identity with their eukaryotic counterparts (131). Additionally, studies in *L. mexicana* have shown that transformation into amastigotes can be triggered by human C-reactive protein and cAMP analogs, further supporting the role of this signaling pathway in stage conversion (133).

Given its involvement in oxidative stress adaptation, intracellular survival, and life cycle progression, receptor adenylate cyclase emerges as a promising drug target. Its structural divergence from mammalian homologs enhances its selectivity, though further studies are needed to characterize its structure, assess immunogenicity, and validate its inhibition in translational models.

2.4.4 Transferrin Receptor

The transferrin receptor (TfR) plays a central role in iron acquisition and homeostasis, a process exploited by *Leishmania* to support intracellular survival. In most organisms, TfR mediates iron uptake via constitutive endocytosis of iron-loaded transferrin (Tf) (134,135). In *Leishmania*, the TfR is a 70 kDa integral membrane glycoprotein that differs

structurally from its mammalian counterpart by existing as a single polypeptide rather than a disulfide-linked dimer (136). This receptor also binds other host proteins, such as lactoferrin and albumin, suggesting broader ligand interactions (137).

Leishmania amastigotes can internalize host transferrin to fulfill their iron requirements during infection (138,139). The critical role of iron in parasite survival is underscored by studies showing that holo-transferrin supplementation promotes intracellular proliferation of *L. donovani*, whereas iron chelation significantly inhibits its growth (140,141). Additionally, *Leishmania* appears to manipulate host iron-regulatory mechanisms. In *L. donovani*-infected mice, an increase in transferrin receptor 1 expression on splenic macrophages correlates with enhanced interaction between iron-responsive proteins (IRPs) and their target mRNAs, suggesting a parasite-driven modulation of host iron metabolism to support infection (140).

Taken together, the parasite's dependency on host-derived iron and the surface accessibility of its TfR highlight this molecule as a potential target for therapeutic and prophylactic interventions. Inhibiting TfR function or modulating host iron availability may impair parasite replication. Furthermore, the structural divergence of the parasite's TfR from mammalian homologs opens possibilities for selective drug targeting. The immunogenicity of this molecule and its regulatory pathway also merit investigation for future vaccine development, particularly under strategies leveraging iron deprivation to modulate immune responses.

2.5 Polytopic membrane proteins

While the preceding sections examined GPI-anchored and single-pass membrane proteins primarily associated with immune modulation and host–parasite signaling, the following sections turn to polytopic membrane proteins, a structurally distinct group characterized by multiple transmembrane domains. These transporters are essential for nutrient uptake, metabolic regulation, and drug efflux, playing central roles in the parasite's adaptation to hostile intracellular environments. Their involvement in glucose and amino acid acquisition, folate and biopterin salvage, and multidrug resistance mechanisms positions them as highly attractive targets for therapeutic intervention and pharmacological inhibition in leishmaniasis.

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2.5.1 Hexose transporters

Hexose transporters are essential for *Leishmania* metabolism, enabling the uptake of fructose, galactose, glucose, and mannose—key substrates for parasite survival and differentiation (142–146). Immunolocalization studies confirm their presence in the plasma membrane (147). Glucose uptake is particularly active in promastigotes, with a 10-fold higher catabolism rate compared to amastigotes (142). While glucose transport occurs optimally at acidic pH in amastigotes, it is more efficient at neutral pH in promastigotes (148,149).

In vitro glucose depletion upregulates transporter activity by ~5-fold in both *L. mexicana* and *Leishmania enriettii* (150). Knockout mutants (Δlmxgt1) unable to uptake glucose can still synthesize glycoconjugates via upregulated gluconeogenesis, especially in amastigotes, which are less responsive to external glucose (147).

LmxGT1, a glucose transporter localized to the flagellar membrane *of L. mexicana*, may function as a sensor. Its expression is upregulated ~50-fold in glucose-deprived media, and Δlmxgt1 mutants lose viability upon glucose depletion, unlike wild-type strains (151). Additionally, *L. donovani* uses a Mg^{2+}-ATPase H^{+}-glucose symport for active glucose accumulation and pH homeostasis (152).

2.5.2 Amino Acid Transporters

During host transition, *Leishmania* must adapt to shifts in nutrient availability. Promastigotes rely on proline for energy, while amastigotes depend on sugars and lipids (153). Amino acid transport is mediated by permeases from the amino acid transporter superfamily-1 and the polyamine-choline superfamily (154).

Key transporters include La-PAT1 (a H^{+}/symporter in *L. amazonensis*) (155) and LdAAP24 in *L. donovani*, which transports proline and alanine and also regulates osmotic balance and non-substrate amino acids like glutamate and arginine (156). L-arginine, critical for polyamine synthesis and parasite replication, is imported via AAP3, whose expression increases under arginine starvation (157). *L. donovani* AAP3 localizes to the flagellar surface and glycosomes, responding to external arginine depletion with increased transcript and protein levels (158,159).

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L. amazonensis also uses a K^+-dependent system for glutamate uptake, with distinct kinetic and inhibition profiles (160). The AAP3 regulatory system mimics mammalian cationic amino acid transporters, reinforcing *Leishmania*'s evolutionary adaptation to host nutrient cues (158). These adaptive mechanisms extend beyond amino acids.

2.5.3 Biopterin/Folate Transporters

Leishmania is auxotrophic for pteridines, relying on host-derived folate and biopterin to synthesize tetrahydrofolate (H4F) and tetrahydrobiopterin (H4B), which are key cofactors in amino acid, purine, and thymidine biosynthesis, as well as parasite differentiation (161–163).

The FBT family comprises 14 transporters; four of them are BT1 (biopterin transporter), FT1 and FT5 (folate transporters), and AdoMetT1 (S-adenosylmethionine transporter) (164,165). BT1 is essential for biopterin uptake, absent in null mutants of *L. tarentolae* and *L. donovani* (166,167). Its activity decreases in stationary and metacyclic stages, suggesting regulatory roles in differentiation (161). FT1 is the primary high-affinity folate transporter, while FT5 has lower capacity (168,169). BT1 is also overexpressed in MTX-resistant strains, albeit with reduced folate transport.

AdoMetT1, though non-essential for growth, mediates sinefungin (SNF) resistance and is also encoded within the FBT cluster (170). Despite progress, most FBT members remain uncharacterized, underscoring the need for further research. Besides nutrient transport, *Leishmania* also employs efflux pumps to resist drugs.

2.5.4 ABC transporters

Therapeutic failure in leishmaniasis has become a critical concern, largely driven by drug resistance mechanisms such as efflux pumps. Among these, ATP-binding cassette (ABC) transporters, which is present in both prokaryotes and eukaryotes, play a central role in the translocation of lipids, steroids, amino acids, xenobiotics, and metals (171,172). In *Leishmania*, these transporters primarily export substrates from the intracellular environment to the extracellular space (173). Structurally, ABC transporters comprise two transmembrane domains (TMDs) and two cytosolic nucleotide-binding domains (NBDs), which hydrolyze ATP to drive substrate translocation (174).

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Of the eight eukaryotic ABC subfamilies (from ABCA to ABCH), four have been identified in *Leishmania*: ABCA, ABCB, ABCC, and ABCG (175). Members of the ABCB, ABCC, and ABCG subfamilies are particularly associated with multidrug resistance, while ABCA proteins are linked to phospholipid transport (176–178).

Two ABCA transporters described in *L. tropica*, LtrABC1.1 and LtrABCA2, are both localized to the flagellar pocket and implicated in phospholipid trafficking. LtrABCA2 is also found in amastigotes and, when overexpressed, reduces infectivity, suggesting a role in membrane remodeling (177,178). Although ten ABCA genes have been identified in *Leishmania*, most remain uncharacterized (176).

Four ABCB genes are known in *Leishmania* (176). LdMRD1, the first described, encodes a P-glycoprotein that confers resistance to vinblastine, puromycin, and anthracyclines (179,180). Homologs such as LaMDR1 in *L. amazonensis* and ABCB4 in *L. enriettii* are associated with resistance to other drugs (181,182). LaMDR2 (ABCB2) confers resistance to 5-fluorouracil (183), while LmABCB3, a mitochondrial transporter in *L. major*, is essential for porphyrin biosynthesis and Fe-S cluster assembly (184). The function of ABCB1 remains unknown.

The ABCC subfamily comprises nine members (ABCC1 to ABCC9), many homologous to mammalian multidrug resistance-associated proteins (MRPs) (176)]. ABCC1, ABCC2, ABCC3 (also PGPA/MRPA), ABCC4, ABCC5, and ABCC7 exhibit MRP-like activity and contribute to resistance by exporting glutathione, sulfate, or glucuronate conjugates (175,185). ABCC3 was first identified in methotrexate-resistant *L. tarentolae* (186) and was later linked to resistance to antimonials and arsenicals (187,188). It transports metal-thiol conjugates and its activity correlates with elevated trypanothione levels, implicating thiol metabolism in antimony resistance (189,190). Notably, ABCC4 and ABCC5 confer SbIII resistance only in sensitive strains, while ABCC7 mediates resistance to both SbIII and pentamidine in multiple species, despite no upregulation in resistant mutants (191–193).

Six ABCG transporters have been identified, three of which, ABCG2, ABCG4, and ABCG6, are implicated in drug resistance. ABCG2 overexpression in *L. major* enhances SbIII efflux and may increase PS exposure, promoting infectivity (194,195). ABCG4 confers resistance to alkyl-phospholipids and sitamaquine in *L. infantum*, while ABCG6

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confers resistance to miltefosine, chloroquine, and other drugs in both *L. infantum* and *L. donovani* (196–198).

The ABCI subfamily includes the recently characterized ABCI4 in *L. major*, which localizes to the plasma membrane and mitochondria. ABCI4 overexpression confers resistance to heavy metals (SbIII, AsIII, CdII, PbII) and toxic porphyrins in both promastigotes and amastigotes (199).

Overall, ABC transporters contribute substantially to drug resistance in *Leishmania*, particularly members of the ABCB, ABCC, and ABCG subfamilies. They confer resistance to key therapeutics, including miltefosine, pentamidine, and antimonials, by reducing intracellular drug accumulation and modulating parasite infectivity (179, 194, 198). Despite these findings, many ABC transporters, especially in the ABCA and ABCC groups, remain poorly understood. Their divergence from mammalian homologs highlights their potential as selective drug targets, reinforcing the importance of further characterization for therapeutic development. Given their complexity, a summary of the ABC transporters is presented in Table 2.

2.5.5 Nucleoside transporter

The existence of nucleoside transporters in *L. donovani* was first demonstrated through the identification of two distinct uptake systems. One system is responsible for transporting adenosine, pyrimidine nucleosides, and analogs such as formycin A and tubercidin and was later designated as LdNT1. The other system, specific for inosine, guanosine, and their analogs like formycin B, was named LdNT2 (200). As *Leishmania* lacks the ability to synthesize purine rings *de novo*, both LdNT1 and LdNT2 are crucial for purine salvage, functioning in coordination with 3′- and 5′-nucleotidases to ensure efficient acquisition of host-derived nucleosides (122).

Further characterization revealed that LdNT1 exists as two isoforms, LdNT1.1 and LdNT1.2, which differ by only six amino acids (201). Both are predicted to contain 11 TMDs and act as high-affinity transporters for adenosine (Km < 1 μM), though LdNT1.2 displays 4- to 7-fold higher Km values for adenosine and uridine than LdNT1.1, indicating differences in substrate affinity (201,202). LdNT2, with 9 TMDs, also exhibits high affinity for inosine (Km = 0.3 μM) and guanosine (Km = 1.7 μM) (203).

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Functional studies with drug-resistant mutants further supported the role of these transporters in antileishmanial responses. The FBD5 mutant, resistant to the inosine analog formycin B, exhibited reduced uptake of inosine and guanosine, linking LdNT2 to formycin B resistance. Similarly, TUBA5, a tubercidin-resistant mutant, showed diminished uptake of adenosine and other pyrimidine nucleosides, implicating LdNT1 in resistance to adenosine analogs (204).

Although classified within the equilibrative nucleoside transporter (ENT) family—whose mammalian members are predicted to function in a passive, bidirectional manner—*Leishmania* nucleoside transporters are electrogenic and proton-coupled, acting as concentrative transporters (203, 205). This distinction is particularly evident in LdNT2, which possesses only nine TMDs and a unique hydrophilic loop between TMDs 5 and 6, a feature absent in mammalian ENTs. These structural and functional differences underscore their potential as selective therapeutic targets.

The parasite-specific features of LdNT1.1, LdNT1.2, and LdNT2, including high-affinity, proton-driven transport, their role in resistance to nucleoside analogs, and structural divergence from host counterparts, highlight their potential as selective targets for antileishmanial drug development (203).

2.5.6 Amastin

Amastins are surface glycoproteins specifically expressed in the amastigote stage of *Leishmania*, with markedly increased expression under acidic conditions, suggesting a role in adaptation to the phagolysosomal environment of host macrophages (206). Although not involved in initial macrophage adhesion, their stage-specific upregulation points to a function in parasite establishment and intracellular survival.

Initially identified in *Leishmania* in 1994 due to homology with *Trypanosoma cruzi* amastins (207), this protein family has since been found to comprise 45 genes in *L. major* and *L. infantum*, and 52 genes in *L. braziliensis* (208,209). These genes are grouped into four subfamilies: α-, β-, γ-, and δ-amastins. In *L. major*, α- and β-amastins are conserved among trypanosomatids and located on chromosomes 28 and 30, respectively. γ-amastins are specific to the *Leishmania* genus and located on chromosome 24, while δ-amastins, the most diverse group, are distributed across various chromosomes (210).

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Structurally, amastins are predicted to possess four hydrophobic TMDs, with both N- and C-terminal regions facing the cytosol (207,208). Although their exact function remains unclear, their localization on the amastigote surface and their intracellular expression pattern suggest potential roles in membrane transport or host–parasite interactions (209).

Functional studies reinforce this hypothesis. *L. braziliensis* mutants lacking δ-amastin exhibited reduced amastigote numbers in infected macrophages and structural abnormalities in the parasitophorous vacuole, including disrupted lipid bilayers and diminished membrane contact sites. These findings support the notion that δ-amastins contribute to intracellular survival and immune evasion (209).

Given their exclusive expression in amastigotes and surface localization, amastins are attractive candidates for vaccine development, particularly through the identification of conserved epitopes across species. The conservation of α- and β-amastins among trypanosomatids, and the genus-specific nature of γ- and δ-amastins, provide a useful framework for rational epitope design. Moreover, their upregulation in acidic environments strengthens their potential as biomarkers for detecting intracellular parasites. Together, these features justify further investigation into amastins as targets for both prophylactic and diagnostic strategies in leishmaniasis.

While amastins exemplify integral membrane proteins with roles in intracellular adaptation and potential immunogenicity, other surface-associated proteins, particularly those with peripheral membrane Anchorage, also contribute significantly to *Leishmania*'s infectivity and immune evasion.

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2.6 Peripheral membrane proteins

2.6.1 Hydrophilic Acidic Surface Protein (HASP)

In general, HASPs are a family of hydrophobic acylated surface proteins expressed predominantly in metacyclic promastigotes of *Leishmania* and amastigotes forms of the parasite; because of that, HASPs are characterized as stage-specific molecules since they do not demonstrate expression in early stages of the promastigote form (211,212). Nowadays, it is known that HASPs are composed of three main and very similar proteins: HASPA1, HASPA2 and HASPB (213). The genomic region encoding HASP proteins has

been identified in multiple *Leishmania* species, including *L. donovani*, *L. infantum*, *L. mexicana*, and *L. amazonensis*. To date, *L. braziliensis* remains the only species in which the absence of this genomic region has been reported (214–216).

HASP proteins exhibit a distinctive characteristic by being secreted via a non-classical pathway. Unlike proteins that follow the conventional endoplasmic reticulum–Golgi route, HASPs lack a signal peptide and reach the parasite surface through alternative mechanisms, suggesting specialized adaptations in *Leishmania* for exporting virulence-associated molecules (217). In this context, the localization of HASPB at the parasite plasma membrane has been associated with dual acylation mechanisms involving co-translational N-myristoylation and post-translational S-palmitoylation at the N-terminal region. The palmitoylation process is dependent on prior myristoylation, and both modifications are required for efficient anchoring of the protein to the parasite membrane. Notably, the presence of myristate alone results in only partial membrane association, highlighting the synergistic role of both acylations in mediating proper membrane targeting (218).

With regard to expression patterns, it has been shown that in *L. major*, HASPA1 is expressed exclusively in intracellular amastigotes, whereas HASPA2 is predominantly expressed in extracellular promastigotes, especially in the metacyclic form. This differential expression highlights the stage-specific regulation of HASP proteins. Moreover, the role of HASPA1 in amastigotes is thought to complement the function of HASPA2 in promastigotes (213). In contrast, HASPB is specifically expressed on the surface of metacyclic promastigotes and has been proposed as a potential marker of metacyclogenesis in *L. major* within the gut of phlebotomine sand flies (211,213).

Interestingly, it has been shown that, upon contact with the host cell, the HASPB protein is detached from the *L. major* metacyclic surface and transferred to the macrophage membrane, where it can be detected on the cell surface for up to 24 hours after the initial interaction. Additionally, HASPB is initially expressed in amastigote forms, but not detected on their surface; furthermore, this detection significantly reduces in both the macrophage and the amastigote after 48 hours. This further suggests that HASPB may play an important role in the parasite-host cell interaction (219).

The role of HASP proteins remains uncharacterized to date, being necessary for more studies to evaluate their interaction with host cell receptors, since they show a well-

characterized stage-specific expression. HASPB stands out in terms of *Leishmania*-phagocyte interaction, as a result of its ability to detach itself from the parasite surface towards the macrophage membrane, indicating an auxiliary role in the vertebrate host's immune system evasion and also supports the infection establishment, together with the other two proteins HASPA1 and HASPA2. To confirm the relationship between HASP and host cell receptors, it is essential to develop new research to understand this interaction. Moreover, it has been demonstrated that HASP proteins are immunogenic. From the serum of patients infected with CL and LV, it was found that these samples recognized the recombinant HASPB protein in a highly sensitive and specific manner (220). This immunogenicity is also verified in cases of canine LV (221). This suggests contact with the vertebrate host defense system, which may involve evasion mechanisms that are directly or indirectly associated with HASP proteins, but which require further studies to elucidate their functions. To consolidate the key functional and translational features of all the surface molecules discussed, Table 3 provides a comparative summary of their biological functions, host interactions, and therapeutic relevance.

3. Therapeutic Targeting of *Leishmania* Surface Molecules and Metabolic Pathways

The search for novel therapeutic targets against leishmaniasis presents opportunities centered on parasite-specific surface molecules and essential metabolic processes. Many of these targets contribute not only to parasite virulence but also to nutrient uptake, immune evasion, and intracellular survival, making them attractive candidates for drug development. GP63 modulates host signaling pathways by cleaving protein tyrosine phosphatases and receptors, impairs inflammasome activation, and is secreted in extracellular vesicles to influence distant immune cells (99,100). Despite its immunogenicity and functional importance, its widespread presence and redundancy within the parasite family pose challenges for selective targeting.

Glycoconjugates such as LPG, GIPLs, and PPGs are also potential therapeutic targets because inhibition of their biosynthetic pathways diminishes parasite virulence and infectivity (37,49,38). However, their structural variability and immune-modulatory duality require rational design of inhibitors to avoid off-target effects and immunopathology. Several enzymes such as ecto-3'-nucleotidases, acid phosphatases,

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and nucleases are absent or functionally divergent in mammalian hosts, making them attractive drug targets (113,126,127). Nutrient transporters for glucose (HT1/HT2), amino acids (arginine, glutamate), and pteridines/folate—especially those involved in nutrient transport across the parasitophorous vacuole—are essential for parasite viability. Knockout and overexpression studies have confirmed their role in parasite growth and virulence, and they have been proposed as potential targets for small-molecule inhibitors or transporter blockers (147–150,165,222). Strategies to inhibit efflux pumps, either directly or via adjuvants that block transporter activity, also offer a promising path to overcome therapeutic failure associated with antimonial and miltefosine resistance (175,176,223).

Finally, the development of new therapeutic options for leishmaniasis depends on the integration of basic scientific research (target identification, molecular characterization, and mechanistic studies) and clinical trials. Global initiatives such as the Drugs for Neglected Diseases initiative have been instrumental in bridging laboratory discoveries and clinical applications, fostering partnerships among academic researchers and the pharmaceutical industry in order to advance through the long and expensive pipeline of drug development. Integrating high-throughput screening, functional genomics, and structural biology to discover and validate new or previously described targets and understand their interactions within both the parasite and host, alongside a continuous effort to increase the number and quality of clinical trials—critical to evaluate the safety and efficacy of promising compounds—will help ensure that discoveries made at the bench ultimately reach the patients who need them most.

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4. CONCLUSIONS AND PERSPECTIVES

The surface architecture of *Leishmania* parasites represents a sophisticated interface that mediates essential interactions with both the insect vector and mammalian host. Throughout this review, we have highlighted key molecular determinants such as LPG, GP63, HASPs, PSA, amastins, nucleotidases, transporters, and others that not only facilitate parasite survival, but also actively modulate host responses and immune evasion strategies. These surface components are not merely structural features but dynamic

elements shaped by the selective pressures of the parasite lifecycle in mammalian and insect hosts.

Importantly, some of these molecules have emerged as promising translational targets. Indeed, LPG and GP63 are widely explored for vaccine and drug development, while nucleotidases and transporters offer opportunities for interference with key biochemical pathways. Nevertheless, significant knowledge gaps remain. Many surface-expressed proteins have yet to be fully characterized in terms of their structure, life-stage-specific expression, post-translational modifications, and precise roles in host-pathogen interactions. Furthermore, species- and strain-specific variations in surface molecules, including those observed in LPG structures and HASP expression, pose challenges for broad-spectrum interventions and underscore the need for comparative studies across diverse *Leishmania* species.

The increasing use of omics technologies, such as single-cell RNA sequencing and high-throughput proteomics, opens new opportunities to identify previously unrecognized surface molecules that may function as virulence factors, biomarkers, or therapeutic targets. Integrating these datasets with functional assays will be essential to validate candidate molecules and to better understand their role within the complex host-parasite interface.

In conclusion, while substantial progress has been made in elucidating the surface biology of *Leishmania*, a deeper mechanistic understanding is still needed. Bridging these gaps may unlock novel strategies for diagnosis, treatment, and prevention of leishmaniasis, a group of neglected diseases that continue to impose a high burden on vulnerable populations worldwide.

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4. CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5. AUTHOR CONTRIBUTIONS

TCR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. ACLY: Writing – original draft. ALDR: Writing – original draft; AMAV: Data curation, Supervision, Validation, Writing – review & editing; NCCC: Data curation, Supervision, Validation, Writing – review & editing. MASG: Conceptualization, Supervision, Validation, Writing – final review.

6. DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE MANUSCRIPT PREPARATION PROCESS

During the preparation of this work, the author(s) used ChatGPT for language editing purposes. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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Table 1. Functional roles of GP63 in host parasite interactions

Function	Host Target/Mechanism	Effect	References
Adhesion and Internalization	Fibronectin receptors; β1 integrins; Complement receptors	Facilitates parasite adhesion and phagocytosis via opsonization and integrin engagement	(224,225)
Complement Evasion	Complement component C3	Cleaves C3b to C3bi, preventing complement cascade activation	(92–94)
Suppression of Innate Immunity	Protein tyrosine phosphatases (PTPs)	Dephosphorylates JAK2 and IRAK-1, downregulating iNOS and NO/ROS production	(95,96,226)
Inflammasome Inhibition	NLRP3 inflammasome complex	Blocks IL-1β and ROS production, dampening inflammatory response	(98)
Chemokine Inactivation	CXCL10	Cleaves CXCL10, impairing T cell chemotaxis via CXCR3	(102)
Phagosome Modulation	VAMP8, SHP-1, Synaptotagmin XI, Syntaxin-5	Disrupts phagosome assembly, antigen presentation, and vacuole expansion	(89,100,101)
Nutrient Acquisition	Poly(rC)-binding proteins (PCBP1/2)	Inhibits ferritin loading, increasing iron availability for parasite growth	(21)

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Table 2. ABC Transporters in *Leishmania*: Localization, Function, and Resistance Phenotypes

Subfamily	Transporter (Gene)	Localization	Function/Substrate	Resistance Phenotype	References
ABCA	LtrABC1.1	Plasma membrane, flagellum	Unknown	Not determined	(177)
ABCA	LtrABCA2	Flagellar pocket	Phospholipid transport	Reduced infectivity	(178)
ABCB	LdMDR1 (ABCB4)	Golgi, ER, lysosome	Drug efflux pump	Vinblastine, puromycin, anthracyclines	(179,180)
ABCB	LaMDR1	Unknown	Drug efflux (presumed)	Actinomycin D, doxorubicin	(181)
ABCB	LtMDR1	Unknown	Drug efflux (presumed)	Miltefosine, edelfosine	(227)
ABCB	LaMDR2 (ABCB2)	Unknown	Drug efflux (presumed)	5-fluorouracil	(181)
ABCB	LmABCB3	Mitochondria	Fe-S cluster transport	Essential, not resistance-related	(184)
ABCB	ABCB1	Unknown	Unknown	Unknown	-
ABCC	ABCC3 (MRPA)	Intracellular vesicles	Metal-thiol conjugate transport	AsIII, AsV, SbIII	(187,189)
ABCC	ABCC4	Tubular organelle	Drug conjugate transport	SbIII (only in susceptible strains)	(193)

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ABCC	ABCC5	Tubular organelle	Drug conjugate transport	SbIII (only in susceptible strains)	(193)
ABCC	ABCC7 (PRP1)	Intracellular vesicles	Drug conjugate transport	SbIII, pentamidine	(191)
ABCC	ABCC1	Intracellular membranes	Not associated	Not associated	(191,193)
ABCC	ABCC2	Intracellular membranes	Not associated	Not associated	(191,193)
ABCC	ABCC6	Intracellular membranes	Not associated	Not associated	(191,193)
ABCC	ABCC9	Unknown	Unknown	Unknown	(193)
ABCG	ABCG2	Plasma membrane	Antimony efflux, PS exposure	AsIII, SbIII	(194,195)
ABCG	ABCG4	Plasma membrane, flagellar pocket	Phospholipid efflux	Miltefosine, edelfosine, perifosine, sitamaquine	(197)
ABCG	ABCG6	Plasma membrane, flagellar pocket	Drug efflux	Miltefosine, edelfosine, sitamaquine, chloroquine, camptothecin	(196,198)
ABCI	ABCI4	Plasma membrane, mitochondria	Heavy metal detoxification	SbIII, AsIII, Cd(II), Pb(II), Zn-protoporphyrin	(199)

Table 3. Key *Leishmania* Surface Molecules and Therapeutic Potential

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Molecule	Stage	Receptor / Interaction	Key Function(s)	Translational Potential
LPG	Promastigote	TLR2, TLR4, CR3	Modulates TLR signaling; inhibits phagosome maturation; protects against oxidative stress	Vaccine candidate; biosynthesis inhibitors as drug targets
PPG	Promastigote / Amastigote	-	Modulates host cytokines; expands phagolysosomal compartments; protects against degradation	N-terminal domain used in DNA vaccine models
GIPL	Promastigote / Amastigote	TLR2, TLR4	Suppresses NO production; promotes Th2 response; masks parasite antigens	Target for immunomodulation; biosynthetic enzymes as drug targets
GP63	Promastigote / Amastigote	CR3, fibronectin, C3b	Facilitates macrophage entry; cleaves complement and host proteins; suppresses immune responses	Vaccine antigen; protease inhibitors as therapeutics
KMP-11	Promastigote / Amastigote	Possible TLR2/4	Induces IL-10 and IFN-γ; modulates macrophage function; triggers antibody response	Included in multi-epitope vaccine constructs
Amastin	Amastigote	-	Expressed under acidic conditions; supports intracellular survival and vacuole integrity	Diagnostic biomarker; potential vaccine target
HASP	Metacyclic / Amastigote	-	Transferred to host cell membranes; modulates early immune responses	Vaccine antigen; recognized by sera of VL patients
ABCC3 (MRPA)	Promastigote	-	Efflux of antimonials and metal-thiol conjugates	Target for reversing drug resistance
ABCG2	Promastigote	-	Mediates SbIII efflux; enhances PS exposure and infectivity	Drug resistance marker and therapeutic target
LdNT1.1 / LdNT2	Promastigote / Amastigote	-	High-affinity, proton-driven nucleoside transport; mediates drug uptake/resistance	Target for nucleoside analog-based therapies

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights:

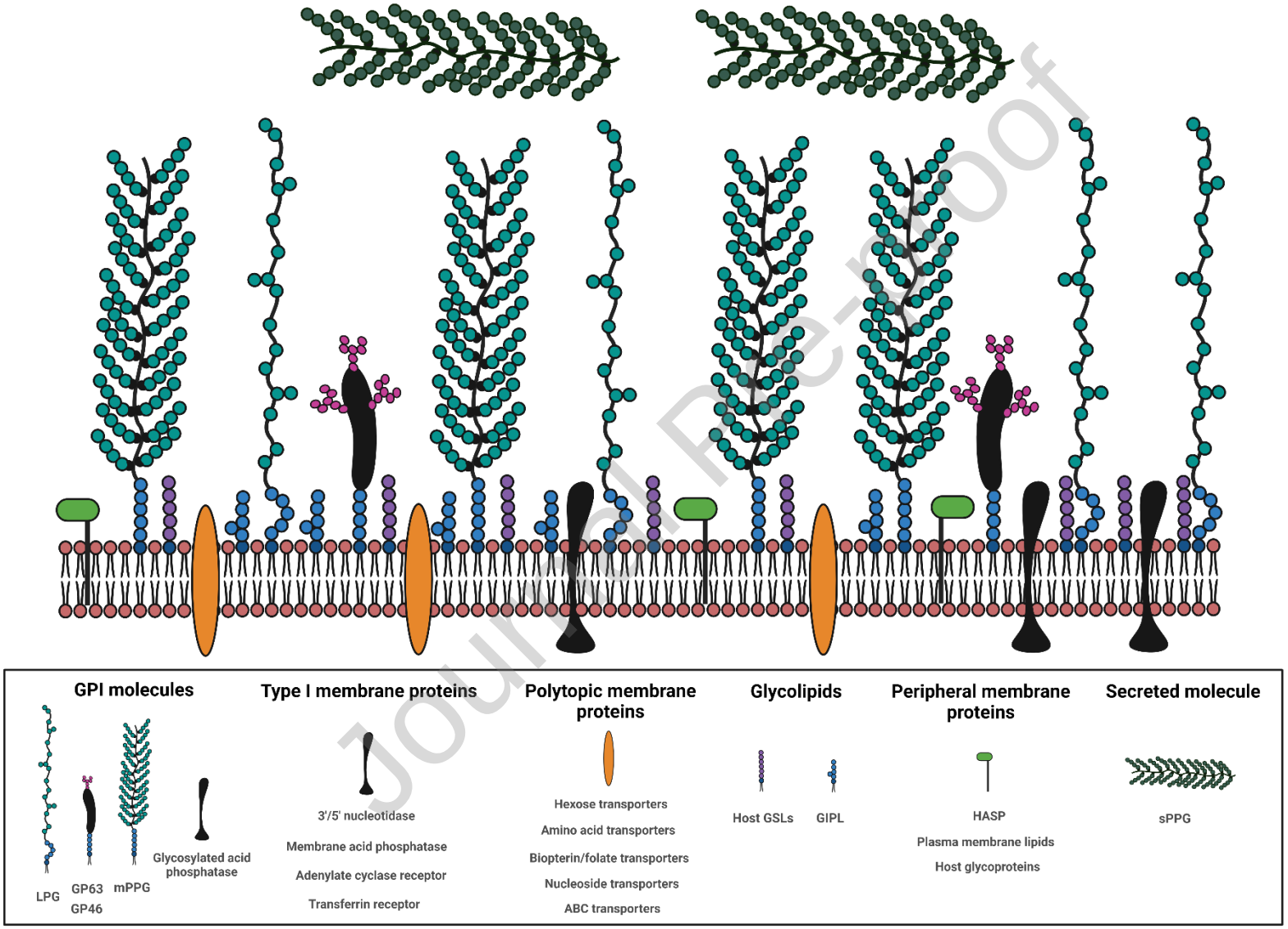
Surface molecules in *Leishmania* are important virulence factors.

Surface molecules are key therapeutic targets due to roles in adhesion and survival.

GP63 and LPG have key biological functions and proven immunogenic properties.

Glycoconjugates highlight novel therapeutic and prophylactic strategies.

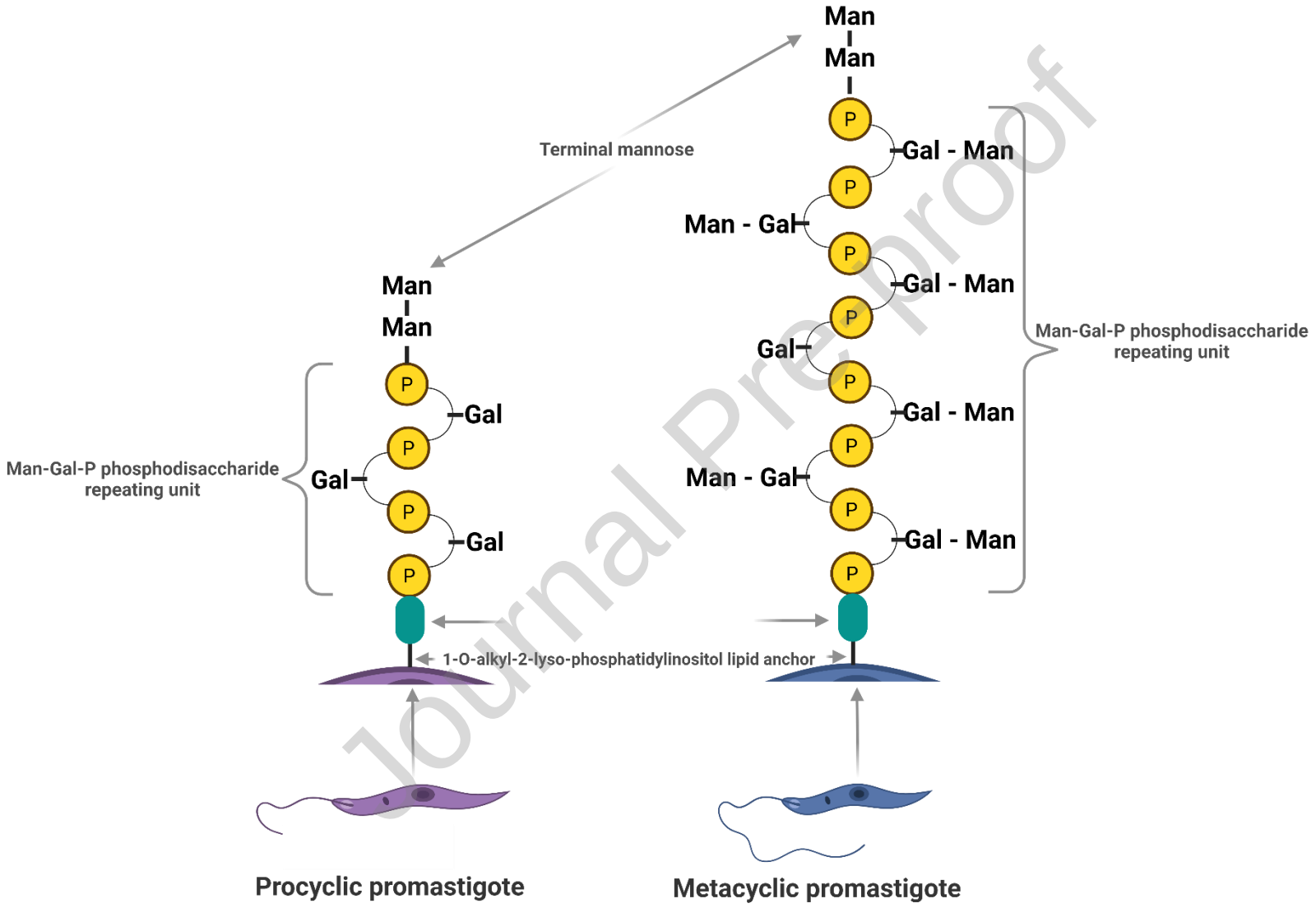
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Figure 1. Schematic representation of the insertion of virulence factors into a general cell membrane of *Leishmania* (Created in https://BioRender.com).

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Figure 2. Schematic representation of the LPG structure in the procyclic and metacyclic promastigote forms, highlighting the structural differences. The LPG is embedded in the membrane by lipid anchors of 1-O-alkyl-2-lyso-phosphatidylinositol, has a heptasaccharide glycan core forming the base, and contains repetitive units of the galactose (Gal) and terminal mannose (Man) phosphodisaccharide (Created in https://BioRender.com).

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