Major Molecular Factors Related to Leishmania Pathogenicity

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Leishmaniasis is a major health problem with 600k - 1M new cases worldwide and 1 billion at risk. It involves a wide range of clinical forms ranging from self-healing cutaneous lesions to systemic diseases that are fatal if not treated, depending on the species of Leishmania. Leishmania sp. are digenetic parasites that have two different morphological stages. Leishmania parasites possess a number of invasive/evasive and pathoantigenic determinants that seem to have critical roles in Leishmania infection of macrophages which leads to successful intracellular parasitism in the parasitophorous vacuoles. These determinants are traditionally known as “virulence factors”, and are considered to be good targets for developing specific inhibitors to attenuate virulence of Leishmania by gene deletions or modifications, thus causing infective, but non-pathogenic mutants for vaccination. Pathway of biosynthesis is critical for keeping the parasite viable and is important for drug designing against these parasites. These drugs are aimed to target enzymes that control these pathways. Accordingly, maintaining low level of parasitic infection and in some cases as a weapon to eradicate infection completely. The current paper focuses on several virulence factors as determinants of Leishmania pathogenicity, as well as the metabolites produced by Leishmania to secure its survival in the host.

Keywords: anti-parasite drugs, macrophages, leishmaniasis, pathoantigenic determinants, infective

INTRODUCTION

Clinical Forms of Leishmaniasis

Leishmaniasis is a serious infectious disease that infects a wide-ranging vertebrates throughout the developing world. It involves a widespread clinical forms ranging from self-healing cutaneous lesions to systemic diseases that are fatal if not treated, depending on the species of Leishmania. It is a major health problem as World Health Organization (WHO) estimated 600k - 1M new leishmaniasis cases worldwide and 1 billion at risk. There are three main types of the disease; the cutaneous leishmaniasis which is the utmost popular form. It begins with a small skin lesion of around 1 cm that increases in size. In most of the cases, when healing occurs, there is 100% immunity against re-infection. In some individuals, failure in cell-mediated immunity causes leishmaniasis diffusa that covers most of the skin surface, just like lepromatous leprosy (1, 2). The second major form of the disease is the mucocutaneous leishmaniasis where there is permanent destruction of the mucous membrane in the mouth, nose and throat cavities. The third main form is the visceral leishmaniasis which is considered to be the most severe form. It is caused by L. donovani, L. infantum, and L. infantum chagasi. This form is fatal if not treated and usually infects the spleen, the liver and the bone marrow. In 20% of the treated patients, a hypo-pigmented skin rash develops
after 6 months or more, usually in the face and the upper parts of the body. This condition is called post Kala-azar dermal leishmaniasis (3–6).

**Molecular Determinants of Leishmania Virulence**

Interestingly, *Leishmania* parasites are able to initiate intracellular parasitism in the parasitophorous vacuoles of the macrophages (7). The key elements that determine parasitism and degree of pathogenicity are mainly molecular determinants of the parasite. These molecules are traditionally called virulence factors. Interactions of these factors determine the degree of pathogenicity (virulence) that is measured as the parasitemia level and/or lesion size (1, 8, 9). These factors enable the parasite to pre-adapt to the mammalian host increased temperature and decreased pH inside the macrophages, required for the initial establishment at the bite site, required for macrophage invasion and for proliferation within the extreme conditions in the phagolysosomal compartments, and are used to avoid the cellular and humoral immune attack of the host (10). It is important to note that these determinants are not direct causative agents of the clinical symptoms of leishmaniasis; this is supported by the fact that direct injection of some virulence factors (e.g. LPG, lipophosphoglycan) into susceptible animals does not cause typical leishmaniasis (5). Since the drugs currently used for leishmaniasis treatment are limited by price, and safety, it is critical to know how the parasite is defending its self in the host cell in order to approach suitable therapeutic treatment.

Elmahallawy and Alkhaldi (11) concluded that *Leishmania* can persist in host cells through influencing the host’s immune system in a variety of ways, including causing immunosuppression and changing the host’s chemokine patterns. Leishmaniasis pathogenesis varies widely depending on a variety of factors, including the infecting species and its virulence factors, as well as the host, all of which influence the disease’s outcome (Figure 1).

**Superoxide Dismutase**

Superoxide dismutase is considered the first line of defence by the parasite by combining superoxide radicals to form molecular oxygen and hydrogen peroxide. This is followed by degradation of the peroxide by catalases or peroxidases to oxygen and water. Inactivation of the toxic peroxidases is catalyzed by catalase enzyme (12).

**Trypanothione Reductase**

Studies have revealed that trypanothione reductase, the enzyme that maintain trypanothione in its reduced form, is important for the parasite to stay alive against the oxidative stress inside the macrophages (13). Knock-out mutants by gene disruption in *L. donovani* and *L. major* strains by means of the selectable markers neomycin and hygromycin phosphotransferases show weakened infectivity and a reduced survival capacity in the macrophages (14). Tunes, Morato (15) revealed that using gold complexes can act against *L. infantum* and *L. braziliensis* intracellular amastigotes by causing mitochondrial damage and oxidative stress due to creation of reactive oxygen species. The author used BALB/c mice infected with luciferase-expressing L. braziliensis or L. amazonensis. These mice were treated with oral administration of 12.5 mg/kg/day of AdT Et or AdO Et. Bioimaging revealed decreased lesion size and parasite burden.

![FIGURE 1](https://example.com/figure1.png)
Glycoconjugates
The parasite surface is mainly occupied by glycoconjugates. The major sugar component of these molecules is mannone. Their abundance and the uniqueness of their chemistry suggest important roles in the parasites virulence and pathogenesis (16, 17). Leishmania possess a variety of glycoconjugates that are essential for parasite virulence and pathogenesis. Of these: lipophosphoglycan or LPG, proteins with GPI anchors such as gp63, a smaller group of glycosinositol phospholipids or GPIPLs, and proteophosphoglycans or PPG. In Leishmania, Mant1-4GlcN-PI is mainly shared between these glycoconjugates, but glycan parts, lipid moieties, and phospholipid precursors are different in the glycoconjugates (18, 19).

Lipophosphoglycan
These are one of the most important glycoconjugates at the exterior of Leishmania species. It is highly expressed in the promastigotes and very little if any is expressed in the intracellular amastigote form. It consists of 15–30 repeating units of phosphorylated oligosaccharides that are linearly connected by phosphodiester bounds. It can be substituted with other sugars depending on the species and is terminated by a capping oligosaccharide. The terminal part of the LPG is coated with a neutral oligosaccharide (19). Although LPG fragments are well-maintained, there are species-specific alterations in the repeated subdivisions. Studies have shown that LPG construction is altered throughout metacyclogenesis and differentiation of L. major promastigotes from a less contagious form in the logarithmic growth phase to a greatly contagious form throughout the stationary growth phase (19). During metacyclogenesis, the normal numeral of the repeated fragments is folded from 14 to 30. Also, the repeated fragments with side chains of beta Gal or Gal beta 1-3Gal beta 1- are decreased in number while the repeat units with side chains of Arap alpha 1-2 Gal beta 1- are increased (19).

LPG mediates the binding of promastigotes to the epithelial cells of the sandfly’s midgut, protects Leishmania during the blood meal digestion in the midgut of the sandfly, acts as acceptor of C3 complement component, inhibits cell signaling for resisting oxidative burst, interferes with the signaling pathway of protein kinase, and inhibits cytokine production in the macrophages (19–21).

In order for a gene to be a virulence gene, it should fulfill the principles of Koch’s postulates on the molecular level. It must be associated with infectivity and pathogenesis. Also, inactivation of the gene must cause loss of virulence. Finally, re-expression of the gene must restore pathogenesis (22). In the case of Leishmania, homozygous null mutants are created by knocking out both of the genes in the instance of L. major were not able to bind to the sandfly midgut and they didn’t survive after a blood meal digestion while $lpg^{-}$ types do (i.e. do have LPG gene). Colonies of Phlebotomus papatasii were infected with $lpg^{-}$ L. major mutants. The mutant parasites persisted and propagated customarily in the fly midgut but they were vanished from the gut more speedily than the wild type parasites after blood meals. According, LPG is not vital for existence of L. major in the early stage of blood-feeding but it is vital to facilitate midgut anchoring and to sustain contagion in the midgut throughout the process of blood digestion. The promastigotes that already invade the macrophages were eliminated in 2 days. Restoration of the LPG gene restored virulence and the amastigotes were able to proliferate in the macrophages. Studies have also revealed that inoculation of 106 promastigotes into the footpad of BALB/c mice (experimental mice) created a wound by day 15 and caused death in due time. On the other hand, inoculation of 106 $lpg^{-}$ parasites revealed delayed wound development and again, restoration of the LPG gene restored virulence (21). A similar study also revealed the same results where Leishmania major $lpg^{-}$ mutants showed reduced virulence and were greatly vulnerable to human complement lysis system (16, 19, 23, 24).

Surprisingly, there was no loss of virulence in the case of L. mexicana. LPG deficient parasites continue to be contagious to macrophages and BALB/c mice. So, LPG is not a virulence factor in case of L. Mexicana (21, 25).

In addition to LPG as a virulence factor, another major surface glycoprotein is a GPI-anchored (glycosyl phosphatidylinositol anchors) zinc metalloprotease of 63 kD. This molecule is usually called GP63 and alternatively called leishmanolysin or major surface protein (MSP). It is the utmost present glycoprotein in Leishmania species. In L. mexicana, there are approximately 5X105 MSP molecules. All Leishmania sp. inspected have numerous tandem genes encoding gp63 (26, 27). Gp63 in ten Leishmania sp. occurs in both amphipilic and hydrophilic forms, encoding the same amino acid sequences. It consists of a predicted protein sequence containing the N-terminal hydrophobic sequence and a propeptide that is detached upon development (28), the later sequence involves a conserved cysteine residue that is shown in L. major to be critical in protecting the parasite from self-destruction due to active protease activity. Differences in gp63 structure among Leishmania sp. include differences in the C-terminal sequence, the 3’ untranslated sequence, and the differential expression in different life stages (27, 29). It is present on surfaces of both promastigotes and amastigotes and has a great role in degrading a range of protein substances and in facilitating attachment to macrophages by acting as opsonin (29). In addition, it inhibits complement-mediated lysis by binding to the complement component C3. It then converts the active C3b molecules into the inactive C3bi (29, 30). Also, GP63 protects the amastigotes from the adverse conditions in the macrophage phagolysosomes; this is evidenced by the point that it was able to protect bovine serum albumin in the same environment. It is suggested that gp63 interferes with immune response of the mammalian host via inhibiting antigen presentation on Class I molecules (31). Additionally, it was revealed that gp63 in L. amazonensis enhanced degradation of the extracellular matrix and basement membrane proteins; this suggests its importance in establishing the infection and migration of the parasite via macrophages circulation to deeper tissues like the spleen and the liver to establish visceral leishmaniasis (32). In vitro degradation of extra-cellular matrix constituents such as collagen, fibronectin, and
laminin by gp63 supports its responsibility in parasite movement and infection creation.

Inhibition of the parasite activity can be achieved by using anti-gp63 monoclonal antibodies. Knock-out parasites for the genes encoding for the gp63 family in L. mexicana showed less virulence than the wild type parasites and were very sensitive to complement mediated lysis. Accordingly, this protein is necessary to support parasite existence. Interestingly, L. major gp63- mutants survived and proliferated normally in the macrophages (31, 33). It is interesting to know that expressions of LPG and GP63 are not related. A study has revealed that LPG1 does not affect the expression of gp63 where crude cell extracts (2.5 X 10⁶ cells) from logarithmic cultures were exposed Western blotting with anti-gp63 antibody. Flow cytometry has also been used for this purpose where the fixed permeabilized parasites are labeled with anti-gp63 antibody. Control parasites were similarly treated except that anti-gp63 antibodies were not used (34).

Interestingly, agglutination experiments using CA7AE antibodies and a lectin were used to select knockout parasites (RCA 120). Five clones were obtained and molecularly analyzed, all of which revealed the expected altered genome as well as the total lack of expression of LPG and PG-containing molecules. Finally, it was discovered that deletion of LPG2 impairs the result of infection in human neutrophils, as evidenced by an 83 percent reduction in intracellular load compared to wild-type parasite infection. The findings support the role of LPG and other PGs in host-parasite interactions as virulence factors (35).

Cysteine Proteinase
In addition, cysteine proteinase (CP) is considered to be a virulence factor in Leishmania sp. It is more expressed in the amastigotes form than the promastigote form. In general, these proteins are believed to have a great role in degrading lysosomal proteases. The cathepsin L-like cysteine proteinases (CPs) of parasitic protozoa are known to influence other vital parasite activities such as nutrition 52 and neutralization of the host immune system (36).

Studies have shown that Lmexicana has cathepsin L-like cysteine proteinase genes that are a multicopy of 19 genes (lmcpb) and two single copy genes (lmcpa and lmcpc). Lmcpb null mutants were able to proliferate and differentiate in vitro, however, infectivity to macrophages was decreased by 80%. The mutants created subcutaneous lesions in mice in a rate less than the wild type parasites. Re-expression of a single copy of Lmcpb restored infectivity (37). The lesions resulting from infection with Acpb appeared slowly at wk 31 and were very minor (mean lesion volume at wk 37 was 3.5 mm³), while injection of Acpa/cpb did not produce lesions (38). In addition, Lmexicana was shown to be sensitive to cysteine proteinase inhibitors in vitro, indicating the importance of CP for Leishmania survival (37).

Studies have also revealed that the main cysteine protease of T. cruzi, cruzain, has been connected to plasma leakage in post-capillary venules and may recruit macrophages for invasion (39).

It was observed that apoptosis (i.e. programmed cell death) in L. donovani involves caspase like activity that can be inhibited using cysteine protease inhibitors. Apoptosis in this intracellular parasite regulate population growth during infection and prolong parasite survival in macrophages. In L. major, however, cathepsin B-like inhibitors reduced DNA fragmentation but did not influence apoptosis. A recent study has identified a gene coding for a protein with high degree of homology to a mitogen-activated protein (MAP) kinase in L. mexicana. A deletion mutant for the gene locus encoding for Secreted Acid Phosphatase (SAP) and containing the intergenic region of ~ 11.5 kb has been prepared. This mutant parasite was not able to produce leishmaniasis in Balb/c mice. However, the infectivity was restored when a 6 kb region of the SAP locus was introduced. This region was shown to contain two Open Reading Frames encoding single copy genes. One of them (ORF1) codes for a protein of 358 amino acids of a molecular weight of 41 kDa, this is called LMPK and is considered to be a homologue of the MAP kinase that is essential for Leishmania differentiation in the macrophages. It is up-regulated in amastigotes as compared to its expression mRNA levels in promastigotes. Polyclonal anti-serum against the C-terminal peptide of LMPK was raised in rabbits and affinity chromatography was used for purification. Immunoblotting of LMPK from cell lysates of both amastigotes and promastigotes has been done (40, 41).

Free Glycoinositol Phospholipids
Free glycoinositol phospholipids (GIPLs) molecules have been also found on the surface of the genus Leishmania; these are not linked to protein or phosphoglycan anchors and are thought to be virulence factors in Leishmania (42, 43). In vitro and in vivo studies have revealed that GIPLs (and LPG) can modify the action of membrane-associated protein tyrosine kinases and protein kinase C in host cells (43, 44). A study has shown that L. mexicana promastigotes synthesize two distinct GIPL lineages, including at least 10 glycolipid species (45). Dolichol-phosphate-mannose synthase (DPMS) is an important enzyme in Leishmania sp., because it stimulates the formation of DPM through the transfer of mannose from GDP-Man to dolichol-phosphate. DPM is the only mannose donor for three mannose residues that structure the trimannose backbone in the GPI protein anchor precursors. A study has shown that creation of Lmexicana null mutants by directed distraction of both alleles of the gene that encodes DPMS, namely lmdpms, caused a augmentation of the chromosomal lmdpms locus indicating that this enzyme is critical for growing due to its role in GIPLs biosynthesis and that GIPLs are essential membrane components in L. mexicana promastigotes (46, 47). The main role of the GIPLs in Leishmania is not very clear. Intracellular partitions containing GIPLs include the megasomes in L.mexicana amastigotes. By similarity with the responsibility of glycosphingolipids in animals, these GIPLs may have a role in establishing a defensive layer of glycoalyx to protect lysosomal membranes from luminal enzymes. Also, the GIPLs may have a role as intermediaries in endogenous signal transduction pathways (48, 49).

In addition, protein phosphorylation is very important in order for Leishmania to proliferate and differentiate in the macrophages. Recently, it was found that Leishmania parasites discharge a range of proteins that are altered by
phosphoglycan fragments analogous to those of the surface glycolipid lipophosphoglycans. These proteins are known as proteophosphoglycans or PPG. These elements contain acid phosphatase manufactured by promastigotes of all \textit{Leishmania} sp. except \textit{Leishmania major}, non-filamentous proteophosphoglycan of \textit{Leishmania mexicana} amastigotes, and a filamentous proteophosphoglycan (IPPG) produced by promastigotes of all \textit{Leishmania} sp. Capped phosphoglycan chains are linked to the polypeptide backbone of these proteins via phosphodiester linkages to serine (50–52).

This mechanism of phosphorylation involves regulation of protein kinases and phosphatases. Secretory acid phosphatase (SAP) is believed to be an important virulence factor in \textit{Leishmania} species. This protein is secreted from the endoplasmic reticulum then it is transferred to the surface or is secreted via the flagellar reservoir (18, 53, 54). Protein Disulfide Isomerase (PDI) of the endoplasmic reticulum plays a critical action in controlling the secretion of acid phosphatase. It catalyzes the oxidation and isomerization of protein disulfide linkages in the endoplasmic reticulum. Studies have revealed the presence of a 12 kDa single thioredoxin-like domain containing PDI in \textit{L. donovani}. Over expression of PDI mutants in \textit{L. donovani} considerably reduced the production of acid phosphatase. In \textit{L. major}, it was observed that highly virulent strains of the parasite contain increased expression of PDI, suggesting a role of PDI and secreted acid phosphatase in supporting the parasite survival in the mammalian host (40).

Investigations by immunofluorescence and immunoelectron microscopes on two \textit{Leishmania/sandfly vector combinations} (\textit{Leishmania mexicana/Lutzomyia longipalpis} and \textit{L. major/Phlebotomus papatasi}) has revealed the presence of a dense three-dimensional network of filaments that surrounds the promastigote cell bodies in a gel-like mass formed mainly by a parasite-derived mucin-like filamentous proteophosphoglycan (IPPG). Accordingly, it was proposed that the constant discharge of PPG by promastigotes in the sandfly gut is an important factor in an efficient transmission of the parasite to the mammalian host (55). The PPG gene has been cloned by antibody screening of a \textit{L. major} genomic expression library, leading to the documentation of repetitive DNA fragments that encode for Ser, Ala, and Pro in ratios in line with the known configuration of IPPG (56).

The non-filamentous proteophosphoglycan were shown to be secreted from the intracellular amastigote form of the parasitic protozoan \textit{Leishmania mexicana}. This high-molecular weight phosphoglycan was purified from a cell-free homogenate of infected mouse tissue and from amastigotes and was shown to consist of serine-rich polypeptide chains and mild acid-labile phosphooligosaccharides capped by mannooligosaccharides. Immunofluorescence and immune-electron microscopy studies suggest that the proteophosphoglycan is secreted in large amounts by amastigotes via their flagellar pockets into the parasitophorous vacuoles of host cells. It is thought that these molecules protect the amastigotes inside these vacuoles (51).

Moreover, N-linked glycans are also thought to be involved in \textit{Leishmania} virulence. One of the most significant purposes of Asn-linked glycans is that they are required for the right folding of polypeptides in the endoplasmic reticulum, this folding is important to transport manufactured proteins to their final destination. A study has used tunicamycin to reveal the importance of these molecules in \textit{Leishmania} parasite (57). Tunicamycin is a specific inhibitor of N-glycan biosynthesis. It was observed that tunicamycin-resistant \textit{Leishmania} lose their virulence in culture more slowly than their non-resistant companions and they showed a high degree of virulence in experimental mice. They also infected macrophages in vitro more efficiently. The ability of the tunicamycin-resistant cells to overcome the inhibitory effect of tunicamycin was resulted from a high level of the glycosyltransferase enzyme that regulates N-glycosylation of leishmanial proteins essential for \textit{Leishmania} to establish intracellular parasitism (58).

An amastigote stage-specific protein termed A2 was first discovered in \textit{L. donovani} and designated as a virulence determinant is \textit{Leishmania sp} (59). It is isolated from subtractive cDNA hybridization libraries as a family of amastigotes specific transcripts of 45-100 kDa proteins encoded by at least 7 genes. These proteins are repetitive sequences (40 to >90 repeats), each contains a secretary leader sequence and 10 amino acids sequence. A2 proteins present mainly in the cytoplasm of the amastigotes and almost absent in the promastigote because more than 90% of serum from visceral leishmaniasis objects contain anti-A2 antibodies. A2 deficient \textit{L. donovani} amastigotes were created by antisense RNA. The resulting mutants were viable in culture but showed a reduced ability to multiply in cultured macrophages. Their virulence in mice was considerably affected and the amastigotes that survived in mice has restored their A2 expression (60). Interestingly, A2 is absent in the genome of \textit{L. major} and \textit{L. tropica} but present in all other \textit{Leishmania} species involving \textit{L. donovani}, \textit{L.chagasi}, and \textit{L. infantum}. More interestingly, \textit{L. major} has non-expressed A2 pseudogenes due to absence of the various repeats in the protein multiple sections of the genome. In depth genetic examination of DNA sequence and gene regulation in \textit{L. major} and \textit{L. donovani} have revealed that phenotypically distinct species have genotypic differences (61).

Studies have revealed that restoring amastigote – specific A2 expression in \textit{L. major} has changed the resulting phenotype of this cutaneous parasite. The \textit{L. major} parasite was not able to cause cutaneous infection in susceptible BALB/c or resistant C57BL6 mice. Also, it had unexpected capability to travel out of the ear dermis, relative to control \textit{L. major}. This phenotype is similar to \textit{L. donovani}. Migration of the parasite to the liver was also observed. Another study has revealed that restoring the A2 expression in \textit{L. major} and infecting BALB/c mice through tail vein injection resulted in splenomegaly, a phenotype typical to \textit{L. donovani} (60, 61). Surprisingly, karyotype analyses in \textit{L. mexicana} complex (\textit{L. mexicana} and \textit{L. amazonensis}) have shown the presence of the A2 coding sequences. This was also supported by Western blot analysis that indicated the presence of three large proteins of > 200 kDa in \textit{L. Mexicana} (62). Although A2 is present in \textit{L. mexicana} complex, these parasites are related to diffuse cutaneous leishmaniasis, but not visceral leishmaniasis. Moreover, there are some visceral leishmaniasis cases reported due to \textit{L. tropica}, a causative agent of cutaneous infection, in...
some soldiers of Operation Desert Storm during the gulf war in 1990. This systemic illness was given the name “viscerotrophic” leishmaniasis to discriminate it from “visceral” leishmaniasis. Accordingly, A2 is not the only responsible factor of visceral leishmaniasis. But in the case of L. donovani, it is very critical as a virulence factor causing the visceral infection. Studies have revealed that this immunization with A2, as protein or DNA, protects against L. donovani infection, this has been used widely in the field of vaccine development against visceral leishmaniasis (63). In addition, another study has examined the significant defensive outcome of immunization with the recombinant A2 (rA2) proteins against L. amazonensis contagion. Protection was linked with the favored and constant induction of a Th1 immune reaction (64–66).

**Metabolic Changes of Host With Leishmania and Its Survival**

Leishmania’s manipulation of host metabolic fluxes is a strategy for circumventing the host immune response, resulting in long-term parasite survival and playing a key role in infection pathology. Specific Leishmania-induced metabolic changes in infected macrophages have been linked to infection resistance or susceptibility. As a result, understanding the multilayer relationships between metabolism and function on innate immune cells during infection has a lot of therapeutic or preventive potential.

In recent years, methods and technology for detecting, identifying, and measuring metabolites within a cell and its surroundings with high sensitivity have vastly improved, spawning the flourishing subject of metabolomics. They may now be used in research on disease agents such as parasites, which helps to better understand their biology while also allowing for better drug discovery, illness diagnostics, and therapy (67, 68). Too far, several research on Leishmania metabolites have been published, offering both precise methods that may be used and insights into the biochemistry and mechanisms of drug resistance in each species (69). It is widely known that one way by which some infections reduce the immune response of their mammalian hosts is by the depletion of amino acids essential to immunological processes (70).

Macrophages probably play a critical role in the Leishmania parasite, both historically and clinically, diagnostically, and immunologically. The first histological account of the pathophysiology of cutaneous leishmaniasis (CL: called “sarsore” in his country) was published in 1898 by Russian-born military doctor Peter Borovsky (1863–1932) from Taschkent, who described the intimate alliance between macrophages and Leishmania. He not only correctly identified the underlying infectious agent as a protozoan parasite, but he also recognized and graphically illustrated its size (on average 1.5 to 2 m) and localization within host cells, which he referred to as “lymphoid and epithelioid cells” because he was presumably unaware of Metschniko’s characterization of macrophages (71). The microscopical detection of oval-shaped Leishmania amastigotes within tissue macrophages (i.e. histiocytes) of cutaneous, splenic, hepatic, or bone marrow biopsies (with the typical disc formed kinetoplast adjacent to the flagellar basal body) is still a central pillar of the microbiological diagnosis of both cutaneous and visceral leishmaniasis.

Activation of macrophages from permissive host cells to leishmanicidal effector cells during Leishmania infection is dependent on cytokines, particularly IFN-γ, which is produced by a variety of cell types (e.g., natural killer [NK] cells, CD4+ or CD8+ T cells, and certain types of NKT cells) and is already released during the early stages of infection (72, 73).

The immunological concept for controlling intracellular Leishmania amastigotes includes a number of components such as reactive oxygen and nitrogen species (ROS and RNS), the impact of microenvironmental and metabolic parameters, and other antileishmanial effectors. Because of their expression of MHC class II and costimulatory molecules, presentation of antigens, secretion of cytokines, and release of RNS and ROS during the acute phases of Leishmania infections, macrophages not only serve as host cells and antileishmanial effector cells, but also as immunoregulatory cells. Infection with Leishmania can alter these processes in either a good or negative way, depending on the parasite species, developmental stage, and experimental setup (74).

Leishmania amastigotes are highly reliant on external supplies of amino acids, which are controlled by the nutrient-sensing pathways previously reported (75, 76). While the defensive response to viruses is heavily reliant on amino acid metabolism, diseases can manipulate this metabolism as a means of spreading throughout the host. The key amino acids arginine, tryptophan, and glutamine are important in immunological control and nutritional competition between the host and pathogens (77). A metabolomic investigation of L. amazonensis-infected macrophages revealed an increase in L-arginine metabolism toward polyamine synthesis, enhancing the intracellular redox balance of infected cells and protecting the parasites from NO and ROS from the host (78). Increased IL-10 production by infected macrophages corresponds with increased Arg-1 activity during Leishmania infection, forming a positive feedback loop that enhances Arg-1 activity (79). The regulation of visceral leishmaniasis relies heavily on glutamine metabolism, and Leishmania amastigotes rely heavily on mitochondrial metabolism for de novo glutamate and glutamine synthesis (80). Glutamine synthetase (GS) is a protein that produces glutamine from glutamate and ammonia, and it has been found in both promastigote and amastigote Leishmania parasites (81). The availability of tryptophan was also discovered to be critical for Leishmania development inside macrophages. Tryptophan depletion caused by idoleamine-2,3-dioxygenase (IDO) activation, a kynurenine pathway enzyme, represents a key antibacterial mechanism during Leishmania infection by lowering tryptophan availability to intracellular amastigotes (82). During infection, tryptophan 2,3-deoxygenase (TDO) was found to compensate for IDO. TDO is identified as a limitation factor in human skin lesions during CL, indicating that its expression may govern parasite growth in lesions, and pharmacological suppression of TDO enhanced parasite load in ex vivo Leishmania major-infected macrophages (82). The
Intracellular Leishmania survival is further influenced by the host’s glycolytic and lipid metabolism. Early after in vitro infection, Leishmania-infected macrophages upregulate the transcription of numerous glycolytic genes (e.g., hexokinase pyruvate kinase isozymes M2, lactate dehydrogenase A), which correlates with intracellular parasite survival (83–86). Infected macrophages were reported to have less intracellular amastigotes when glycolysis was inhibited with 2-deoxyglucose (2-DG) (86). In addition, Leishmania infection reduces the sensitivity of mitochondria to apoptotic stimuli, implying a relationship between mitochondria and parasite persistence (82). Overall, glycolysis was found to be crucial in the early stages of Leishmania spp. infection in macrophages and neutrophils, whereas enhanced mitochondrial metabolism was revealed to be important in the late stages of infection (87, 88).

We can deduce from these data that metabolic reprogramming of Leishmania-infected macrophages is a driving factor for Leishmania parasite infection and immune evasion by reducing the ability of infected cells to elicit robust immunological responses. Thus, regulating the host nutrient-sensing pathways (AMPK, mTOR, and HIF-1α), which affects amino acid, lipid, and glucose utilization, appears to be a crucial regulator of Leishmania infection, while the molecular mechanism behind such alterations is unknown. Overall, the evidence presented here suggests that modulating host metabolism during infection could be a promising treatment approach for leishmaniasis (74).

CONCLUSION

To conclude, Leishmania parasites have a variety of invasive/evasive and pathogenic factors that appear to be relevant for Leishmania infection of macrophages and intracellular parasitism. These determinants are known as “virulence factors” and are thought to be ideal targets for designing particular inhibitors to decrease Leishmania sp (64), virulence through gene mutations, resulting in infectious but non-pathogenic mutants for vaccine immunization. Hence, biosynthetic pathways are essential for the survival of any parasite and for the production of anti-parasitic drugs that target enzymes involved in parasite establishment. As a result, parasite infection is kept at a low level, and in some situations, it is used as a weapon to totally eradicate infection. Future research on the virulence factors of distinct Leishmania species could aid in the development of a novel vaccine to treat the disease by providing a better understanding of the disease’s etiology.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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