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REVIEW

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Leishmania and the macrophage: a multifaceted interaction

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ABSTRACT *Leishmania*, the causative agent of leishmaniases, is an intracellular parasite of macrophages, transmitted to humans via the bite of its sand fly vector. This protozoan organism has evolved strategies for efficient uptake into macrophages and is able to regulate phagosome maturation in order to make the phagosome more hospitable for parasite growth and to avoid destruction. As a result, macrophage defenses such as oxidative damage, antigen presentation, immune activation and apoptosis are compromised whereas nutrient availability is improved. Many *Leishmania* survival factors are involved in shaping the phagosome and reprogramming the macrophage to promote infection. This review details the complexity of the host–parasite interactions and summarizes our latest understanding of key events that make *Leishmania* such a successful intracellular parasite.

KEYWORDS
• GP63 • *Leishmania*
• lipophosphoglycan
• macrophage
• phagocytosis
• phagosome

Leishmaniases encompass a spectrum of human diseases caused by the protozoan parasites of the genus *Leishmania*. Three major forms of leishmaniases are delineated from the symptoms and clinical manifestations caused by the various *Leishmania* species. Cutaneous leishmaniasis is characterized by the presence of ulcerative lesions of the skin, which, in most cases, are self-healing. Mucocutaneous leishmaniasis, a variant form of cutaneous leishmaniasis, is accompanied by the destruction of the oro-naso-pharyngeal tissues. Visceral leishmaniasis is a chronic infection affecting internal organs such as the liver, the spleen and the bone marrow. This disease is fatal if left untreated.

Although *Leishmania* infection frequently presents itself as devastating disease, many *Leishmania* species establish asymptomatic long-term parasitism, which may eventually lead to disease following perturbations in host–parasite interactions and an increase in immune responses leading to tissue damage. The complexity of host–parasite interactions is well demonstrated in *L. braziliensis* infection of humans, which causes parasite- and immune-driven disease symptoms ranging from mild cutaneous lesions to severe mucosal ulceration of the oro-naso-pharyngeal tissues, sometimes with asymptomatic phases lasting for several years [1]. While parasite persistence correlates with the ability of the parasite to adapt to its environment and to counter host defenses, disease progression is the product of disruption of the dynamic equilibrium between the parasite and the host. Understanding host–parasite interactions is pivotal to addressing disease prevention and improving disease outcomes.

Transmission of the parasite is mediated by female sand flies of either the genus *Phlebotomus* or the genus *Lutzomyia*. In the midgut of the sand fly the parasites replicate as promastigotes, which are 10–20 µm in length and 2 µm in width with a long anterior flagellum. Fully infective metacyclic promastigotes are found in the most anterior part of the mid-gut embedded within a parasite-derived gel

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composed of proteophosphoglycans, which promotes regurgitation by the sand fly during feeding [2]. Delivery of infectious promastigotes into the vertebrate host occurs when an infected sand fly takes a bloodmeal. The vertebrate hosts of *Leishmania* parasites parallel those on which the sand fly relies for feeding, and range from wild rodents and canids to humans. Promastigotes are rapidly internalized by phagocytes, where they transform into nonmotile amastigotes, which proliferate within the acidic and hydrolase-rich phagolysosomal compartment.

To persevere within the hostile environment of the macrophage, *Leishmania* evolved various strategies aimed at countering the microbicidal power of the macrophage and the mounting of an effective immune response. In this review, we will discuss the latest research findings in the mechanisms used by *Leishmania* to establish infection and survive within macrophages. In the broad overview of various aspects of *Leishmania* infection of macrophages, the latest studies are comprehensively described and highlight the complexity of *Leishmania*–macrophage interactions in different settings for a range of *Leishmania* species. Consideration is given to the *Leishmania*–macrophage relationship as a multifaceted interaction. Better knowledge of *Leishmania*–macrophage interactions will further our understanding of the key stages in the parasite life cycle and the disease pathogenesis.

Inttial events in parasite uptake by the macrophage

• Recognition & uptake

During transmission of the parasite from its vector to the vertebrate host, macrophages and neutrophils are rapidly recruited to the site of the sand fly bite [3]. Proteophosphoglycans secreted by the parasite in the sand fly's midgut and inoculated into the host during blood meal are powerful stimulators of macrophage recruitment, as shown in studies with *L. mexicana* and *L. infantum* [4,5]. Initially, the parasites are mainly found in neutrophils and later in macrophages. Promastigotes survive in neutrophils but they do not differentiate into amastigotes. Thus, neutrophil infection is transient and following neutrophil apoptosis, the parasites may subsequently infect macrophages [6–8]. Thus, the macrophage is an important host cell for the establishment of infection and persistence of the parasite.

Initial interaction of the promastigote with the macrophage occurs via the parasite flagellum. This may trigger release of intracellular survival factors by the parasite and subsequent modulation of macrophage phagocytic activity [9]. *L. donovani* promastigotes killed by glutaraldehyde fixation or parasites with pharmacologically inhibited motility did not get internalized by murine bone marrow-derived macrophages [10], highlighting the importance of the parasite flagellum in the initial stages of macrophage parasitism.

Different species of *Leishmania* rely on a range of macrophage receptors, including complement receptors (CRs), mannose receptors, fibronectin receptors and Fcγ receptors (FcγRs) [11]. The choice of receptor may impact the course of infection. CR-mediated uptake via CR3 and CR1 inhibits inflammation and superoxide burst, as well as accumulation of the lysosomal markers LAMP1 and Cathepsin D. This may create more favorable conditions for the parasite within the macrophage phagosome. However, in conjunction with fibronectin receptors, more inflammatory conditions may result in parasite clearance. Mannose receptor signaling may trigger inflammatory pathways and more efficient delivery of hydrolytic enzymes into the phagolysosome. FcγR-mediated phagocytosis leads to enhanced activation of NADPH oxidase on the newly formed phagosome [11].

Whereas phagocytosis of infective metacyclic *L. donovani* promastigotes by murine bone marrow-derived macrophages occurred within 10–20 min after parasite attachment [10], the downstream events in phagosome biogenesis depended on the macrophage receptors used in the recognition of the parasite. In CR3 (*CD11b*$^{-/-}$) and FcγR (common chain -/-) deficient macrophages, phagosome fusion with lysosomes occurred significantly faster following *Leishmania* phagocytosis than in wild-type macrophages (1 vs 5 h, respectively), as assessed by recruitment of lysosome-associated proteins. Interestingly, uptake or viability of the *L. donovani* or *L. major* parasites by murine bone marrow-derived macrophages were not affected by the receptor choice [12], pointing to the complexity of *Leishmania*–macrophage interactions.

Following recognition at host cell surface, promastigotes can be internalized via caveolae that are composed of cholesterol-rich membrane lipid microdomains, as shown for *L. chagasi*

infection of murine bone marrow-derived macrophages [13]. Indeed, it has been recently shown that membrane cholesterol is required for *L. donovani* uptake by J774A.1 macrophage-like cell line [14,15]. Cholesterol depletion and subsequent lipid microdomain disruption by methyl-β-cyclodextrin compromised *Leishmania* promastigote uptake via nonopsonic pathways. Uptake of opsonized *Leishmania,* however, was not affected. Interestingly, phagocytosis of amastigotes proceeded independently of lipid microdomains [13]. Likewise, internalization of *Escherichia coli* was not affected by cholesterol depletion, highlighting the significance of the lipid microdomain-mediated pathway for *Leishmania* promastigote internalization [16,17].

The significance of lipid microdomains in *Leishmania* infection is also emphasized in recent studies, which demonstrate that infective *Leishmania* promastigotes promote lipid microdomain formation through activation of host acid sphingomyelinase. This enzyme converts sphingomyelin to ceramide, which is a key component of lipid microdomains [18]. Translocation of acid sphingomyelinase to the cell membrane led to ceramide generation and enhanced parasite uptake. However, at later stages of infection, the parasite also induced *de novo* ceramide synthesis, which in excess might displace cholesterol, disrupt lipid microdomains and impair antigen presentation [18]. Hence, the fine tuning of lipid microdomains is likely to be one of the key players in host–parasite interactions in *Leishmania* infection.

In addition to receptor- and lipid microdomain-regulated uptake, *Leishmania* infection also depends on actin-mediated uptake, and the integrity of the actin cytoskeleton of the host macrophage has recently been shown to be essential for *L. donovani* infectivity [15]. Actin cytoskeleton destabilization induced by cytochalasin D treatment led to a reduction in promastigote attachment to macrophages and the concomitant reduction in intracellular amastigote load. Cellular F-actin levels strongly correlated with the reduction in *Leishmania* attachment and load in macrophages. In contrast, uptake of *E. coli* was unaffected following actin disruption [15].

• Phagosome maturation & parasite differentiation

Following phagocytosis, *L. donovani* promastigote-containing phagosomes of murine bone marrow-derived macrophages failed to acidify and were characterized by a reduced fusogenicity towards late endosomes and lysosomes [19,20]. LAMP1 was recruited to the parasitophorous vacuole (PV) with delayed kinetics [19]. The parasite was shown to subsequently reorient itself with the cell body pointing towards the cell nucleus and the flagellum towards the cell periphery. The parasite promoted outward movement of the PV, opposite to the inward cellular forces. Such opposing motions resulted in cell injury and prompted lysosome docking and exocytosis. The authors proposed that, at this stage, some lysosomes may fuse with the PV and promote promastigote-to-amastigote differentiation, whereas cellular injury may impact plasma membrane integrity and host capacity to combat infection [10].

Promastigote-to-amastigote differentiation is believed to be triggered by the increase in temperature and a decrease in pH. Additionally, iron uptake and subsequent generation of hydrogen peroxide by *L. amazonensis* has been shown to be a major trigger in parasite differentiation [21,22]. Iron mediates generation of reactive oxygen species (ROS), which are normally deleterious for pathogens, but were proposed to act as a signaling molecules regulating parasite differentiation. The *Leishmania* iron transporter LIT1 mediated iron acquisition by the parasite, which led to parasite growth arrest and differentiation. In contrast, LIT1-deficient promastigotes had reduced iron levels, sustained their growth, but eventually died in iron-poor medium. Importantly, LIT1 triggered iron superoxide dismutase to convert ROS to hydrogen peroxide, the presence of which alone was sufficient to trigger differentiation in both wild-type and LIT1-deficient promastigotes. In contrast, the ROS-generating drug menadione could only trigger differentiation in wild-type but not LIT1-deficient cells, implicating LIT1 in the ability of the parasite to generate hydrogen peroxide and the role of the latter in parasite differentiation [21,22]. Interestingly, iron uptake by the parasite was upregulated in iron-deficient conditions, consistent with the low iron levels that the parasite encounters in PVs, where differentiation takes place [23].

Promastigote-to-amastigote differentiation is associated with a reduction in growth rate and the induction of a distinct metabolic state characterized by a decrease in uptake and utilization of glucose and amino acids, reduced

organic acid secretion and increased fatty acid beta-oxidation. Catabolism of hexose and fatty acids provide substrates for glutamate synthesis, which is essential for amastigote growth and survival. Notably, *in vitro* differentiated amastigotes displayed a metabolic profile similar to that of lesion-derived amastigotes, suggesting its coupling to differentiation signals rather than nutrient availability [24]. Such changes likely facilitate amastigote survival in the nutrient-poor intracellular niche.

Both promastigotes and amastigotes are able to divert the classical phagosome maturation pathway, which occurs via a set of highly regulated fusion and fission events with vesicles including endosomes and lysosomes, and form PVs of very specific phenotypes. Fusion is strictly species- and stage-dependent. This is neatly demonstrated in a recent study by Real and colleagues, in which PVs containing *L. major* promastigotes, but not *L. major* amastigotes, could fuse with pre-established *L. amazonensis* amastigote PVs in murine bone marrow-derived macrophages [8,25]. Furthermore, the *L. major* promastigotes within the pre-established *L. amazonensis* amastigote-harboring PVs failed to differentiate, suggesting that *L. amazonensis* niche may not provide an appropriate environment for *L. major* differentiation. *L. amazonensis* differentiation in dendritic cells correlated with an increase in ERK1/2 phosphorylation, the levels of which remained low in *L. major* infection [26], suggesting important differences in key cellular events during differentiation of the two parasite species. These observations signify the unique nature of host–parasite interactions for different parasite species and their vulnerability to perturbations. Major events in *Leishmania* PV formation are depicted in **Figure 1** and are discussed in more detail below.

The PVs of *L. amazonensis* and *L. major* amastigotes show distinct dynamics with the former favoring fusion of individual PVs to form spacious communal vacuoles and the latter promoting fission of the PVs of dividing parasites. These differences are only recently being investigated in greater detail and highlight the complexity in PVs formation and maintenance [27].

Intracellular parasite growth

• Membrane contribution from the endoplasmic reticulum

Once *Leishmania* establish infection within macrophages, their subsequent multiplication requires a source of nutrients and additional membrane for phagosome expansion. For this, the amastigote PV remains a highly dynamic organelle and interacts with the secretory pathway, which contains endoplasmic reticulum (ER) proteins destined for other organelles [28]. RAW 264.7 macrophages infected with *L. donovani* or *L. pifanoi* axenic amastigotes for 6 h to establish PVs and incubated with ricin were able to deliver ricin to *Leishmania* PVs, and this activity was abolished following brefeldin A treatment, which blocks transport from the ER to the Golgi [29]. Importantly, recent studies by Canton *et al.* demonstrated that disruption of *Leishmania* PV fusion with ER vesicles resulted in control of *L. amazonensis* infection of RAW 264.7 macrophages [30]. Lysosome recruitment to PVs has been implicated in recent findings [10], and warrants further studies to address membrane contribution from lysosomes to *Leishmania* PVs.

Further characterization of *Leishmania* PVs in RAW 264.7 macrophages revealed that phagosomes containing *L. donovani* and *L. pifanoi* promastigotes recruit the ER proteins calnexin and Sec22b very early during PV maturation, whereas zymosan-containing phagosomes do not [29]. Loss of the ER SNAREs Sec22b or its cognate partners D12 or Syntaxin 18 or knockdown of Syntaxin 5 had very little effect on the ER or secretion but led to a significant reduction in PV size, as well as a reduction in parasite replication [31]. A similar effect was achieved upon disruption and redistribution of Syntaxin 5 following treatment with the small organic molecule Retro-2 [31,32]. These findings highlight the role of the ER SNAREs Sec22b and Syntaxin 5 in the delivery of ER content to *Leishmania* PVs that supports the infection. Interestingly, Sec22b was also required for cross-presentation and its presence in the phagosome may play a role in parasite control [33].

• Nutrient acquisition

Intracellular *Leishmania* parasites have complex nutritional requirements, with amino acids and polyamines being important carbon sources and growth-limiting nutrients. Long-term survival of the parasite within macrophages requires nutrient availability within the intracellular niche. The parasite may achieve some of its nutrient requirements through parasite-driven PV fusion with endosomes and ER-derived Sec22b- and calnexin-positive vacuoles [29]. Actively scavenged metabolites include hexoses, amino acids,

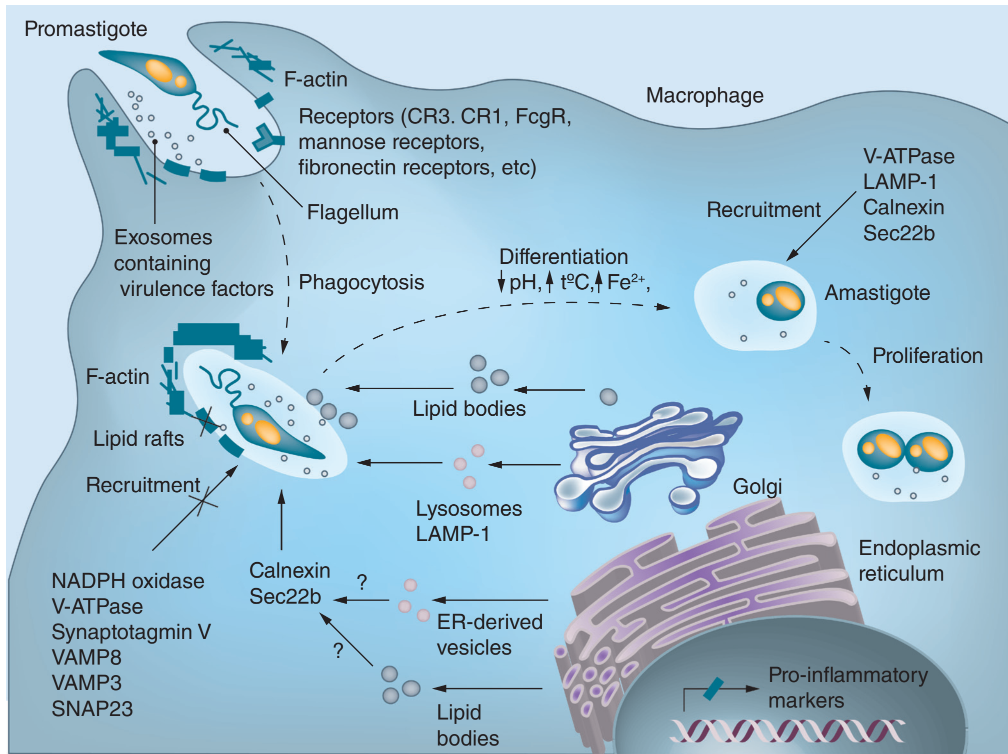

Figure 1. Formation of the *Leishmania* parasitophorous vacuole in macrophages. *Leishmania* promastigote interacts with the macrophage via its flagellum and a number of macrophage receptors. This interaction triggers parasite phagocytosis and the release of *Leishmania* intracellular survival factors, which modify phagosome biogenesis and inhibit induction of proinflammatory pathways. F-actin assembly around the phagosome and the disruption of lipid rafts inhibit recruitment of SNAREs Synaptotagmin V, VAMP8, VAMP3 and SNAP23, and assembly of NADPH oxidase and V-ATPase. The PV is positive for the endoplasmic reticulum markers Calnexin and Sec22b and for the lysosomal marker LAMP1, suggesting that it interacts with the endoplasmic reticulum secretory pathway and selective endolysosomal vesicles. Lipid bodies are present around the PV, although the extent of this interaction is unknown. Low pH, increase in temperature and increase in ferrous iron uptake trigger promastigote-to-amastigote differentiation and acidification of the PV. The amastigote PV remains a highly interactive and dynamic organelle, in order to acquire nutrients and additional membrane from the host cell, to allow for parasite replication.
PV: Parasitophorous vacuole.

polyamines, purines, vitamins, sphingolipids, heme and cations Fe^{2+}, Mg^{2+}, among others. Amastigotes also produce amino acid permeases and cysteine proteases to generate alternative sources of amino acids [34]. Interestingly, classically activated macrophages may have depleted sources of arginine, as it is used in nitric oxide (NO) production, as well as other amino acids that are essential for *Leishmania,* such as tryptophan [35]. Such nutrient limitations may contribute to the infection control by the classically activated macrophage. Conversely, alternatively activated macrophages have increased availability of ornithine and urea for polyamine biosynthesis and hence may promote amastigote growth [35].

Additionally, lipid bodies (LBs) in the macrophage may fulfill some of the parasite's nutrient requirements. LBs are organelles with a core of neutral lipids mainly triacylglycerol and sterol esters, which may act as nutrient sources for parasites. LBs have been shown to be induced in murine thioglycolate-elicited peritoneal macrophages infected with *L. amazonensis* stationary-phase promastigotes [36]. Transcriptomic changes in murine bone marrow-derived macrophages following *L. major* promastigote infection point to highly perturbed carbohydrate and lipid metabolic pathways. The reduced cholesterol efflux and enhanced triacylglycerol synthesis may increase intracellular lipid availability

and hence facilitate foamy macrophage formation [37]. The LB profile of dendritic leukocytes infected with *L. amazonensis* amastigotes was transcriptionally distinct from that of lipid overloaded cells following oleate treatment and presented with larger and more numerous LBs [38]. Interestingly, LB accumulation in peritoneal macrophages was enhanced by the saliva of the sand fly *Lutzomyia longipalpis*, the vector of *L. chagasi*, further promoting foamy cell generation [39]. The LBs interact, associate and sometimes fuse with phagosomes containing zymosan, silica beads or pathogens [40,41]. As well as containing energy-rich nutrients, LBs also bear Rab GTPases, ER proteins and molecular chaperones, and may provide means for acquisition of phagosomal proteins such as Rab5 and Rab7. Thus, LB interaction with parasite PVs may play a role in phagosome maturation [41] and fusion with other organelles, potentially providing additional nutrient sources. Indeed, LBs induced by *L. amazonensis* amastigote infection of dendritic leukocytes were observed to be in close apposition to the PV membrane [38]. The extent of interaction of LBs with *Leishmania* PVs in macrophages has not yet been determined, and the contribution of LBs to *Leishmania* phagosome biogenesis and to host–parasite interactions remains to be explored.

• Iron acquisition

Iron is an essential element for most organisms, including parasites such as *Leishmania*. This nutrient is required for *Leishmania* growth and survival. Iron acquisition by *Leishmania* is facilitated via the parasite ferric iron reductase LFR1, ferrous iron transporter LIT1 and heme transporter LHR1. The three mediators of iron uptake are upregulated in response to low iron. LHR1 is essential for *Leishmania* viability whereas LFR1 and LIT1 are required for intracellular survival [42]. *LHR1*-null mutants have disrupted heme uptake and are nonviable. Heterozygous mutants ($LHR1^{+/-}$) were attenuated in heme-deficient medium, however differentiated normally into amastigotes but did not replicate in macrophages, unless under iron overload conditions [43]. This suggests that iron availability is essential for parasite growth in the form of heme or high concentrations of labile iron. The host responds by restricting iron availability to intracellular *Leishmania* by expressing the NRAMP1 iron efflux pump in maturing phagosomes and lysosomes. The parasite responds by upregulating LIT1 to counter diminishing phagosomal iron availability in the presence of NRAMP1, as observed in infection of murine bone marrow-derived macrophages by *L. amazonensis* amastigotes [44,45], highlighting the importance of iron availability to *Leishmania*.

The host has a complex set of iron homeostatic pathways to maximize iron availability to metabolizing cells and at the same time minimize the undesired oxidative properties of excess iron. During infection, macrophages play a central role in withdrawing iron from the circulation and limiting iron to infectious agents. The systemic iron regulator, hepcidin, facilitates iron sequestration within macrophages by mediating cell surface degradation of the iron exporter ferroportin. Such host defense tactics may actually benefit *Leishmania* as an intracellular parasite of macrophages. Indeed, it has recently been demonstrated that *L. amazonensis* axenic amastigotes causes TLR4-dependent hepcidin upregulation, which triggers ferroportin degradation in murine bone marrow-derived macrophages. Hepcidin deficiency or overexpression of mutant ferroportin that is resistant to hepcidin-induced degradation inhibited parasite replication. Exogenous hepcidin or expression of dominant-negative ferroportin enhanced parasite growth and restored growth of parasites defective in iron acquisition [46], highlighting the role of the hepcidin-ferroportin axis in macrophage–*Leishmania* interactions and the infection outcome.

Alongside promoting iron uptake by the macrophage, *L. donovani* stationary phase promastigotes depleted labile iron to activate iron regulatory proteins IRP1 and IRP2 in primary murine splenic macrophages [47]. These transcription factors promote increased iron uptake in macrophages through increased expression of the transferrin receptor TfR1. Holotransferrin (Tf-Fe) supplementation increased and iron chelation decreased intracellular *Leishmania* growth in J774A.1 macrophage-like cells, signifying the importance of transferrin receptor-mediated iron uptake into macrophages for *Leishmania* survival [47]. Iron acquisition by intracellular *Leishmania* is summarized in **Figure 2**.

Macrophage defenses

• Oxidative damage

One of the major tactics used by macrophages to incapacitate pathogens is the generation of ROS and reactive nitrogen intermediates (RNI). Multiple approaches are used by the macrophage

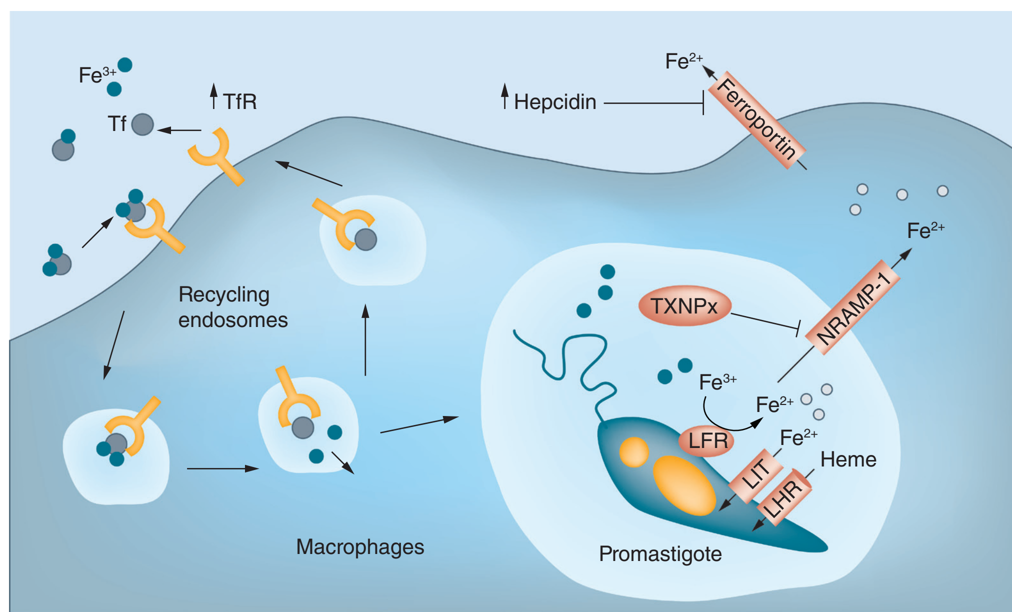

Figure 2. Iron acquisition by *Leishmania* within macrophages. *Leishmania* expresses the heme transporter LHR and ferrous iron transporter LIT to scavenge iron inside the PV within macrophages. Additionally, the *Leishmania* ferric reductase LFR converts ferric iron to ferrous iron to facilitate its transport by LIT. *Leishmania* also produces TXNPx, which inhibits iron export out of the PV by the cation transporter NRAMP-1, thereby augmenting phagosomal iron availability to the parasite and depleting cytosolic iron stores. Depleted intracellular iron triggers upregulation of TfRs and enhances uptake of Tf-bound ferric iron via the endosomal network, with which the PV interacts. Furthermore, elevated expression of the iron regulator hepcidin in infected macrophages causes degradation of the iron exporter ferroportin and leads to iron retention inside the macrophage, increasing iron availability to *Leishmania*.
PV: Parasitophorous vacuole; Tf: Transferrin; TfR: Transferrin receptor; TXNPx: Tryparedoxin peroxidase.

to tightly control production and elimination of these deleterious species, from global macrophage activation to responses localized to pathogen-containing phagosomes. As discussed previously, NADPH oxidase assembly at *Leishmania*-containing phagosome stimulates ROS production and superoxide burst localized to the phagosomal lumen. Additionally, recent findings have demonstrated that NO was produced following Nlrp3 inflammasome assembly in murine bone marrow-derived macrophages infected with *L. amazonensis* metacyclic promastigotes and helped control the infection [48]. Activated Nlrp3 drove IL-1β production, which through IL-1R and MyD88 induced NOS2 to produce NO [48]. ATP-activated purinergic receptor P2X7 induced inflammasome assembly and participated in the subsequent restriction of *L. amazonensis* promastigote growth in bone marrow-derived macrophages. Interestingly, P2X7 was also able to induce leukotriene B4 production, which led to a reduction in parasite load [49]. Overall, these findings indicate a concerted effort by multiple macrophage defense mechanisms to induce oxidative damage to the parasite and compromise its ability to survive.

• Macrophage activation

The macrophage is an extremely plastic cell equipped with homeostatic functions of clearing dead cells and debris in its resting state and microbicidal and antigen presentation tasks following its activation. Classical activation by IFN-γ leads to inflammatory responses and inhibits *Leishmania* growth, whereas alternative activation by IL-4 inhibits inflammation through IL-10 production and stimulates *Leishmania* growth [50]. The latest studies addressing macrophage activation following *Leishmania* infection and its effect on *Leishmania* growth are discussed below.

Peritoneal resident and inflammatory macrophages infected with *L. major* promastigotes

showed increased expression of FasL, TNF, IL-6, and other proinflammatory markers following induction of a cellular stress response in macrophages, via the SAPK/JNK activation [51]. Interestingly, the cellular stress response also promoted parasite survival and replication in macrophages [51]. Inflammation-induced IFN-γ led to the activation of members of the PKC family of protein kinases, which were critical for macrophage activation and parasite killing [50,52].

Induction of proinflammatory functions could be further stimulated by NRAMP-1-mediated cation transport, which led to a reversible inhibition of protein tyrosine phosphatases (PTPs), via direct PTP-metal interaction and/or ROS-dependent PTP oxidation. The resulting lower PTP activity led to induction of proinflammatory pathways and lower survival of *L. donovani* stationary phase promastigotes in RAW 264.7 macrophages [53]. In the absence of the PTP SHP-1, phagosome acidification was impaired, and pro-Cathepsin D was not processed to the active enzyme [54]. This is consistent with the phagosomal profile of activated macrophages, in which phagosomal degradative capacity is decreased to promote more efficient antigen presentation [55].

Recently, IGF-1, negatively regulated by IFN-γ and macrophage activation, has been implicated in the control of *L. major* promastigote infection in RAW 264.7 macrophages. IGF-1 was expressed in macrophages and colocalized with parasites. IGF-1 production was inhibited by IFN-γ stimulation, which led to a reduced parasite load. Addition of extrinsic IGF-1 reversed the reduction in parasite load completely [56]. IGF-1-mediated mechanisms of parasite growth control remain to be explored.

• Foamy cell formation

As discussed above, *Leishmania* infection of macrophages is frequently associated with an increase in LBs in infected macrophages and foamy cell formation. Although from the nutrient perspective, LB induction may potentially be beneficial to the parasites, foamy cells may also form part of host defense against the parasite. LBs are the principal storage organelle for arachidonic acid, which is a paracrine mediator of cell activation. Arachidonic acid can promote phagosome maturation and pathogen killing through ROS production and phagosome–lysosome fusion [41]. Also present in LBs are proinflammatory mediators such as cyclooxygenases, lipoxygenases, leukotriene C4 and MAPK (ERK1, ERK2, p85 and p38) [41]. Irgm, the ER protein involved in phagocytic MHC class I presentation is also present in LBs and may facilitate cross-presentation to other immune cells [57].

Interestingly, the high concentration of prostaglandin E2 (PGE2) as a product of eicosanoid production within LBs in macrophages acts as a potent inhibitor of NO production, and exogenous PGE2 increased parasite load in peritoneal inflammatory macrophages infected with stationary phase *L. amazonensis* promastigotes [36,41]. Hence, the exact contribution of the presence of LBs in macrophages to *Leishmania* intracellular survival may rest on the composition of the LBs, which in turn may be governed by additional factors, such as activation status of the macrophage.

Leishmania evasion of host defenses

• Curbing inflammation

Leishmania employs a number of intervention mechanisms to counter host defenses. Molecular targets and mechanisms for the evasion of macrophage defenses by *Leishmania* are summarized in **Table 1**. *Leishmania* targets multiple signaling pathways in the macrophage to reduce infection-induced inflammation. Even as early as during inoculation of the parasites by the sand fly vector, the parasite-produced proteophosphoglycan-rich secretory gel enhances alternative activation and arginase activity of host macrophages to promote *L. mexicana* survival [4]. Infection of murine peritoneal macrophages with *L. amazonensis* stationary phase promastigotes led to suppressed LPS-induced inflammatory responses, such as the production of IL-12, IL-17 and IL-6. Interestingly, *Leishmania* also augmented LPS-induced proinflammatory cytokines IL-1α, TNF, MIP-1α and MCP-1 and the anti-inflammatory cytokine IL-10 [58]. Hence, *Leishmania* may possess selectivity over manipulation of certain cytokines in order to stimulate a unique activation state in the macrophage suitable for the parasite survival.

Recently, *L. donovani* promastigote infection of murine peritoneal macrophages was shown to induce expression of host PPARγ, which is known to curb inflammation and protect the host from excessive injury. Inhibition of PPARγ facilitated removal of the parasite [65]. *Leishmania* also induced host PTP activation, including PTP1B, TC-PTP, PTP-PEST and SHP-1. Activation of PTPs leads to a number of events

Table 1. Macrophage defenses against *Leishmania* infection and *Leishmania* evasion mechanisms and intracellular survival factors that counter them.

Macrophage defense	Molecular mediators targeted for evasion	Mechanism(s)	*Leishmania* factor(s)	Ref.
Inflammation	IL-12	Activation of PTPs (PTP1B, TC-PTP, PTP-PEST, SHP-1)	GP63, CPB, LPG	[50,58,59,60,61]
	TNF			
	Phagolysosomal maturation			
	MHC II presentation			
	TLR4 signaling	↓ TRAF3 ↓ neutrophil elastase	ISP	[62,63]
	IL-17	Unknown	Unknown	[58]
	IL-6	↓ Protein Kinase R	ISP	[58,64]
Classical activation	iNOS	↑ PPAR-γ	Unknown	[65]
Oxidative damage	ROS	↓ mitochondrial UCP-2	Unknown	[66]
	ROS	↓ NADPH oxidase assembly	LPG, GP63	[19,67,68]
	NO	↑ autophagy, ↑ PPAR-γ	Unknown	[36,69]
Immune recognition	MHC II presentation	lipid microdomain disruption	GP63, LPG	[20,67,70,71]
	MHC I presentation	↓ VAMP8 ↓ NADPH oxidase	GP63	[67,72,73]

For each macrophage defense, the upstream molecular pathways known to be perturbed in Leishmania infection are identified. Mechanisms of disruption of these pathways are described, including participating Leishmania defense factors where known.
CPB: Cysteine protease b; ISP: Inhibitor of serine peptidase; LPG: Lipophosphoglycan; NO: Nitric oxide; PTP: Protein tyrosine phosphatase; ROS: Reactive oxygen species.

favorable for the parasite, such as the reduction of proinflammatory processes, a reduction in IL-12, NO, TNF, phagolysosomal maturation and MHC class II antigen presentation [50,59]. TRAF3 is yet another recently identified target of *L. donovani* promastigotes. The parasite inhibited TRAF3 degradation in order to impair TLR4-mediated inflammatory host response in RAW 264.7 cells and in bone marrow-derived macrophages. TRAF3 degradative ubiquitination is required for TLR4 activation. Reduction in TRAF3 by shRNA decreased parasite burden [62]. The above studies reveal the multitude of host targets that *Leishmania* exploits in order to evade macrophage activation and the accompanying proinflammatory response.

As *Leishmania* establishes infection inside the macrophage and proliferates, the macrophage may eventually undergo apoptosis. The parasite delays macrophage apoptosis but ultimately exploits the apoptotic host cell to spread to neighboring uninfected macrophages, with minimal exposure to extracellular immune recognition systems. Cell-to-cell transfer of *L. amazonensis* amastigotes, which were isolated from BALB/c mice and used to infect bone marrow derived macrophages, was mediated by parasitophorous extrusions, enriched in lysosomal enzymes. The PV components such as LAMP1/2 and Rab7 were shown to be internalized by recipient macrophages together with the rescued parasite and stimulate production of the anti-inflammatory cytokine IL-10 by the recipient macrophage [74]. Thus, even at the most vulnerable stages of its life cycle, *Leishmania* successfully manipulates its host to avoid immune recognition and subsequent inflammation.

• Interfering with host cell signaling

Macrophages infected with *Leishmania* are defective in the expression of activation-associated functions and are unresponsive to IFN-γ [75]. Studies with *L. donovani* revealed that this parasite targets distinct steps along the JAK-STAT pathway. Upon contact with macrophages, *L. donovani* promastigotes activated the PTP SHP-1, which dephosphorylated JAK2. In addition, proteasome-mediated degradation of STAT1 was rapidly induced, preventing its nuclear translocation. *L. donovani* promastigotes were also reported to downregulate the IFN-γ receptor and to induce the expression of the

suppressor of cytokine signaling SOCS3 [76]. *L. donovani* promastigotes can thus efficiently shut off the predominant signaling cascade of one of the most important macrophage activators. Similar to promastigotes, *L. donovani* amastigotes inhibited IFN-γ-induced expression of MHC class II and iNOS. However, infection with *L. donovani* amastigotes downregulated IFN-γ-induced gene expression without affecting STAT1 activation. Rather, amastigotes inhibited IFN-γ-induced STAT1 nuclear translocation by blocking the interaction of STAT1 with the karyopherin importin-α5 [77]. The underlying mechanisms remain to be elucidated.

• Avoiding oxidative damage

Leishmania responses to oxidative stress vary greatly depending on *Leishmania* species and host cell type [78]. For example, *L. major*-infected thioglycolate-elicited peritoneal macrophages infected with *L. major* stationary phase promastigotes produced ROS, whereas in *L. amazonensis*-infected cells ROS production was inhibited [79]. Various approaches are used by different *Leishmania* species to counter oxidative stress. For example, *L. donovani* axenic amastigotes were able to impair cellular and mitochondrial ROS via the induction of mitochondrial uncoupling protein 2 (UCP2). *L. donovani* degraded the transcription factor USF-1, hence facilitating recruitment of the transcription factors SREBP2 and Sp1 to the UCP2 promoter, UPC2 upregulation and inhibition of ROS [66].

Leishmania is also able to avoid oxidative damage by preventing NADPH oxidase assembly at the phagosomal membrane and generation of ROS within the PV. A recent study by Matheoud and colleagues has demonstrated that *L. donovani* and *L. major* stationary phase promastigotes achieve this by cleaving the host SNARE VAMP8, which was necessary for NADPH oxidase recruitment to the phagosome of bone marrow-derived macrophages [67]. Disruption of lipid microdomains by insertion of the surface glycolipid lipophosphoglycan (LPG) in the phagosomal membrane by the parasite may also inhibit recruitment of the cytosolic components of the NADPH oxidase to the PV [19]. In a different approach, *L. mexicana pifanoi* axenic amastigotes recruited the immature 65-kDa form, but not the mature 91-kDa form, of the gp91^{phox} subunit of the NADPH oxidase complex to the PVs by disrupting gp61^{phox} maturation in RAW 264.7 macrophages. Heme-dependent maturation of gp91^{phox} was inhibited by the parasite through upregulation of host heme oxygenase 1 and heme degradation [80].

As well as harming the parasite directly, oxidative damage by ROS induces apoptosis in macrophages, which destroys the replicative niche of the parasite. Apoptosis was suppressed by *L. donovani* stationary phase promastigote infection of RAW 264.7 macrophages via the induction of the suppressors of cytokine signaling SOCS1 and SOCS3, which enhanced parasite survival [81].

• Countering antigen presentation

Antigen cross-presentation is a critical process for immunity against pathogens. It involves presentation of foreign proteins derived from phagocytosed cargo on MHC class I for detection by cytotoxic CD8$^+$ T cells and for orchestration of a systemic immune response. As a professional antigen presenting cell (APC), the macrophage participates in cross-presentation of *Leishmania*-derived proteins. *Leishmania* evades host immunity by inhibiting antigen cross-presentation through cleavage of the SNARE VAMP8 in murine bone marrow derived macrophages infected with *L. major* or *L. donovani* stationary phase promastigotes but not *L. donovani* amastigotes isolated from spleens of infected hamsters. Disruption of VAMP8 prevented NADPH oxidase assembly which led to more efficient phagosomal acidification and proteolysis, thereby inhibiting MHC class I presentation and T cell activation [67,72,73]. Both VAMP8 and VAMP3 were excluded from *Leishmania* PVs. The consequences of VAMP3 exclusion from *Leishmania* PVs are unknown. Interestingly, MHC class II-dependent antigen presentation was also compromised in *Leishmania* infection but in VAMP8-independent manner [67,70].

Inhibition of antigen cross-presentation was also achieved via disruption of membrane lipid microdomains by the parasite [71]. Indeed, membrane cholesterol levels were found to be reduced in infected cells and the antigen presentation defect could be corrected with liposomal delivery of exogenous cholesterol. Liposomal cholesterol was also found to promote ROS and RNI, proinflammatory cytokine expression and intracellular parasite killing, and was implicated in cellular stress and ROS-induced apoptosis of peritoneal exudate cells infected with *L. donovani* promastigotes [82]. Hence, lower cholesterol levels, whether through dysregulated macrophage

lipid metabolism or *Leishmania*-driven cholesterol displacement or depletion, may favor *Leishmania* survival.

- **Induction of autophagy**

Induction of autophagy in bone marrow-derived macrophages or peritoneal exudate cells by *L. amazonensis* amastigotes isolated from mouse footpad and stationary phase promastigotes, respectively, enhanced intracellular parasite survival. Autophagy inhibitors, such as 3-methyladenine (3MA) or wortmannin, reduced parasite load whereas autophagy inductors such as rapamycin or starvation did not alter or increased parasite load [36,69]. Induction of autophagy was associated with NO reduction and highlights the role of this pathway in the outcome of infection [36].

Leishmania intracellular survival factors

A number of essential surface molecules protect the parasite from oxidative damage and hydrolytic activity within the phagolysosome. Other survival factors are secreted and directly interact with macrophage proteins within specific signaling pathways to modulate phagosome biogenesis, macrophage defense mechanisms and systemic inflammation. Among others, the most studied *Leishmania* factors that modulate host cell physiology include LPG, glycosylinositol phospholipids, proteophosphoglycans, cysteine proteases, secreted acid phosphatases and the zinc-dependent metalloprotease GP63 [19,78]. The better studied *Leishmania* factors are discussed below, and their role in host–parasite interactions summarized in **Table 2**.

Leishmania expresses multiple intracellular survival factors, which vary according to the species and life cycle stage. For example, amastigotes lack LPG but retain a glycocalyx of parasite-synthesized glycosylinositol phospholipids and host-derived glycosphingolipids, which may protect the parasite from hydrolases and MHC class II presentation [35]. Promastigotes can release microvesicles, or exosomes, into the extracellular milieu to deliver macrophage-modulating molecules into nearby cells before internalization of the parasite. Exposure of macrophages to exosomes induced IL-8 secretion but not TNF. This may modify parasite uptake by the macrophage as well as further downstream events during established infection [104–80].

Exosome-based secretion pathway is responsible for *Leishmania* protein export into macrophage cytosol. Exosome composition is governed by external cues such as temperature and pH. Exosome release is upregulated at 37°C and low pH, the conditions the parasite encounters

Table 2. *Leishmania* intracellular survival factors and their role in *Leishmania*–macrophage interactions.

Name	Description	Role in host–parasite interactions	Ref.
LPG	Lipophosphoglycan	Activates MAPK, disrupts lipid rafts, ↑ TNF, ↑ IL-1β,↑ IL-6, ↓ TLR9, ↓ recruitment of Syntaptotagmin V, NADPH oxidase and V-ATPase to PV, scavenges ROS, ↑ HO-1	[19–20,60,68,83–88]
GP63	Zinc-dependent metalloprotease	Activates PTPs, p130Cas, Cortactin, Caspase 3 ↓ miRNA-122, ↓ TNF, ↓ IL-12, ↓ NO, ↓ mTOR, ↓ AP-1	[50,61,67,89–92]
ISP	Inhibitor of serine peptidase	↓ Neutrophil elastase, ↓ trypsin, ↓ chymotrypsin, ↓ TLR4 activation, ↓ Protein kinase R activation	[63–64,93]
Prohibitin	Prohibitin ortholog	Interacts with host HSP70, ↑ parasite uptake	[94]
PKC-like	Protein Kinase C ortholog	↑ Parasite phagocytosis	[95]
ISCL	Inositol phosphosphingolipid phospholipase C-like	↑ Survival and replication	[96]
Aldolase	Fructose-bisphosphate aldolase	Activates SHP-1, ↓ acidification	[54,97]
MsrA	Methionine sulfoxide reductase A	↑ Resistance to ROS/RNI	[98]
ALO	Arabino-1,4-lactone oxidase/vitamin C biosynthesis	↑ Resistance to ROS/RNI, ↓ IL-12, ↓ TNF	[99]
TXNPx	Tryparedoxin peroxidase	Detoxifies ROS/RNI, ↓ NRAMP-1, Fe redistribution	[100,101]
Thioredoxin	ROS scavenging enzyme	Stabilizes PTPs, ↓ proinflammatory pathways	[81]
CPB	Cysteine protease	Activates PTPs, ↓ activation, ↓ NO	[102]
MIF	Macrophage migration inhibitory factor ortholog	Activates MAPK, ↓ apoptosis	[103]

Names of Leishmania survival factors are listed alongside their descriptions. Their effects on macrophage defense pathways and Leishmania survival are described.
LPG: Lipophosphoglycan; NO: Nitric oxide; PTP: Protein tyrosine phosphatase; PV: Parasitophorous vacuole; RNI: Reactive nitrogen intermediates; ROS: Reactive oxygen species.

following inoculation by the sand fly into a mammalian host, as observed with *L. donovani* stationary phase promastigotes [105]. Within 4 h of temperature shift from 26°C to 37°C, a rapid increase in protein release was induced in *L. mexicana* promastigotes via the exosomes budding from the parasite surface. *Leishmania*-secreted molecules disrupted macrophage intracellular signaling pathways, including cleavage of PTPs, altered translocation of NF-κB and AP-1 in macrophages and inhibition of NO production [106,107]. Thus, exosomes provide a means for the parasite to efficiently deliver effector molecules to macrophages and modify their behavior to benefit parasite survival.

• Lipophosphoglycan

LPG is the most abundant surface glycolipid of promastigotes and is one of the best studied *Leishmania* molecules. LPG exhibits wide variation in sugar composition between and within species. For example, LPG from *L. braziliensis* is devoid of oligosaccharide side chains whereas LPG from *L. infantum* contains side chains, and they both trigger distinct immune responses in macrophages. *L. braziliensis* LPG results in higher levels of TNF, IL-1β, IL-6 and NO production and a stronger but more transient MAPK activation than *L. infantum* LPG, as observed in thioglycolate-elicited peritoneal macrophages infected with late-log phase promastigotes [60].

LPG may protect the parasite by scavenging ROS and inhibiting NADPH oxidase assembly at the phagosome [68]. LPG accumulated in lipid microdomains during phagocytosis and interfered with vesicle attachment and fusion and recruitment of host mediators of phagosome maturation. For example, LPG of *L. donovani* late stationary phase promastigotes excluded Synaptotagmin V at the phagocytic cup, resulting in decreased promastigote internalization [19–20,83]. Impaired recruitment of Synaptotagmin V by LPG also excluded V-ATPase from the phagosomes and prevented their acidification [20]. LPG was also the cause of periphagosomal F-actin accumulation, characteristic of the *L. donovani* PV, believed to play a role in phagosome remodeling. Disruption of lipid microdomains by cholesterol depletion abolished the effects of LPG on phagosome maturation and periphagosomal F-actin accumulation [84].

As well as causing local modifications in macrophage behavior that are restricted to individual phagosomes, LPG has a more global effect on macrophages by targeting intracellular signaling pathways. Thus, *L. mexicana* LPG activated ERK and p38 MAPK through their phosphorylation and led to the production of TNF, IL-1β, IL-12p40, IL-12p70 and IL-10 in ERK/ p38 MAPK-dependent manner, as observed in human periferal blood monocyte-derived macrophages infected with *L. mexicana* stationary phase promastigotes [85]. Production of these cytokines was also TLR2/4-dependent, and LPG has been shown to interact with TLR2 [85]. The changes in cytokine levels affect the activation status of the macrophage as well as the more systemic inflammatory pathways. TLR9 activation has been shown to protect the host, however recent findings reveal that LPG interacts with TLR2 to decrease TLR9 to favor survival of *L. braziliensis* and *L. major* promastigotes in bone marrow-derived macrophages and thioglycolate-elicited peritoneal macrophages, respectively [86,87]. *L. chagasi* LPG upregulated heme oxygenase-1 (HO-1), a key enzyme triggered by cellular stress, which was associated with diminished production of TNF and ROS and enhanced parasite survival [88].

Differentiation of promastigotes into amastigotes is accompanied by the loss of flagellum and a 1000-fold downregulation in LPG levels, underlying major physical differences between the two life cycle stages and the resulting differences in the two intracellular niches [10].

• GP63

GP63 is a GPI-anchored metalloprotease predominantly expressed by promastigotes and is thought to be released from the parasite via exosomes [78,105]. In infected cells, this intracellular survival factor colocalized with ganglioside GM1-positive lipid microdomains, possibly via its GPI anchor [53,59]. However, disruption of lipid microdomains did not impair GP63-dependent downstream events, such as TC-PTP cleavage, suggesting that additional mechanisms for entry into the host cell cytosol exist. GP63 also interacts with the complement component C3b so it can be taken up directly into cells [59]. Indeed, it has been proposed that parasite phagocytosis is not required for GP63 uptake and subsequent intracellular modifications, suggesting that *Leishmania* can modulate cell behavior prior to parasite uptake by the macrophage [89].

Once inside macrophages, GP63 cleaves host proteins, including phosphorylated adaptor

protein p130Cas, PTP-PEST, cortactin, TC-PTP and caspase-3, as observed in primary embryonic fibroblasts infected with *L. major* promastigotes and with recombinant GP63 and host proteins [90]. GP63 also participates in p38 MAPK inactivation, through cleaving TAB1 [90]. Modulation of PTPs by GP63 led to the inhibition of MAPK activation and downregulation of proinflammatory cytokine production [50]. Additionally, studies in B10R macrophage cell line infected with *L. major* promastigotes showed that GP63 cleaves the transcription factor AP-1, which regulates proinflammatory cytokine and NO production [89]. Furthermore, GP63 expressed by *L. major* stationary phase promastigotes was able to repress induction of type I IFN responses in B10R cells at translational level by targeting mTOR, the negative regulator of translation initiation by the eukaryotic initiation factor 4F [91]. Manipulation of the macrophage by the parasite via GP63 leads to a reduction of TNF, IL-12 and NO production among other changes geared to protect the parasite and promote its survival [61].

GP63 also targets pre-microRNA processor Dicer1 to downregulate microRNA-122, which plays a role in regulation of lipid metabolic genes. Restoration of microRNA-122 or Dicer1 increased serum cholesterol and reduced parasite burden in *L. donovani*-infected mouse liver [92]. GP63 is also responsible for the cleavage of VAMP8 in murine bone marrow-derived macrophages infected with *L. major* promastigotes [67]. As mentioned above, this SNARE is responsible for recruiting NOX2 to phagosomes and its disruption leads to reduced ROS and compromised MHC class I presentation. GP63 also plays a role in MHC class II presentation, although the mechanisms of such effects are yet to be determined.

• Inhibitors of serine peptidase

Leishmania produces molecules known as inhibitors of serine peptidase (ISP), which inhibit a number of host enzymes, including neutrophil elastase, trypsin and chymotrypsin. This was demonstrated in murine peritoneal macrophages infected with *L. major* stationary-phase metacyclic promastigotes enriched by agglutination with peanut lectin [63]. Inhibition of neutrophil elastase prevented TLR4 activation and promoted parasite survival. ISP2/3 mutants could differentiate but failed to divide in the absence of serine peptidase inhibition [63]. ISP2/3 deficiency in parasites led to unregulated activity of neutrophil elastase and enhanced parasite uptake and killing rates following increased superoxide burst [93]. *Leishmania* ISP is also involved in preventing activation of the host protein kinase, PKR. PKR is a serine/threonine kinase normally activated in response to dsRNA, such as during viral infections, but also to LPS via the TLR2/4 signaling pathway. PKR regulates NF-κB, TNF and IL-6 production. Disruption of PKR activity by *L. major* purified metacyclic promastigotes prevented activation of bone marrow-derived macrophages and killing of the parasite [64].

• Other intracellular survival factors

Several other *Leishmania* molecules contribute to parasite fitness and survival inside macrophages. For instance, mammalian ortholog proteins such as prohibitin and the PKC-like enzyme play a role in parasite uptake. Prohibitin interacts with host HSP70 on the macrophage surface and possibly forms part of a recognition complex, which is required for parasite binding to macrophages. Overexpression increased infectivity, whereas antibody treatment led to lower infectivity of purified metacyclic *L. donovani* promastigotes in J774A.1 cells [94]. *L. mexicana* PKC-like enzyme is expressed during the infective stationary phase, exhibits external Ca^{2+} and phosphatidylserine-dependent PKC activity in murine resident peritoneal macrophages, and plays a role in parasite internalization [95].

L. amazonensis promastigotes produce the mitochondrial enzyme inositol phosphosphingolipid phospholipase C-like (ISCL), which is responsible for sphingolipid degradation, and mutants in this enzyme were severely attenuated in low pH medium and in bone marrow-derived macrophages [96]. This suggests that ISCL is required for parasite survival in its macrophage replicative niche. *L. donovani* produces aldolase, which binds and activates the PTP SHP-1 in RAW 264.7 macrophages [97]. This may lead to impaired phagosome acidification [54] and may help the parasite avoid the hostile environment of the macrophage phagolysosome.

Other factors help the parasite counter oxidative stress inside the macrophage. *L. major* produces Methionine Sulfoxide Reductase A, which is required for resistance to oxidative stress. Mutants in this enzyme exhibited increased sensitivity to hydrogen peroxide and a reduced proliferation in RAW 264.7 macrophages. Interestingly, this enzyme was not essential for *in vivo* lesion formation [98]. *L. donovani*

produces the ALO enzyme, which is involved in vitamin C biosynthesis. ALO-deficient stationary phase promastigotes induced IFN-γ, IL-12 and TNF production and were susceptible to ROS and RNI in J774A.1 cells [99]. *L. donovani* and *L. pifanoi* secrete tryparedoxin peroxidase (TXNPx), which is then trafficked out of PVs in vesicles with distinct morphologies, into the cytosol and nucleus, where it acts as an antioxidant that detoxifies peroxides, ROS and RNI [100]. *L. donovani* peroxidase has peroxidoxin-like peroxidase activity and also downregulates NRAMP1 expression in peritoneal macrophages, possibly to redistribute iron to PVs and dampen immune responses, such as the production of IFN-γ, IL-12 and TNF [101]. Another ROS scavenging enzyme, *Leishmania* thioredoxin, is induced during infection and is also involved in PTP stabilization [81].

The ability of *Leishmania* to alter macrophage signaling and counter inflammation is also mediated by the cysteine protease CPB [102]. *L. mexicana* promastigotes and amastigotes activate host PTPs, including SHP-1, in B10R cells. Interestingly, PTP-1B is activated by promastigotes but not amastigotes. Both activate STAT-1α and AP-1. Promastigotes cleave p65 subunit of NF-κB to p35, while amastigotes fully degrade p65. All of these events are mediated by CPB. As a result, IFN-γ-mediated activation is suppressed and NO production is blocked [102]. *L. major* produces an ortholog of macrophage MIF, which binds MIF receptor, and like its mammalian counterpart it induced ERK1/2 MAPK activation and inhibited activation-induced apoptosis in murine bone marrow-derived macrophages [103]. Overall, such *Leishmania* tactics create an intracellular niche more conducive to their survival and replication.

Conclusion

As an intracellular parasite of macrophages, *Leishmania* has to find ways for efficient uptake into the host cell, and subsequently remodel the hostile environment of phagolysosome. *Leishmania* uses multiple macrophage receptors for recognition and is well adapted to stimulate phagocytosis with the help of the flagellum. Even before the phagosome has fully formed, the macrophage elicits an anti-microbial response through the recruitment of NADPH oxidase and V-ATPase complexes to the phagosomal cup. These measures would normally result in oxidative damage to the pathogen and promote acidification of the newly formed phagosome, as well as hydrolysis following the delivery of lysosomal enzymes to the phagosome. As well as playing a central role in pathogen destruction, the phagosome also functions in immune recognition by providing substrates for antigen presentation. *Leishmania* has evolved evasion strategies, such as the expression of LPG, to selectively modify the recruitment of various phagosome maturation factors and the fusion with lysosomes. The resulting PV protects the parasite from oxidative damage and immune recognition by antigen presentation. Furthermore, *Leishmania* releases intracellular survival factors, such as GP63, to target host signaling and cause global cellular modifications, including suppressed immune activation, retention of iron, foamy cell formation and enhanced autophagy. Such changes enhance nutrient availability to the parasite and prevent stimulation of proinflammatory responses. Multiple other defense factors have been shown to contribute to the intra-macrophage survival of different *Leishmania* strains and life stages, helping the parasite modify and adapt to its niche and making it a highly successful parasite.

Future perspective

The recent studies of *Leishmania*-infected macrophages highlight the vast complexity of host–parasite interactions, with many of the described pathways overlapping or interacting with each other. We have learned that the LPG-mediated disruption of lipid microdomains prevents microbicidal events, such as the NADPH oxidase recruitment to the phagosomal cup, but at the same time parasite uptake by the macrophage is compromised. Lower NADPH oxidase activity in the phagosome protects the parasite from ROS damage and also results in suppressed antigen presentation. LBs may simultaneously act as nutrient sources for *Leishmania* and as providers of arachidonic acid and other proinflammatory factors for the macrophage. LPG-mediated TLR2/4 activation may improve iron availability to the parasite, through iron retention by the macrophage, but it may also lead to macrophage activation. Many proinflammatory pathways are in turn selectively countered by GP63, ISP, CPB and other *Leishmania* defense molecules.

Although a lot of progress has been made in *Leishmania* research in the past few years, much remains to be explored in the area of *Leishmania*–macrophage interactions. What is

the trigger for parasite differentiation? What is causing foamy cell formation? What other macrophage functions are compromised? How do these findings translate to in vivo research and to humans? As we learn more about the changes exerted on the macrophage by *Leishmania* infection, we will gain greater appreciation for key macrophage functions and their role in immune response. As more *Leishmania* defense molecules are discovered, their importance in intracellular parasite survival is determined, and mechanisms for their actions are described, we will have a more comprehensive understanding of strategies employed by the parasite to survive in

EXECUTIVE SUMMARY

Initial events in parasite uptake by the macrophage

- *Leishmania* is phagocytosed by the macrophage via a range of receptors. Receptor choice affects phagosome biogenesis. Macrophage interaction with the parasite flagellum may trigger the release of parasite intracellular survival factors that modulate macrophage phagocytic activity.
- *Leishmania* phagosome maturation is modified by the mechanical action of the flagellum and a restricted fusion with vesicles of the endosomal pathway. Phagosomal pH and iron availability trigger promastigote-to-amastigote differentiation.

Intracellular parasite growth

- *Leishmania* growth depends on its interaction with ER-derived vesicles, presumably as a source of nutrients and additional membrane for the parasitophorous vacuole (PV).
- *Leishmania* acquires nutrients from vesicles of the endolysosomal and ER pathways, and possibly lipid bodies, following their fusion with the PV.
- *Leishmania* acquires iron through heme and ferrous iron transporters. The parasite upregulates iron uptake and retention by the macrophage to enhance iron availability.

Macrophage defenses

- The macrophage promotes assembly of NADPH oxidase and Nlrp3 inflammasome complexes, which produce reactive oxygen species (ROS) and reactive nitrogen intermediates to impair the parasite.
- The infected macrophage induces inflammation and IFN-γ-mediated activation, leading to enhanced ROS production, phagosome maturation and antigen presentation.
- The macrophage modulates lipid metabolism to generate lipid bodies, which produce arachidonic acid and other proinflammatory mediators.

Leishmania evasion of host defenses

- *Leishmania* upregulates host PPAγ and protein tyrosine phosphatases, which leads to suppressed inflammation.
- *Leishmania* interferes with the JAK/STAT pathway, preventing macrophage activation.
- *Leishmania* inhibits ROS generation by preventing NADPH oxidase assembly.
- *Leishmania* inhibits MHC class II expression and modulates the phagosome proteolytic function to suppress substrate production for efficient antigen presentation.
- *Leishmania* induces autophagy in macrophages, which is associated with nitric oxide reduction.

Leishmania intracellular survival factors

- Lipophosphoglycan scavenges ROS and disrupts lipid microdomains to suppress phagosome maturation. Lipophosphoglycan also interacts with TLR2/4 to interfere with host cell signaling.
- GP63 is released via exosomes and cleaves multiple host proteins, including protein tyrosine phosphatases, leading to downregulation of proinflammatory responses and antigen presentation.
- Other defense factors promote phagocytosis, modulate PV biogenesis and counter macrophage defenses including oxidative stress, activation, apoptosis and inflammation.

macrophages. As the macrophage responds to infection, tissue damage often occurs, contributing to clinical manifestations of Leishmaniasis disease. Advancing our understanding host-driven immune responses to the infection will support our efforts in minimizing symptoms of this devastating disease.

Expanding our knowledge of macrophage functions and *Leishmania* survival strategies will help us make more informed decisions in vaccine and drug development efforts. Currently, there are twelve million people infected with *Leishmania*, with two million new cases a year, however there is no available vaccine and drug resistance is emerging. *Leishmaniasis* is prevalent in 98 countries in Asia, Africa, South and Central America and southern Europe, with at least 17 different species of *Leishmania* causing the disease. The inter-species variation in drug sensitivity often means limitations in drug choice. Additionally, most available drugs have severe side effects, further complicating treatment. Manipulation of the immune response by the parasite makes it difficult to design an effective vaccine. Enhanced understanding of essential interaction pathways in *Leishmania* infection of macrophages will help design new drugs to disrupt interactions that favor the parasite, boost macrophage microbicidal and immune functions that support parasite elimination and inhibit *Leishmania* molecules that are essential for intracellular *Leishmania* survival.

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