Subversion and utilization of host innate defense by *Leishmania amazonensis*

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Infection with *Leishmania amazonensis* and other members of the *Leishmania mexicana* complex can lead to diverse clinical manifestations, some of which are relatively difficult to control, even with standard chemotherapy. Diffuse cutaneous leishmaniasis (CL) is a rare but severe form, and its clinical hallmark is excessive parasitic growth in infected cells accompanied by profound impairments in host immune responses to the parasites. Since these parasites also cause non-healing CL in most inbred strains of mice, these animals are valuable models for dissecting the mechanisms of persistent infection and disease pathogenesis. In comparison to other *Leishmania* species, *L. amazonensis* infections are most remarkable for their ability to repress the activation and effector functions of macrophages, dendritic cells, and CD4^{+} T cells, implying discrete mechanisms at work. In addition to this multilateral suppression of host innate and adaptive immunity, the activation of types I and II interferon-mediated responses and autophagic/lipid metabolic pathways actually promotes rather than restrains *L. amazonensis* infection. These seemingly contradictory findings reflect the remarkable adaptation of the parasites to the ancient defense machinery of the host, as well as the complex parasite–host interactions at different stages of infection, which collectively contribute to non-healing leishmaniasis in the New World. This review article highlights new evidence that reveals the strategies utilized by *L. amazonensis* parasites to subvert or modulate host innate defense machinery in neutrophils and macrophages, as well as the regulatory roles of host innate responses in promoting parasite survival and replication within the huge parasitophorous vacuoles. A better understanding of unique features in host responses to these parasites at early and late stages of infection is important for the rational design of control strategies for non-healing leishmaniasis.

Keywords: *Leishmania amazonensis*, innate immunity, parasite adaptation, immunopathogenesis

INTRODUCTION

Leishmania amazonensis is a member of the *Leishmania mexicana* complex and the etiological agent for a broad-spectrum of leishmaniasis in South American countries. It can cause mild cutaneous leishmaniasis (CL), diffuse CL (DCL), and deadly visceral leishmaniasis (Silveira et al., 2004). DCL is a rare but severe form and is not limited to patients in South America. In patients with *Leishmania tropica* and *Leishmania major* infections in India and Africa, DCL is also considered a clinical indicator of HIV co-infection (Niamba et al., 2007; Khandelwal et al., 2011). Generally speaking, patients with DCL share some clinical characteristics. For example, they have defective responses for the leishmanin skin test and poor antigen-stimulated T cell proliferation *in vitro*, although some patients remain responsiveness to other antigens such as

tuberculin and lepromin (Silveira et al., 2004). The mechanisms responsible for this antigen-specific T cell anergy remain obscure, although it is likely due to alterations in activation of dendritic cells (DC) and macrophages (MΦ) following infection by *L. amazonensis*. These profound impairments in cell-mediated immune responses partially explain the relatively poor responses of DCL patients to available anti-*Leishmania* chemotherapy.

Mouse models are important in understanding the biology of leishmaniasis. The Th1/Th2 polarization of IFN-γ/IL-4 production seen in *L. major* infection between resistant C57BL/6 and susceptible BALB/c mice has provided valuable insights into protective and pathogenic mechanisms; however, this dogma is not fully applicable to human diseases and to some other *Leishmania* infection models (McMahon-Pratt and Alexander, 2004). *L. amazonensis* infections in mice have several distinguished features. All inbred strains of mice are genetically susceptible to *L. amazonensis*, developing progressive non-healing lesions that contain characteristic parasitophorous vacuoles (PV) and heavy parasite loads (Soong et al., 1997). This generalized susceptibility is not linked to a polarized expansion of IL-4-producing Th2 cells, but rather to

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Abbreviations: Atg, autophagy-related genes; CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; IFNAR, type I interferon-α receptor; ISCL, inositol phosphosphingolipid phospholipase C-like; *LYST/Beige*, lysosomal trafficking regulator gene; PKR, dsRNA-dependent protein kinase; PS, phosphatidylserine; PV, parasitophorous vacuoles; SOD1, superoxide dismutase 1; TCR, T cell receptor.

the activation of pathogenic CD4$^+$ T cells that produce relatively low levels of IFN-γ, IL-10, IL-17, and other cytokines (Soong et al., 1997; Xin et al., 2007). While knockout of endogenous IFN-γ or IL-10 alone is insufficient to change host susceptibility to this infection, depletion of endogenous CD4$^+$ T cells or adoptive transfer of primed CD4$^+$ T cells can deprive or exacerbate lesion pathogenesis, respectively (Soong et al., 1997; Xin et al., 2007). The low and mixed Th1/Th2 responses seen in *L. amazonensis*-infected mice are very similar to those observed in human infections (Silveira et al., 2009), validating the biological relevance of these mouse models for the study of human diseases.

Among the *Leishmania* species that are pathogenic to humans, only *L. amazonensis* and other members in the *L. mexicana* complex are capable of forming huge communal PV. It has been proposed that the large PVs may serve as a "shield," by diluting the leishmanicidal effects of nitric oxide (NO) and reactive oxygen species (ROS) in infected cells (Wilson et al., 2008); however, additional studies are needed to verify this possibility. Comparative studies with *L. amazonensis* promastigotes generated from DCL and CL patients have revealed that DCL-derived parasites tend to form big PVs with heavy infection in mouse MΦs, suggesting a positive correlation between parasite virulence and PV sizes (Franca-Costa et al., submitted). Under the cell-free condition, *L. amazonensis* parasites (especially the amastigote form) are also more resistant to neutrophil- or MΦ-derived leishmanicidal molecules than are their counterparts of other species (Mukbel et al., 2007; Wang et al., 2011), possibly indicative of biological features that are unique and intrinsic to these parasites.

The purpose of this review is not to provide a comprehensive summary for the biology of *Leishmania* parasites or the pathogenic roles of adaptive immune responses, as there are excellent reviews covering these areas in this Special Topics issue for leishmaniasis. Instead, this review will focus on new evidence that reveals the strategies utilized by *L. amazonensis* parasites to down-modulate and subvert host innate defense machinery in neutrophils and MΦ, as well as the regulatory roles of host innate responses in promoting parasite survival and infection in the host. These studies indicate complex roles of host immune responses to *Leishmania* infections and open areas for further investigation.

L. AMAZONENSIS INFECTION CAN TRIGGER BROAD-RANGE IMMUNOSUPPRESSION

As successful intracellular parasites that have a unique replication niche, it is not surprising that *Leishmania* have developed multifaceted strategies to subvert or suppress MΦ's defense machinery (Osorio y Fortea et al., 2007; McConville and Naderer, 2011). It is commonly thought that *L. amazonensis* parasites have evolved additional mechanisms, some of which account for their uncontrolled replication in humans and in various strains of mice. Although promastigotes of *L. amazonensis* can transiently trigger DC and MΦ activation, the magnitude of cell activation, and the spectrum of cytokine production are often much lower than those triggered by promastigotes of *L. major* and *Leishmania braziliensis* (Xin et al., 2007; Vargas-Inchaustegui et al., 2008). Since these species-related differences are evident even at 12 h of infection *in vitro* (Xin et al., 2007) or at 1 week of infection in mice (Ji et al., 2003), several scenarios may account for the

deficient immune responses against *L. amazonensis*: these parasites are either intrinsically potent in immune repression, antigenically weak in immune activation, or both. These hypotheses are supported by the findings that promastigote antigens of *L. amazonensis* (but not *L. braziliensis*) markedly inhibit mitogen- and anti-CD3-induced T cell proliferation, and that such T cell anergy cannot be reverted by exogenous cytokines (Pinheiro et al., 2004). Likewise, vaccination with antigens of *L. amazonensis* (but not *L. braziliensis*) preferentially stimulate IL-10 and TGF-β production, leading to an increased susceptibility of vaccinated BALB/c mice to *L. amazonensis* infection (Pinheiro et al., 2005). The mechanisms underlying the promastigote species-specific differences remain ambiguous.

The *in situ* and *in vivo* measurement of a large panel of cytokines and chemokines and their corresponding receptors during *L. amazonensis* infection has confirmed that the early immunosuppressive status in antigen-presenting cells sets the stage for impaired inflammatory responses and T cell responses. For example, IL-1-related cytokines (IL-1α, IL-1β, and IL-1Rα) are rapidly induced and tightly regulated in *L. major*-infected C57BL/6 (selfhealing) mice; however, these cytokines are barely induced even at 4 weeks of infection with *L. amazonensis* (Ji et al., 2003). Surprisingly, while IL-1β treatment *in vitro* promotes the activation of *L. amazonensis*-infected DCs and CD4$^+$ T cells, such treatment actually exacerbates lesion progression and parasite growth in mice (Xin et al., 2007), suggesting that the pathogenic condition may not be readily reprogrammed by exogenous cytokines. To better understand these pathogenic T cells, the Soong group compared the frequency of cytokine-producing cells and their T cell receptor (TCR) repertoires for CD4$^+$ T cells isolated from non-healing (*L. amazonensis*-infected) and self-healing (*L. braziliensis*-infected) mice during primary and secondary infections. These different sources of CD4$^+$ T cells differ at the level of IFN-γ, IL-10, and IL-17 production by the same TCR Vβ-barring subsets, implying that the low magnitude of CD4$^+$ T cell activation (rather than the altered TCR diversity) is the determining factor for non-healing disease caused by *L. amazonensis* (Xin et al., 2011).

Leishmania amazonensis amastigotes are superior in silent invasion, forming huge PV, and preventing the activation of NK, DC, and MΦ triggered by exogenous stimuli such as LPS (Soong, 2008; Bhardwaj et al., 2010). The intracellular events triggered by *L. amazonensis* infection in MΦs have been described in detail (Bhardwaj et al., 2010; McConville and Naderer, 2011). The suppression of host cell activation can be initiated at the stage of amastigote–host contact. On one hand, *L. amazonensis* amastigotes can infect human DCs through multiple receptor–ligand interactions such as antibodies/Fc receptors, complement components/component receptors, and proteoglycans/heparin-binding proteins (Bosetto and Giorgio, 2007), leading to altered DC activation and impaired responsiveness to exogenous stimuli through ERK/MAP kinase-mediated mechanisms (Boggiatto et al., 2009). On the other hand, *L. amazonensis* amastigotes can expose phosphatidylserine (PS) on their surface, leading to enhanced parasite growth and host suppression through IL-10 and TGF-β production by host cells (Wanderley et al., 2006). The levels of PS exposure are relatively high on lesion- and MΦ-derived amastigotes, but nearly absent on axenic amastigotes, and are positively correlated to host immune

responses (unpublished data). Although the origin of and the conditions inducing PS exposure on amastigote surface are matters of investigation, modulating PS exposure is fundamental for repressing host immune responses and granting survival for *Leishmania* parasites (Wanderley and Barcinski, 2010). This "apoptotic mimicry" strategy is also important for other pathogens, including *Toxoplasma gondii* and *Vibrio cholerae* (Thanawastien et al., 2009; Dos Santos et al., 2011). No doubt, *L. amazonensis* can elicit a broad-spectrum suppression against host innate and adaptive immune responses, but low levels of host immune responses are required for disease progression.

L. AMAZONENSIS PARASITES ARE PROFICIENT IN UTILIZING HOST INNATE IMMUNE RESPONSES FOR THEIR OWN GROWTH
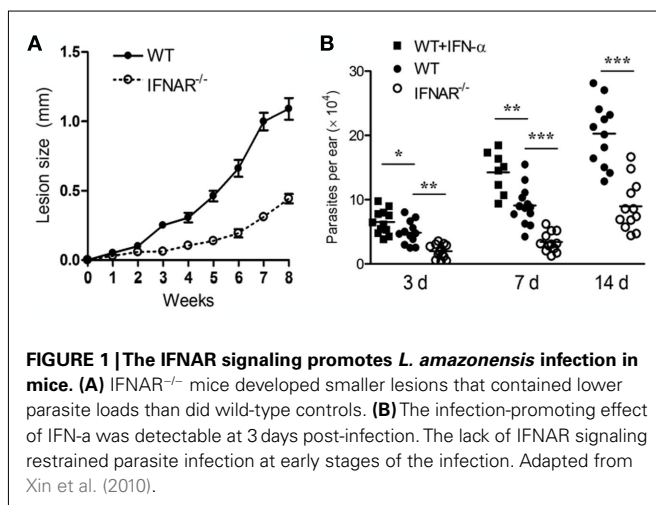
Several lines of new evidence have revealed that *L. amazonensis* parasites take advantage of host innate defense machinery in neutrophils and MΦs for their own infection. The first one is related to the utilization of host type I interferons (IFN). The Soong group has shown that signaling through type I IFNs and IFN-α receptor (IFNAR) actually favors *L. amazonensis* infection, rather than promotes its control (Xin et al., 2010). As shown in **Figure 1**, *L. amazonensis*-infected IFNAR$^{-/-}$ mice developed much smaller lesions that contained lower numbers of parasites than did wild-type controls. Even at 3 days post-infection, parasite growth was restrained in IFNAR$^{-/-}$ mice but exacerbated in IFN-α-treated mice, implying a regulatory role for type I IFNs in this infection model. Along the same line, it is known that IFNAR$^{-/-}$ mice are also more resistant to intracellular bacteria such as *Listeria monocytogenes* and *Mycobacterium tuberculosis* (Rayamajhi et al., 2010a,b). In the case of *L. amazonensis* infection, the disease-promoting effect of type I IFNs is multifarious, correlating with the functions and interactions of neutrophils and MΦs. For instance, IFN-α prolongs the life span of un-infected and infected neutrophils, whereas the deletion of IFNAR decreases the survival of neutrophils (and most likely the survival of promastigotes; Xin et al., 2010). Importantly, the lack of IFNAR signaling promotes the differential recruitment and activation of neutrophils and inflammatory monocytes, as well as restrains parasite infection in MΦs that are co-cultured

with neutrophils (Xin et al., 2010). Therefore, New World leishmaniasis caused by *L. amazonensis* and *L. guyanensis* infections are exacerbated by the production of type I IFNs (Xin et al., 2010; Ives et al., 2011). During *Leishmania* infection, type I IFN production can be triggered by TLR9-dependent responses to *Leishmania* genomic DNA or TLR3-dependent responses to *Leishmania* RNA virus-1 or exposure to other dsRNA viruses (Liese et al., 2007; Ives et al., 2011). The pathogenic role of type I IFNs is supported by the findings that exogenous IFN-β stimulates superoxide dismutase 1 (SOD1) and impairs the superoxide-dependent killing of *L. amazonensis* and *L. braziliensis* in human MΦ, playing a deleterious role in CL (Khouri et al., 2009). Since type I IFNs are known to play a protective role in infections against *L. major*, *L. braziliensis*, and *Leishmania infantum* infections (Liese et al., 2008), careful evaluation is needed for the functions of type I IFNs in murine and human leishmaniasis.

The second line of evidence is related to the utilization of TLR2-mediated responses for parasite growth. While MyD88 is required for the effective control of *L. amazonensis* and *L. braziliensis* infections, signaling through TLR2 seems to have a regulatory role, favoring these infections (Vargas-Inchaustegui et al., 2009; Guerra et al., 2010). In the absence of TLR2, DC, and CD4$^+$ T cells display an elevated activation and enhanced production of IL-12, IL-10, and IFN-γ cytokines. Lesions of TLR2$^{-/-}$ mice had reduced numbers of neutrophils and MΦs, but increased numbers of eosinophils. As a consequence, TLR2$^{-/-}$ mice have attenuated lesions following infection with *L. braziliensis* (Vargas-Inchaustegui et al., 2009) and *L. amazonensis* (Guerra et al., 2010; unpublished data).

Mechanistically, the disease-promoting effects of IFNAR and TLR2 appear to be linked to the initial intracellular events triggered by parasite infection. The elegant studies conducted by the Lopes group have shown that infection with promastigotes of *L. amazonensis* leads to the activation of an interferon-dependent expression of dsRNA-dependent protein kinase (PKR), a classic antiviral protein, and that treatment of infected mouse MΦs with the synthetic dsRNA poly(I:C), a potent PKR inductor, increases parasite intracellular growth through an IL-10-mediated mechanism (Pereira et al., 2010). More recently, the same group also found that *L. amazonensis* and lipophosphoglycan, the major surface component of promastigotes, induce the activation of the PKR promoter by a TLR2-dependent manner, and that TLR2$^{-/-}$ MΦs express low IFN-β and PKR levels with reduced parasite loads, while IFN-α treatment promotes the growth of *L. amazonensis* promastigotes (de Carvalho Vivarini et al., 2011). Importantly, lesions of DCL patients displayed higher levels of PKR/type I IFN-expressing cells compared with those from single CL patients, indicating an important and regulatory role for type I IFNs and TLR2 in human leishmaniasis (de Carvalho Vivarini et al., 2011).

How type I IFNs and TLR2 execute their regulatory roles at the early and late stages of *L. amazonensis* infection remain unclear. In the cases of *L. monocytogenes* and *M. tuberculosis* infections, host susceptibility is known to be associated with the antagonistic crosstalk between type I and type II IFNs, as IFN-α/β can impair MΦ activation and their responsiveness to IFN-γ (Rayamajhi et al., 2010b). Also, type I IFN singling can influence the phenotypes of MΦ subsets, as well as their responsiveness to a range of cytokines

FIGURE 1 | The IFNAR signaling promotes *L. amazonensis* infection in mice. (A) IFNAR$^{-/-}$ mice developed smaller lesions that contained lower parasite loads than did wild-type controls. **(B)** The infection-promoting effect of IFN-a was detectable at 3 days post-infection. The lack of IFNAR signaling restrained parasite infection at early stages of the infection. Adapted from Xin et al. (2010).

that use STAT1 (IFN-γ, IL-10, IL-6, M-CSF, CCL5, and CXCL10; Fleetwood et al., 2009). Given that exogenous pro-inflammatory cytokines (IFN-α/β, IFN-γ, IL-1β), regulatory cytokines (TGF-β, IL-10), and insulin-like growth factor-1 all have the potential to promote *L. amazonensis* infection *in vitro* and/or in mice (Vendrame et al., 2007; Wanasen et al., 2007; Xin et al., 2007), it is reasonable to speculate that the low level and crosstalk of these host cytokines create a microenvironment, which is exploited by these parasites for efficient entry and intracellular growth.

L. AMAZONENSIS PARASITES CAN EXPLOIT HOST'S LIPID METABOLISM AND ENDOCYTIC/AUTOPHAGIC PATHWAYS

Autophagy is an ancient mechanism for recycling aged organelles or proteins, and an intrinsic antimicrobial defense for pathogen killing and antigen presentation. Signaling pathways triggered through TLR3/4/7/8 and pro-inflammatory cytokines (IFN-α/β/γ, TNF-α, IL-1β) can induce canonical macro-autophagy characterized by the double-membrane autophagosome, leading to the clearance of most viruses and bacteria and the presentation of microbial antigens (Delgado et al., 2009; Deretic, 2011). However, some pathogens can override or exploit this innate defense machinery for their favored growth (Deretic and Levine, 2009). The successful intracellular pathogens often have multipronged strategies to avoid autophagolysosomal degradation and/or dampen autophagy-dependent activation of host immune responses. In *Leishmania* research, autophagy is known to be critically important for parasite stage differentiation and development (Besteiro et al., 2006; Williams et al., 2009); however, it is largely unclear as to how host cell autophagy affects *Leishmania* infection and how replicating parasites manipulate host autophagic pathways. Several recent studies have suggested a unique ability of *L. amazonensis* to circumvent the host's lipid metabolism and endocytic/autophagic pathways.

First, *L. amazonensis* parasites can selectively manipulate host cell lipid biosynthesis pathways. Wilson et al. (2008) have reported that *L. amazonensis* promastigote infection can up-regulate the lysosomal trafficking regulator gene (*LYST/Beige*) in MΦs, and that up- or down-regulation of the *LYST/Beige* gene in MΦs and fibroblasts directly modulates PV sizes and parasite growth. At present, the definitive roles for and regulation of *LYST/Beige* expression during *in vivo* infection have not been demonstrated. Recently, Osorio y Fortea et al. (2009) conducted large-scale, transcriptional signature analyses of amastigote-free MΦ and amastigote-harboring MΦ at 24 h after the uptake of nude mouse-derived *L. amazonensis* amastigotes. Their data indicate that amastigote infection preferentially up-regulates the function of lipid metabolism, especially the canonical metabolic pathway of biosynthesis of steroids, as well as the polyamine pathways. Therefore, while these replicating amastigotes repress host cell activation in general, they can selectively exploit the host cell's lysosomal trafficking and sterol biosynthesis machinery for their own cell membrane sterols and for the sterol-dependent remodeling and expansion of the PV membrane. However, these transcriptomes profiles represent early host–parasite interactions in the absence of host cytokines and components that are often detected in the lesion microenvironment or on the amastigote surface.

To address the functional role of host phospholipids and ceramides in parasite infection, the Zhang group generated *Leishmania* parasites that are deficient in the degradation of host sphingolipid, due to the deletion of the inositol phosphosphingolipid phospholipase C-like (ISCL) protein. They found that the *iscl*-null mutants of *L. major* completely abolished their virulence in BALB/c mice, partially due to parasite's defects in survival under an acid pH condition; however, a low number of mutant parasites could persist in the injection site for several months (Zhang et al., 2009; Xu et al., 2011). In sharp contrast, the *iscl*-deficient *L. amazonensis* are fully virulent in BALB/c mice, but are partially attenuated in C57BL/6 mice (K. Zhang, personal communication). The striking differences in disease outcomes following infection with *iscl*-deficient *L. major* and *L. amazonensis* in BALB/c mice suggest that ISCL is an essential virulence factor for *L. major*; however, *L. amazonensis* may have additional, ISCL-independent mechanisms for survival within the acidified PV.

Second, *L. amazonensis* amastigote-harboring PVs are enriched with nutrients, but are not augmented with microbicidal function of the lysosome. Osorio y Fortea et al. (2009) reported the infection-induced gene expression of eight isoforms of the V0 and V1 sub-units of the MΦ vacuolar proton ATPase, supporting the notion that amastigote-harboring PVs are highly acidified and optimal for amastigote nutrient acquisition (McConville and Naderer, 2011). Most notably, *L. amazonensis* amastigote-harboring PVs are quite special, as they can fuse with vacuoles that carry *L. major* promastigote but not amastigotes. *L. major* promastigotes within these chimeric PVs can temporarily survive and multiply, but fail to differentiate into amastigotes (Real et al., 2010). Therefore, the mature PV of *L. amazonensis* is not the compartment with a license to kill. Furthermore, *L. amazonensis* amastigote-harboring PVs can efficiently fuse with vacuoles that carry *Coxiella burnetii*, the causative agent of human Q fever. Importantly, *L. amazonensis* amastigotes, as well as wild-type and mutant *C. burnetii* deficient in their ability of lysosomal renovation, can replicate comfortably within these chimeric vacuoles (Rabinovitch and Veras, 1996; Beare et al., 2011). The findings that *L. amazonensis* and *C. burnetii* replicate in fusogenic vacuoles are intriguing. It is plausible that both pathogens can block the microbicidal function of the lysosome and acquire nutrients by continuous fusion and/or membrane transport with other compartments in the secretory and endolysosomal systems (McConville and Naderer, 2011; Newton and Roy, 2011). At this stage, it remains to be a big challenge and a big prize to cell biologists to isolate and study the protein and lipid composition of *Leishmania* PV. One hypothesis may be incompleteness of PV membrane for ROS producing machinery after *L. amazonensis* infection. An assessment of absolute NO or ROS levels in *Leishmania*-carrying PVs versus lysosomes and phagosomes formed after engulfment of bacteria and latex beads will shield some light in this area.

Lastly, factors that induce MΦ autophagy can promote *L. amazonensis* infection. In mammalian cells, autophagy induction is regulated by sequential activation of regulators, the formation of Atg complexes and autophagosomes, and the maturation of autolysosomes. Although IFN-γ treatment (10 ~ 100 ng/ml) is known to increase PV sizes and *L. amazonensis* amastigote loads in mouse MΦs in an arginase-independent manner (Wanasen et al.,

2007), the underlying mechanism is ambiguous. Recently, Pinheiro et al. (2009) have shown that autophagy induction via IFN-γ or starvation increased *L. amazonensis* but not *L. major* infection in BALB/c MΦs; however, this was not the case in C57BL/6 MΦs. Since *L. amazonensis* amastigotes do not co-localize with IFN-γ-induced, double-membrane vacuoles (Pinheiro et al., 2009), it is plausible that IFN-γ, starvation, or autophagy-inducing drugs may create a vacuolar environment that is generally permissive for engulfment and replication of the parasites. Information on whether and how *Leishmania* parasites alter, modulate, and utilize host autophagic pathways is needed. Additional studies with mutant parasites, in the context of genetic and/or pharmacological perturbation in the host autophagic pathway and lipid metabolism, will uncover the parasites' intrinsic ability of utilizing host cell resources and exploiting host defense mechanisms for their favored intracellular growth.

CONCLUDING REMARKS

The emerging evidence from recent studies has uncovered the complex host–*Leishmania* interactions. A detrimental regulatory loop at very early stages of the infection with *L. amazonensis* can lead to the activation and expansion of pathogenic CD4^{+} T and B cells and non-healing lesions. **Figure 2** is a schematic diagram, illustrating key regulatory events at the level of neutrophils and MΦs that collectively contribute to the exacerbated *L. amazonensis* infection. Firstly, the IFNAR-mediated signaling augments the differential recruitment and activation of neutrophils and inflammatory monocytes, as well as the prolonged survival of neutrophils, the potential Trojan horses for parasite spread to MΦ (Jochim and Teixeira, 2009). Secondly, type I IFNs contribute to parasite infection by impairing the superoxide-dependent killing of parasites in MΦ and simulating PKR-mediated production of IL-10 in infected MΦs. Thirdly, pro-inflammatory cytokines such as IFN-γ can turn on the host cell autophagic pathway and lipid metabolism, thereby creating a microenvironment that favors parasite entry or replica-
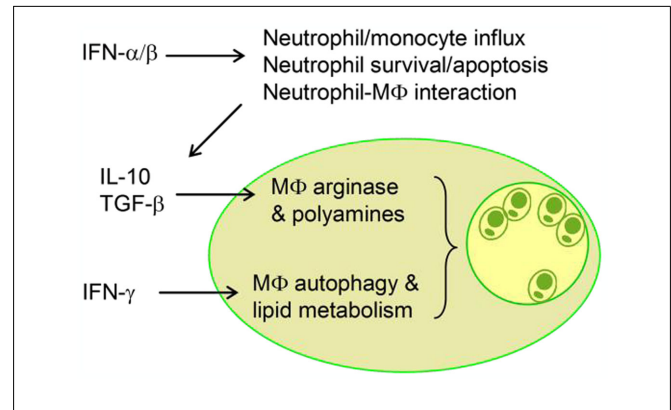
FIGURE 2 | A schematic diagram illustrates the interplays between innate immune cells and the regulatory roles of key cytokines during infection with *Leishmania amazonensis*. Modulation of intracellular pathways by host and/or parasite factors can lead to enlarged vacuoles and enhanced parasite growth.

tion. Further validation of the cross-talking by using immunological, genetic, and pharmacological approaches will be important. For the study of innate immune responses *in vivo*, another issue of consideration is the differences in local responses triggered by needle- versus sand fly-delivered parasites (see other reviews in this Special Topic). A better understanding of how different species and stages of *Leishmania* parasites can selectively utilize host innate defense machinery and adoptive immunity for immune evasion, intracellular growth, and immunopathogenesis will lead to the rational design of control strategies for this and other related infections.

ACKNOWLEDGMENTS

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