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How to master the host immune system? *Leishmania* parasites have the solutions!

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Abstract

Infection by protozoan parasites of the genus *Leishmania* results in the development of leishmaniasis, an increasingly prevalent group of diseases affecting over 12 million people worldwide. Leishmaniasis can have very different outcomes ranging from cutaneous lesions, mucosal lesions to visceralization depending on the species of the infecting parasite and on the immune response developed by the host. As an obligate intracellular parasite, residing within macrophages, *Leishmania* evolved in strict contact with the host immune system, developing different mechanisms to evade or modulate the immune response. Various types of immune responses are observed during different *Leishmania* spp. infections, resulting in parasite clearance but also contributing to the pathogenesis, thus increasing the complexity of the course of the disease. Interestingly, depending on the type of leishmaniasis developed, opposite treatment strategies, which either boost or inhibit the inflammatory response, have shown efficacy. In this review, we summarize the contribution of different immune cell types to the development of the anti-leishmanial immune response and the parasite strategies to evade and modulate host immunity. Further, we discuss the involvement of co-infecting pathogens in the determination of the outcome of leishmaniasis and on the effectiveness of treatment and the implication of the immune response for treatment and vaccine development.

Keywords: co-infection, immune modulation, leishmaniasis

Introduction

Infection with the protozoan parasite *Leishmania* can lead to the development of leishmaniasis, a prominent but neglected disease affecting over 12 million people worldwide, with an increasing geographical extension, limited treatment options and still no human vaccine (1, 2). Depending principally on the species of the infecting parasite, leishmaniasis can have a wide spectrum of manifestations (2). Cutaneous leishmaniasis (CL) is most frequently displayed as ulcerating skin lesions at the site of the sand fly bite, and caused by species such as *L. major*, *L. braziliensis* or *L. guyanensis* (2, 3).

CL can evolve into mucocutaneous cutaneous leishmaniasis (MCL) or disseminated cutaneous leishmaniasis (DCL), two more severe forms of the disease characterized by parasite migration to secondary sites and the formation of metastatic lesions (4, 5). Interestingly, these secondary lesions often present very low parasite numbers, but concomitant exaggerated inflammation provokes tissue destruction. This dissemination and re-activation of the infection at secondary sites can occur years after the primary infection and are

observed with species such as *L. braziliensis*, *L. guyanensis* and *L. aethiopica*. Despite being less frequent than CL, visceral leishmaniasis (VL), caused by *L. donovani* and *L. infantum*, is the most dangerous manifestation and is characterized by parasite dissemination to the liver and spleen that can be fatal if left untreated.

Aside from the species of the infecting parasite, the outcome of leishmaniasis is mainly determined by the host immune response and several immune-related genetic polymorphisms have been associated with the severity of the disease. *Leishmania* being obligate intracellular parasites, after the transmission to humans by a sand fly bite, they need to rapidly locate their host cells. In the mammalian host, *Leishmania* reside mainly in long-lived resident macrophages where they differentiate from promastigotes to amastigotes and then survive in the hostile, acidic and higher temperature environment of a phago-lysosome-like organelle. To establish the infection, some (but not all) *Leishmania* species require the recruitment of inflammatory monocytes, macrophages

and neutrophils at the infection site. Once inside its host cell, *Leishmania* hide from the immune system to survive. Thus, *Leishmania* had to develop different strategies to shape the immune response from its very beginning, affecting both innate and adaptive immunity to promote its own survival.

Considering the abundance of excellent recent reviews on leishmaniasis, we will focus our review on some of the actual knowledge of innate and adaptive immune responses to *Leishmania* and parasite evasion techniques, then address the effect of co-infections on leishmaniasis and finally analyze the implications for treatment and vaccine development.

Innate immunity to *Leishmania*

The first hours after the infection are crucial for obligate intracellular *Leishmania* parasites to rapidly localize phagocytes to colonize its host. At this initial stage, *Leishmania* benefit from pro-inflammatory properties of the sand fly saliva that plays an essential role in phagocyte chemo-attraction, being thus an important determinant of infection outcome (6). The early phases of *Leishmania* infection are also affected by the dual role of complement, as complement-mediated lysis eliminates parasites whereas opsonization promotes parasite up-take by phagocytes, thus favoring the infection. The *Leishmania* lipophosphoglycan (LPG) coat protects the parasite from complement-mediated lysis and at the same time induces complement activation, thus promoting parasite phagocytosis (7). Moreover, leishmanial LPG delays the formation of phago-lysosomes and allows *Leishmania* parasites to differentiate into its intracellular form, the amastigote (8).

Leishmania and macrophages

The long-lasting *Leishmania* infection is established within macrophages (phagocytic cells usually specialized in the elimination of intracellular pathogens) confining the parasite in a phago-lysosome organelle filled with lytic enzymes and low pH. In the first hours of the infection, the most effective anti-*Leishmania* response of the macrophages is the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (9, 10), a tightly regulated process that aims to kill any invading pathogen without damaging the host cell. This process is in part activated by phagocytosis and involves several enzymes. The two major producers of reactive species in macrophages are NADPH oxidase 2 (NOX2) and inducible nitric oxide synthase (iNOS), which produce superoxide (O_2^-) and nitric oxide (NO), respectively (11, 12).

Evidently, *Leishmania* parasites have developed various strategies to interfere with this process (13) (see Fig. 1), such as the LPG shield that protects parasites from ROS and RNS and subsequently delays the assembly of NOX2 at the surface of the phago-lysosome in the phagocytic cup, thus suspending the production of O_2^- (14). This process is further exploited by amastigotes, because of a thick layer of LPG (15). Further, *Leishmania* parasites can secrete or induce macrophages to produce arginase, which competes with iNOS for arginine and produces essential nutrients for the parasites such as L-ornithine for the synthesis of polyamines and urea, and at the same time diminishes the production of parasitotoxic NO (16). Oxidative stress could also be further

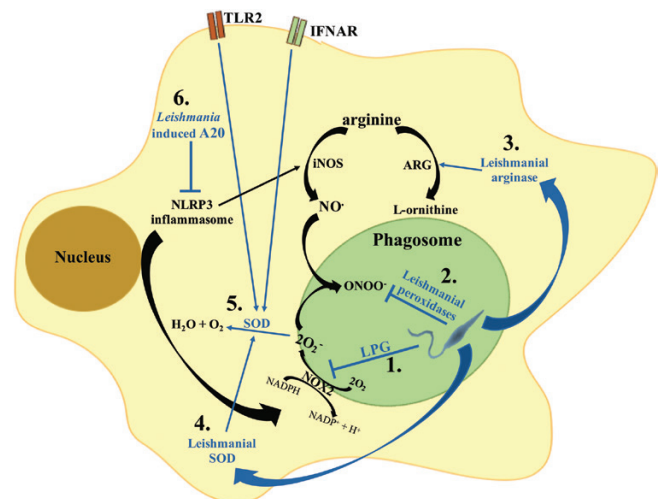


Fig. 1. Mechanisms of *Leishmania* interference with the macrophage oxidative stress response. *Leishmania* parasites have developed different strategies to interfere with the macrophage oxidative stress response. (1) Early after phagocytosis, the leishmanial LPG coat inhibits the assembly of the NOX2 complex at the phagosome membrane, thus blocking superoxide production (O_2^-). (2) *Leishmania* secrete a series of peroxidases which detoxify parasitotoxic superoxide. In addition, *Leishmania* parasites can secrete arginase (3), which promotes the conversion of arginine into urea and ornithine used for parasite metabolism, and secrete SOD homologues (4), which increase the detoxification of parasitotoxic superoxide. (5) Additionally, stimulation of TLR2 or the type I interferon receptor (IFNAR) during *Leishmania* infection induces macrophages to up-regulate SOD expression. (6) NLRP3 inflammasome activation was shown to promote the production of ROS and RNS; *Leishmania* inhibits inflammasome activation via the induction of the deubiquitinating protein A20, which inhibits IL-1 β maturation.

inhibited by the leishmanial metalloprotease gp63, which interferes with macrophage signaling pathways leading to NOX2 and iNOS induction (17).

Interestingly, *L. braziliensis* and *L. amazonensis* have been shown to increase macrophage superoxide dismutase (SOD) expression, through the induction of type I interferons, thus favoring parasite survival (18), possibly acting in concert with various leishmanial antioxidant analogues present in the parasite (19–21).

In conclusion, *Leishmania* parasites actively master the oxidative stress from their initial entry to their survival in the macrophage.

Leishmania and neutrophils

Beside macrophages, *Leishmania* parasites can also be trapped by neutrophils that are rapidly recruited to the infection site, where they produce 'neutrophil extracellular traps' (NETs). These are a network of extracellular fibrils composed of DNA associated with antimicrobial proteins such as elastase, histones and toxic granules.

Despite usually being very short-lived immune cells, the neutrophil life-span highly expands during inflammation. However, the impact of neutrophils on leishmaniasis outcome is likely species-specific (22). In fact, while *L. amazonensis* parasites are efficiently killed by NETs, *L. donovani* and *L. infantum* are resistant and may even benefit from NETs formation (23). Moreover, the rapid recruitment of neutrophils

during *L. mexicana* infection contributes to disease progression and chronicity (24). Further, an increased recruitment of neutrophils was observed in mice co-infected with *L. major* and lymphocytic choriomeningitis virus (LCMV), which developed more severe lesions compared to controls infected with just *L. major* (25). The molecular signatures or mechanisms underlying these differences in the relation between neutrophils and different *Leishmania* species are not yet known and would deserve thorough investigations.

Leishmania and inflammatory monocytes

Not only resident macrophages and neutrophils, but also inflammatory monocytes, recruited via platelet activation very early at the infection site, can be infected by *Leishmania* parasites, as was shown for *L. major* (26). In general, recruitment of inflammatory monocytes correlates with increased resistance to *L. major* infection (26). Inflammatory monocyte recruitment depends on chemokine (C-C motif) ligand 2 (CCL2/MCP-1), which is produced by cells at the infection site upon activation via platelet-derived growth factor (26). The importance of CCL2 and recruitment of inflammatory monocytes at the site of infection was investigated in C-C chemokine receptor type 2 (CCR2)⁺ mice infected with *L. major*, where a non-healing phenotype was observed in a mouse strain usually controlling *Leishmania* infection. Thus, recruitment of inflammatory monocytes correlates with increased resistance to *L. major* infection (26).

Interestingly, monocytes are crucial to control the re-infection of resistant mice with *L. major*, as they clear the parasites through the production of ROS and RNS following activation by resident memory CD4⁺ T cells (27, 28). However, this phenotype is parasite species specific, as shown following infection with *L. amazonensis*, where the recruitment of inflammatory monocytes was associated with increased

parasite burden and pathology (29). Similarly, infection of human monocytes by different *Leishmania* species alters their adherence to connective tissues (30) and could favor dissemination of the infection.

The paradoxical role of pattern recognition receptors

A common characteristic of the different innate immune cells is the expression of pattern recognition receptors (PRRs), capable of recognizing pathogen-associated molecular patterns (PAMPs). These latter are broadly conserved motifs covering bacterial endotoxins, lipopeptides and peptidoglycans, together with single-stranded (ss) and double-stranded (ds) nucleic acids. These PAMPs are recognized via Toll-like receptors (TLRs), cytosolic DNA sensors (CDSs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and C-type lectin receptors (CLRs). Because of the numerous ligands expressed by the parasite (31, 32), the most studied PRRs are TLRs, which play different and often species-specific roles during *Leishmania* infection, adding complexity and diversity to their role in leishmaniasis but possibly explaining different clinical outcomes ranging from clearance to exacerbated pathology (see Fig. 2).

For example, TLR2 promotes the clearance of *L. major*, *L. aethiops* or *L. mexicana* infection by promoting NO⁻ and ROS production, therefore favoring the development of a protective immune response in mice (33–35). However, TLR2 stimulation has also been shown to promote the persistence of *L. braziliensis* and *L. amazonensis* infection by inducing the expression of SOD1 through a PKR–IFN-β axis (36). These contradictory roles allegedly depend either on the thickness of the LPG coat, which vary considerably between parasite species or on other molecules still to be identified.

One report suggests that TLR3 is required for the phagocytosis of *L. donovani* and the subsequent production of parasitotoxic NO⁻ and TNF-α by macrophages, thus

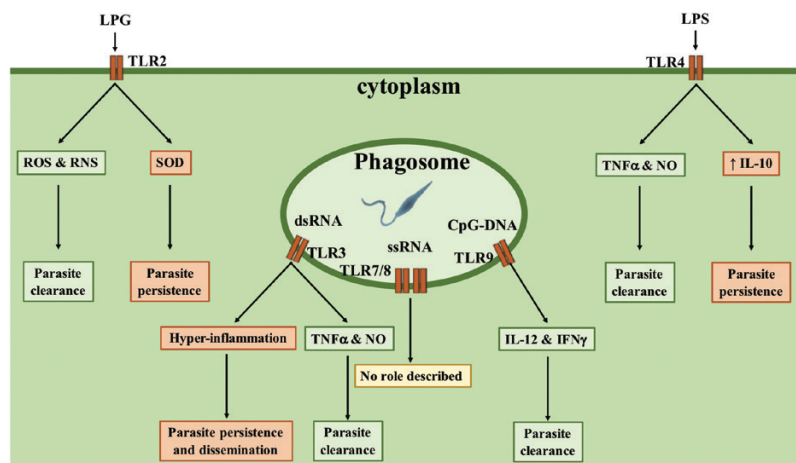


Fig. 2. TLRs' influence on the outcome of *Leishmania* infection. Several TLRs are stimulated during *Leishmania* infection and can have opposite consequences on the outcome of the infection depending on parasite species and timing of stimulation. During *L. major* infection, TLR2 activation increases the production of ROS and RNS, thus favoring parasite clearance, whereas in *L. braziliensis* infection it promotes the expression of SOD, leading to parasite persistence. TLR4 stimulation post-infection promotes TNF-α and NO production, increasing parasite killing, whereas stimulation prior to infection increases IL-10 production, favoring parasite persistence. TLR3 stimulation increases host resistance to *L. donovani* infection, by promoting TNF-α and NO production, whereas it promotes *L. guyanensis* virulence, by provoking a hyper-inflammatory response and the dissemination of parasites. TLR9 is the only TLR member which was solely associated with host protection, as it promotes IL-12 and IFN-γ production by dendritic cells, thus favoring the development of a protective T_H1 immune response.

promoting parasite clearance despite the source of the ligand being unknown (37). In contrast, TLR3 stimulation was shown to induce a hyper-inflammatory response associated with increased symptoms and parasite burden during infection with *L. guyanensis* (38). This exacerbated phenotype is associated with the presence of an endosymbiotic virus offering its dsRNA genome to induce TLR3-dependent hyper-inflammation, suggesting that TLR3 activation by viral dsRNA genomes present in co-infection could exacerbate *Leishmania*-dependent pathology.

TLR4 also shows a dual role, depending on the time of stimulation. Protective TNF- α and NO⁻ production is observed when TLR4 is stimulated at the same time or after *Leishmania* infection (39, 40), whereas stimulation prior to infection was associated with increased IL-10 production and parasite persistence (41).

Thus far, only TLR9 has been systematically associated with resistance to various *Leishmania* species infections, possibly by stimulating IL-12 production by plasmacytoid dendritic cells, which in turn induces IFN- γ production by NK cells (42–46). IFN- γ then acts on infected macrophages and induces the production of parasitotoxic nitrites.

In conclusion, taken together, information gathered recently on the role of TLRs suggests that they can play opposite roles in *Leishmania* infections, depending on the infecting species and possibly on additional ligands of bacterial or viral origins acting before, during or after the infection.

While currently there is no evidence of an involvement of other TLRs, RLRs and CDSs in *Leishmania* infection, the studies on NLRs and CLR are still rare and present controversial results. In fact, NLRP3 activation and IL-1 β production were shown to protect the host against *L. donovani*, *L. amazonensis* and *L. braziliensis*, via induction of parasitotoxic oxidative stress (47). Interestingly, *L. donovani* promotes macrophage expression of A20 (48) which inhibits NLRP3 inflammasome activation (49) and the production of IL-1 β (see Fig. 1). On the other hand, NLRP3 activation is detrimental in mouse infection with a unique *L. major* strain isolated from a chronic CL patient, as it promoted the development of chronic infection by increasing neutrophil recruitment at the site of infection and inducing the inflammasome and the production of IL-1 β (50). One should be, however, careful in the interpretation of several studies implying a role for the inflammasome since, in several instances, inflammasome activation was obtained by lipopolysaccharides (LPS) stimulation concomitant with *Leishmania* infection.

Regarding CLRs, Dectin-1 and mannose receptor stimulation were shown to promote macrophage microbicidal oxidative stress production, favoring the clearance of *L. infantum* infection (51). In contrast, SIGNR3 was shown to promote *L. infantum* infection, by inhibiting IL-1 β , whereas Mincle was shown to promote the infection of *L. major*, by dampening dendritic cell priming (51, 52). Defining the molecular pathways downstream of the CLRs will be challenging but could help to further define their relevance.

Adaptive immune responses to *Leishmania*

The early phase after *Leishmania* infection is crucial to determine the outcome of the disease. In fact, once the parasite is phagocytized, innate immune cells react by producing

cytokines that shape the development of adaptive immunity, thus generating either a protective or detrimental type of response. Interestingly, despite being indispensable for the complete clearance of the infection and to establish a long-lasting memory response conferring protection against re-infection, adaptive immune cells have also been shown to participate in the pathogenesis of leishmaniasis, causing tissue destruction and inducing disease relapse.

CD4 T cells

Interestingly, the murine model of *L. major* infection was originally used to define T_h1/T_h2 T-cell polarization. In fact, T_h1 response, characterized by the production of IFN- γ and TNF- α which induce the activation of the macrophage oxidative stress machinery, induces host resistance, whereas T_h2 response, characterized by IL-4 and IL-13 production, leads to a susceptible phenotype, unable to control *Leishmania* infection. However, in the last years, it became clear that this correlation is less strict in humans, and a more complex scenario has emerged also in the mouse model. In fact, DCL patients often show high levels of TNF- α in their lesions, suggesting that a too robust T_h1 response and the consequent hyper-inflammatory state can favor the development of metastatic infection (53).

Moreover, other subsets of T_h cells have been implicated in leishmaniasis outcome, such as the IL-17-producing T_h17 T cells, which have a controversial, species-specific role. Often involved in autoimmune disorders, T_h17 T cells have been associated with healing in *L. infantum* and *L. braziliensis* infection (54, 55), whereas they worsen the outcome of *L. major*- and *L. guyanensis*-induced leishmaniasis (56, 57). Interestingly, in this latter case, IL-17 is associated with parasite dissemination and the metastatic infectious lesion formation in the absence of IFN- γ , both in humans and mice and is correlated with the presence of a viral endosymbiont. Finally, IL-10-producing T_{reg} cells have been associated with parasite latency, treatment resistance and disease relapse in *Leishmania* infection, both in mice and in humans (58–61).

CD8 T cells

The role of CD8 T cells during *Leishmania* infection seems to depend on the form of leishmaniasis, and ranges from protective to disease exacerbating (62). In the murine model of CL induced by *L. major*, CD8 T cells are essential to control low-dose infection, whereas they are non-essential after high-dose inoculation (63). In humans, higher numbers of CTLs have been observed during the acute and healing phase of *L. major* infection (64–66), allegedly promoting parasite clearance through the higher production of IFN- γ . CD8 T cells have a protective role also against VL caused by *L. donovani* and *L. infantum*, both in the murine model and in humans, as CTLs were shown to be essential to the proper formation of granulomas. Further, CD8 T cells were associated with resistance to *L. infantum* re-infection, implying a potential use for vaccine development.

Contrarily, higher numbers of IL-10-producing CD8 T cells have been observed in DCL and post-kala-azar dermal leishmaniasis (PKDL) patients (67–69), suggesting their involvement in the dissemination of the disease. Similarly, high numbers of granzyme-positive and IL-10-refractory CTLs

were associated with tissue destruction and disease progression in MCL patients (70, 71). Moreover, in the murine model, CTLs have been recently associated with increased severity and infectious metastasis development during *L. braziliensis* infection (72), and NKG2D⁺ CD8 T cells have been shown to increase the severity of *L. major* infection following LCMV co-infection (25, 73).

B cells

B cells are thought to play a negligible role in the pathology of leishmaniasis as *Leishmania* are obligate intracellular parasites. However, a few studies have reported a disease exacerbatory role of B cells during *L. donovani* and *L. amazonensis* infection (74, 75). In fact, IgM transmembrane domain deficient (μ MT) mice—which lack mature B cells—clear *L. donovani* infection faster than wild-type (WT) mice do, because of enhanced granuloma formation and an increased T_h1 immune response (74, 76). Nevertheless, μ MT mice show more severe liver necrosis compared with WT mice, because of increased recruitment of neutrophils. Similarly, B-cell-deficient mice develop delayed and less severe lesions following *L. amazonensis* infection (75). In contrast, thus far, B cells play no role during *L. guyanensis* infection (77).

Leishmania and co-infection

Differently from the highly sterilized animal models used in research, the human body is constantly in contact with a multitude of microorganisms, which shape the immune response. In fact, commensal bacteria modulate *L. major* infection, as germ-free mice developed a higher parasite burden but reduced lesions, through an IL-17-dependent mechanism (78), whereas the disruption of the microbiota skin flora exacerbates inflammation and lesion development during *L. major* infection (79).

The most intriguing pathogen-*Leishmania* co-infection is the presence of *Leishmania* RNA virus (LRV) within certain species of *Leishmania* parasites, which was recently described to modulate the severity of CL induced by *L. guyanensis* and *L. braziliensis* (5, 38). In fact, LRV has been associated with the development of DCL, as viral dsRNA recognition by TLR3 improves infected macrophage survival by phosphorylation of the pro-survival kinase Akt (77) and is the driving force in the dissemination of the infection (57). The presence of a second species of LRV within *L. major* and more recently in *L. aethiopica*, another species known to cause DCL, suggests an even greater role of LRV in the determination of *Leishmania* infection (80). Moreover, the pathology-increasing effect of LRV completely relies on production of type I interferons, implying that other viral co-infections could have the same effect (81).

Of great interest are also the reports of sand fly bearing both *Leishmania* parasites and different *Phleboviruses*, which could thus be transmitted together in a simultaneous co-infection (82–84). Interestingly, co-infection with the *Toscana* phlebovirus increases both parasite burden and lesion size in a murine model of *L. guyanensis* infection (81). Interestingly, the presence of Narna-like virus 1—a virus infecting *Leptomonas seymouri* (protozoan parasites also transmitted

by sand flies)—has recently been reported in *L. donovani* isolates from India, underscoring the existence of triple co-infection and suggesting its involvement in the development of PKDL (85).

Currently, HIV is still the most studied co-infecting pathogen as it worsens both VL and CL, inducing the development of atypical symptoms and increasing treatment resistance and disease relapses (86–88). In fact, HIV infection decreases CD4 T-cell numbers, favors a T_h2 immune response and impairs macrophages' functions, thus promoting parasite persistence and disease development (89–91). Interestingly, mice co-infected with LCMV, both before or after *L. major* infection, develop bigger leishmanial lesions, through a mechanism involving the increased inflammation generated by bystander CD8 T-cell activation (25, 73). Moreover, LCMV co-infection increases lesion size and parasite burden, and promotes parasite dissemination and disease relapse of *L. guyanensis*-induced leishmaniasis (81). In this second case, the mechanism completely relies on production of type I interferons and is associated with the inhibition of macrophage responsiveness to IFN- γ .

Implications for treatment and vaccine development

The current leishmaniasis treatment strategy focuses solely on parasitotoxic drugs, neglecting the importance of the host immune response. As leishmaniasis is an immune-mediated disease, immunotherapy could be crucial for the development of new treatments or to improve the efficacy of the current ones. In particular, because of the central role of TLRs in the initiation of the anti-leishmanial immune response, the use of TLR agonists or antagonists could help to improve the efficacy of current drugs. For instance, combination treatment with the synthetic bacterial lipopeptide Pam3Cys (a TLR2 ligand) was shown to improve the efficacy of miltefosine in a murine model of VL, by promoting a T_h1 immune response and ROS and RNS production (92). Further, TLR9 stimulation with D-type CpG reduced lesion severity and parasite burden in *Leishmania*-infected rhesus macaques (93).

Interestingly, D-type CpG treatment protected also simian immunodeficiency virus (SIV)-infected rhesus macaques from *Leishmania* infection, suggesting its possible application in *Leishmania*–HIV co-infected human patients. Moreover, the use of a topical formulation of Imiquimod (a TLR7 ligand that induces a potent pro-inflammatory response) in combination with antimony was shown to reduce the healing time and improve wound repair in Peruvian CL patients (94), setting the stage for the simultaneous use of TLRs agonists and anti-*Leishmania* drugs as CL therapeutics. Paradoxically, a completely opposite strategy—the use of pentoxifylline (a TNF- α inhibitor used to treat autoimmune disorders) in combination with antimony—improved healing and reduced the relapse rate in MCL patients (95). However, the use of TNF- α blockers was also associated with disease re-activation in VL patients, and thus should be carefully monitored as a therapeutic approach (96).

The development of a leishmanial vaccine remains a major challenge. Despite the efforts and different strategies tested, ranging from leishmanization to recombinant or DNA vaccines, there is still no effective vaccine, with the possible

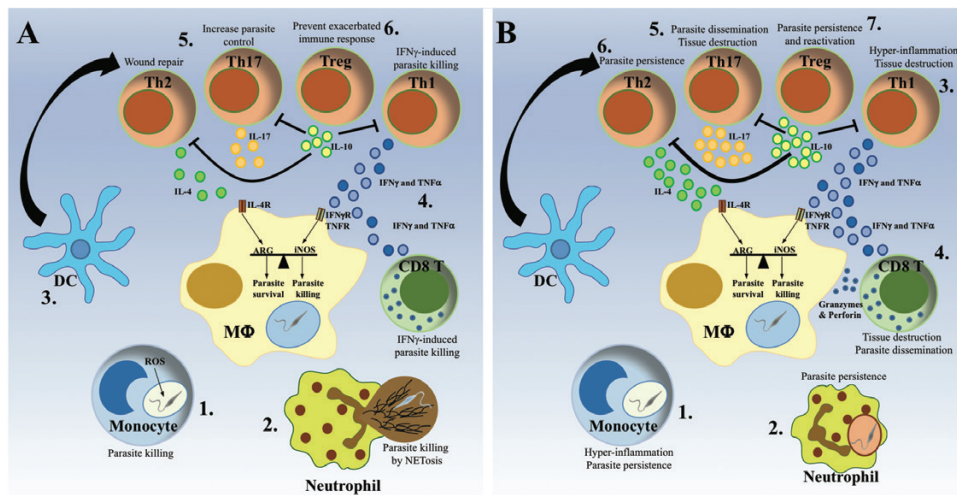


Fig. 3. Protective and pathological anti-*Leishmania* immune responses. The same cell types have been associated with either host protection or disease progression depending on parasite species and host immune-competence. (A) The protective immune response. Monocytes (1) and neutrophils (2) are rapidly recruited to the infection site, where they clear *Leishmania* parasites via ROS production or NET formation, respectively. Subsequently, dendritic cells (DC) migrate to the lymph node (3), where they activate and polarize T cells into different T_h populations. The development of a predominant T_h1 immune response, characterized by production of IFN- γ and TNF- α by CD4 $^+$ and CD8 $^+$ T cells (4), activates infected macrophages to kill intracellular parasites via production of NO, while a moderate T_h2 and T_h17 response has been shown to promote the control of the parasite and the resolution of the infection (5). The subsequent development of IL-10-producing T_{reg} cells ensures the dampening of the immune response, avoiding the development of chronic inflammation (6). (B) The pathological immune response. The excessive recruitment of cytokine-producing monocytes causes hyper-inflammation, which favors parasite persistence (1). Inefficient parasite killing by neutrophils hides parasites from macrophages, interfering with the development of the immune response and favoring parasite persistence (2). Exaggerated IFN- γ and TNF- α production by CD4 $^+$ T_h1 cells causes hyper-inflammation resulting in tissue destruction (3), whereas the addition of cytotoxic granzymes and perforin by CD8 $^+$ T cells promotes parasite dissemination (4). Similarly, a disproportionate T_h17 response induces tissue destruction and parasite dissemination, resulting in the formation of metastatic lesions (5). In contrast, an excessive T_h2 response promotes parasite persistence and re-activation following the resolution of the disease (7). While high numbers of IL-10-producing T_{reg} cells promote parasite persistence and re-activation following the resolution of the disease (7).

exception of Leishmune® used for dogs. Also in this case, the central role of TLR stimulation in the determination of the immune response makes TLRs an important candidate not only for immunotherapy, but also for vaccine development. For instance, TLR4 and TLR9 ligands have been used as adjuvants in vaccine formulations, showing promising results in the murine model. In fact, the TLR4 agonist ONO-4007 was shown to boost the efficacy of *L. amazonensis* antigens, inducing protection in BALB/c mice, by promoting the production of IL-12 and IFN- γ (97).

Furthermore, the TLR9 agonist CpG was used in many vaccine formulations and was shown to improve vaccine efficacy by promoting IL-12 production, whereas the combination between TLR4 and TLR9 agonists increased their adjuvant potency, thus showing a synergizing effect between the two TLRs (98). Interestingly, mice immunized with a recombinant LRV capsid in combination with CpG are protected against the increased pathology of LRV-bearing *L. guyanensis* infection (99). Despite not conferring complete protection, LRV-based vaccination could be the first pre-emptive agent against disseminated leishmaniasis.

Conclusions

Here, we summarized some of the recent knowledge on the host immune response to *Leishmania* and the parasite immune evasion strategies. Several aspects of the anti-leishmanial immune response have been disclosed in the

last 30 years. We now understand many more facets of the immune response to *Leishmania* than the oversimplified, bi-dimensional T_h1/T_h2 dogma. Despite the increasing complexity of anti-leishmanial immunity, a mountain of data underline the importance of the balance between the different types of immune responses rather than the development of a unique and specific response (see Fig. 3).

Moreover, the involvement of bacterial and viral co-infection (often overlooked in the current murine models of infection) in the determination of the outcome of leishmaniasis highlights the need for new research models, better replicating the human immune system and taking into account the human microbiome which, on the basis of mouse models, is likely to be relevant to control or exacerbate *Leishmania* infection in the case of dysbiosis. Another aspect not to underestimate is the cohabitation between leishmaniasis and autoimmune disorders. The two diseases require completely opposite treatment strategies; thus the adjustment of an appropriate therapy could be particularly difficult, resulting in a vicious cycle of relapse of one or the other disease.

Despite the increased knowledge of the immunology of *Leishmania* infection, the treatment options for leishmaniasis are still limited and rely on old drugs often adopted and repurposed from other diseases, while an effective human vaccine has still to be developed. As the outcome of *Leishmania* infection is influenced by multiple factors, which could vary extremely between individuals, the use of

immunotherapy to improve leishmanial treatment should also be adapted as a sort of precision medicine. Similarly, the parasite species specificity of the immune response required to clear *Leishmania* infection does not facilitate the generation of a universal vaccine. Thus, despite the recent discovery of an immunodominant antigen conserved in many *Leishmania* species (100), the combination with different adjuvants to develop many species-specific formulations could be required.

Finally, as the immune responses developed during the different types of leishmaniasis are drastically divergent, the generation of different outcome-specific anti-leishmanial treatments or vaccines might prove more effective than a unique solution for leishmaniasis.

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