The Battle between *Leishmania* and the Host Immune System at a Glance

(June 2015)

Santos-Mateus D1**, BScMicr, MSc, Passero F2**, BioSc, PhD, Rodrigues A1, BioSc, MSc, Valério-Bolas A1, BioSc, MSc, Silva-Pedrosa R1, BHSc, Pereira M1, DVM, MSc, Laurenti MD2, MSc, PhD, Santos-Gomes G1* BioSc, PhD

Abstract — *Leishmaniasis* is a neglected parasitic disease whose diverse clinical manifestations are dependent on the interrelations between intrinsic and extrinsic factors. The infecting species of *Leishmania*, the parasite’s ability to evade mammal immune response and the host genetic background seems to pre-determine the degree of resistance and sensitivity to infection, regulating the disease outcome. The introduction of metacyclic promastigotes into the dermis of the mammal host by the sand fly originates an unspecific immune response that can difficult the parasite replication and dispersion or, by the contrary favor the selection of fit parasites, assuring the parasite survival and the disease onset. This review aims to provide a comprehensive outline of the immune response displayed against *Leishmania* parasites by the host and the strategies exhibited by the parasite to subvert the host immune mechanisms. Emphasis is given to the early contact of the parasite with the immune system of the host, as this is a crucial time-point for parasite control that might be explored for the development of new and more efficient control measures. The role of neutrophils, macrophages and dendritic cells when facing different species of *Leishmania* are examined as well as the link of immediate innate immune response with the late acquired immunity.

Keywords — *Leishmania* spp., Clinical manifestations, Host immune response, Parasite survival strategies, Phagocytic cells, Antigen presenting cells, Lymphocytes

This work was supported the Portuguese Foundation of Science and Technology through GHTM–UID/Multi/04413/2013 and by FAPESP (SP, Brazil) through project 2013/16297-2.

1Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal.

2Departamento de Patologia da Faculdade de Medicina da USP, São Paulo/SP, Brazil

** These authors contributed equally to this paper

*Correspondence to G. Santos-Gomes (e-mail: santosgomes@ihmt.unl.pt).

DOI: 10.18281/iti.2016.1.3

I. INTRODUCTION

*Leishmaniasis* comprises a variety of clinical syndromes caused by different *Leishmania* species. Although considered a rare disease in Europe (ORPHA Number 507) where has been mainly associated with travelers and cases of immunosuppression, worldwide, there are 350 million people at risk of getting infected and approximately 2 million of new cases each year, mainly affecting tropical and sub-tropical regions. In those regions leishmaniasis is considered one of the most neglected diseases strongly associated with poverty.

There are no available vaccines and the best way to reduce the incidence of this disease and increase the wellbeing of human population is getting a successful treatment. However, antileishmanial drugs are costly, far from satisfactory and, in some areas, their application is threatened by the emergence of resistant parasites, stressing the importance of understanding the host immune mechanisms directed to *Leishmania*.

Inside the host, immediately after inoculation by sand flies, *Leishmania* promastigotes exposed to extracellular environment have to resist to the defensive immune mechanisms, assure internalization by macrophages, undergo morphological differentiation in the amastigote form and guarantee their own replication inside the nasty phagolysosome compartment (acidic and rich in proteolytic enzymes). Dissemination and persistence of parasites in immunocompetent host and even in cases of clinical healing are dependent of parasite continuous strategies able to circumvent innate and adaptive immune response.

Geographical distribution and clinical manifestations vary with the *Leishmania* species and the immune competence of the host. In the New World, cutaneous leishmaniasis (CL) is caused by species of subgenus Viannia (V.) (e.g. *L. braziliensis*, *L. guyanensis*, *L. shawi*) and *Leishmania* (L.) (e.g. *L. mexicana*, *L. amazonensis*, *L. mexicana*) and the rare but disfiguring mucocutaneous leishmaniasis is mainly caused by *L. braziliensis*. *L. (L.) major*, *L. (L.) tropica* and *L. (L.) aethiopica* are the etiological agents of CL in the Old World and *L. (L.) donovani* is the causative agent of anthroponotic visceral leishmaniasis (AVL). Post-kala-azar dermal leishmaniasis is another clinical syndrome that may upsurge following AVL treatment. Chronic and anergic diffuse forms of CL are caused by *L. aethiopica* that occur mainly in Ethiopia and Kenya and by *L. mexicana* and *L. amazonensis* in Central.
and South America. Zoonotic visceral leishmaniasis (ZVL) caused by *L. (L.) infantum* (syn. *L. (L.) chagasi*) is typically a pediatric disease that can be found in Central and South America, South Europe, North Africa, Middle East and China.

While the immune response to *Leishmania* infection has been extensively characterized in rodent models, specific descriptions of the human immune response are scarcely reported. Therefore, this work aims to critically review the most relevant aspects of *Leishmania* interaction with the human immune system, reflecting on the translation of important evidences obtained in animal models for the development of more efficient prophylactic and therapeutic strategies. In order to understand how the immune system exerts their action, a brief overview describing the general infection process is provided together with the current understanding of the balance between host immune mechanisms preventing severe immunopathology and the pathways used by the parasite to subvert immune functional activity, favoring the existence of chronic protective infections in the immunocompetent host.

II. INNATE IMMUNE RESPONSE

After be regurgitated by the sand fly into the dermis of mammals, promastigotes activate the complement cascade by any of the three activation pathways (classical, alternative or lectin pathway). However, *Leishmania* can inhibit and modulate these pathways in order to survive. Complement activation leads to formation of chemotactic elements, like C3a and C5a that attract macrophages to the inoculation site. C3a can be proteolytic cleaved by C3 convertases, producing C3b molecules that bind covalently to the *Leishmania* surface, aiming the assembly of the membrane attack complex (MAC), which is responsible for parasite lysis. In the attempt to avoid MAC assembly, this parasite possesses membrane antigens, such as the metalloproteinase of 63 kDa (gp63) that are able to inactivate C3b (iC3b). Promastigotes of logarithmic phase of growth and gp63-mutated parasites are highly susceptible to the complement-mediated lysis1. Therefore, the most infectious parasites can survive to first line of attack of host immune defense and be opsonized by C3bi that facilitates the phagocytosis process. Multiple host cell receptors, such as the complement receptors (CR) type 1 and type 3, the mannose-fucose receptor, fibronectin receptor, and fragment crystallizable (Fc) region receptor are involved in parasite phagocytosis1. Macrophages which internalize iC3b-opsonized parasites did not trigger respiratory burst, evidence low capacity to promote their destruction (Fig. 1), assuring parasite viability and disturbing the activation of acquired immunity and, consequently affect the infection outcome.

Parasites that resist to these non-specific immune mechanisms will be processed by antigen-presenting cells (APC), such as resident dendritic cells (DC). After contact with invading pathogens, tissue resident DC undergo maturation characterized by increase of MHC and co-stimulatory molecules and decrease of phagocytosis. In order to enhance the migration process for lymphoid organs, changes in chemokine receptors and chemokine production also occur.

![Fig. 1. Interaction between the promastigote parasite, the complement system and macrophages (MØ). C3b molecule is inactivated by gp63 to iC3b that binds to gp63 or LPG antigens aiding the process of phagocytosis. CR1-complement receptor 1; CR3-complement receptor 3; FcR – fragment crystallizable receptor; Fn – fibronectin; FnR – fibronectin receptor; LPG – Leishmania promastigote LPG. MPR – mannose-fucose receptor.](http://www.researchpub.org/journal/iti/iti.html)
MHC and, sequester of *Leishmania* antigens inside the endocytic compartment.

II. VISCERAL LEISHMANIASIS

More than 90% of all cases of visceral leishmaniasis occur in only six countries: India, Bangladesh, Sudan, Southern Sudan, Brazil and Ethiopia. It is the most severe and fatal form of the disease, comprising a broad range of clinical manifestations. Parasite invades and replicates in the organs of mononuclear phagocyte systems such as the spleen, liver and lymph nodes and the symptoms are characterized by prolonged and irregular fever, splenomegaly, lymphadenopathy, hepatomegaly, pancytopenia, progressive anemia, weight loss and hyper gamma-globulinemia with hypoalbuminemia. In many cases, the infection does not take an acute or chronic course, remaining asymptomatic or subclinical and can even lead to a self-healing scenario.

Human disease by *L. infantum* occurs in susceptible individuals. In endemic areas, the majority of infected adults is resistant, thus active disease reflects an imbalance between host and parasite. Studies performed in adults living in the northeastern region of Brazil, employing delayed hypersensitivity (DHT) or serological tests as immune markers of infection, identified two forms of symptomatic infection, classical and oligosymptomatic ZVL, and also the immune resistant asymptomatic form. Studies using DHT and indirect immunofluorescence assay (IFA) associated with the clinical status identified a wider immune spectrum of adults with this infection. This spectrum includes the asymptomatic infection (AI) characterized by mild to intense DTH and negative IFA, the symptomatic infection (SI) and the oligosymptomatic infection (OSI) presenting negative DTH and moderate to intense IFA, the subclinical resistant infection (SRI) evidencing mild to intense DTH and moderate to mild IFA and, the initial indeterminate infection (III) showing negative DTH and moderate to mild IFA. The AI profile, supported by a strong DTH response is related to a resistant genetic background while the SI is linked to the genetic character of susceptibility. OSI and SRI profiles represent a borderline genetic background between susceptibility and resistance. The OSI, denotes an initial manifestation of susceptibility (fever, asthenia, pallor, and moderate splenomegaly) followed by the spontaneous evolution for clinical cure in two to three months. Nonetheless, the III profile of major importance for epidemiological surveillance since III-patients can develop immune resistance (Al) or, by the contrary immune susceptibility (SI). Recently, it was described that 3 to 5 % of these individuals showing detectable levels of anti-*Leishmania* IgM will develop symptomatic infection. Other atypical clinical features caused by *L. infantum*, such as non-ulcerated skin lesions in adolescents and young adults are also described in Central America.

A. Parasite immune evasion

*L. infantum* prevents complement-mediated lysis by reducing the levels of C3b deposition on the cell membrane and by inactivating C3b (iC3b). *L. donovani* does not present reduced levels of C3b deposition, but instead possesses high proteolytic activity able to inactivate C3b. Gp63 seems to be the molecule responsible for the adhesion of C3b to the parasite surface as well as the responsible for its degradation into iC3b. The presence of iC3b on the parasite surface does not lead to MAC activation, but the opsonic activity of the molecule remains the same and facilitates parasite internalization by macrophages. This way of getting an intracellular position is beneficial for parasites since it does not induce the superoxide production.

It was shown that one of the strategies used by *L. donovani* to assure its own survival is to delay the fusion of phagosome and lysosome. This delay appears to be directly related to the quantity of LPG present in the parasite surface.

Few hours post-infection, neutrophils are the first cells to be recruited and reach the site of inoculation. In the same way as macrophages, neutrophils are able to phagocytose and eliminate invading parasites. However, it was shown that parasites can survive, but not multiply, inside neutrophils. This step can be seen as an indirect way of silent delivery of the parasites to macrophages. Chemokines of the CXC family, like interleukin (IL)-8, seem to be mainly responsible for the recruitment of neutrophils. This chemokines are secreted by epithelial cells, keratinocytes, fibroblasts and endothelial cells, as well as by neutrophils. IL-17 and tumor necrosis factor (TNF) are also involved in recruitment of neutrophils. *Leishmania* is able to release the granulocyte chemotactic factor (GCF) which is chemoattractant to polymorphonuclear leukocytes (PMN) and induces PMN to produce IL-8. Interaction of GCF with the chemokine receptor lipoxin A4 was proven to increase parasite phagocytosis and promote their survival inside neutrophils through the inhibition of oxidative mechanisms. Phosphatidylserine is known to be a marker of apoptotic cells and a promoter of transforming growth factor (TGF)-β. Infected neutrophils are recognized as apoptotic cells by macrophages, not leading to the activation of antimicrobial mechanisms.

It was also shown that *L. donovani* has the ability to inhibit the fusion of lysosome inside neutrophils, staying in compartments that display endoplasmatic reticulum features, resting protected from degradation. This inhibition appears to be mediated by the promastigote surface LPG. Programmed cell death is also delayed in neutrophils presenting parasites in those compartments, pointing to another survival strategy used by the parasite. It was demonstrated that the most fit *L. infantum* parasites can downregulate the same receptor gene expression of neutrophils and the production of chemokines and cytokines responsible for migration and activation of other immune cells.

Neutrophils are also able to eliminate invading parasites by secreting chomatins, granules and cytoplasmic proteins, generating web like structures called neutrophil extracellular traps (NET). *L. donovani* and *L. infantum* trigger NET release, however *L. donovani* is able to resist the microbicidal activity due to the presence of LPG. To a lesser extent than *L. donovani*, *L. infantum* is able to survive NET activity. This seems to be due to a membrane-anchored enzyme responsible for the cleaving of DNA and RNA.
The interaction between neutrophils and macrophages is also an important factor that determines the immune response of the host to parasite infection. In susceptible mice, the contact between apoptotic neutrophils and macrophages induces the release of large quantities of TGF-β and prostaglandin E2 (PGE2), leading to Leishmania survival and increase of parasite burden. On the other hand, in resistant mouse strains the interaction between neutrophils and macrophages increases TNF production, leading to parasite destruction. The production and release of neutrophil elastase (NE) as a consequence of activation of macrophage toll-like receptor (TLR) 4 downstream pathway is also crucial for parasite control at the early phase of infection. Contrary to L. major infection, where NE release was shown to be dependent on the susceptibility of the host, in L. infantum infection this release is always triggered by the presence of the parasite.

The activation of the immune system to fight Leishmania infection is also dependent on cytokines and chemokines. However, the parasite can regulate their production. TGF-β and IL-10 are two cytokines with the ability to suppress natural killer cells and the macrophage effector functions. It was reported that both L. infantum and L. donovani infections lead to IL-10 increase by CD4+ and regulatory T cells. L. infantum also has the ability to induce TGF-β production by macrophages. Studies also showed the ability of L. infantum and L. donovani to downregulate the production of IL-12 which is important for the differentiation of interferon (IFN)-γ-producing CD4+ T cells, and of TNF-α that participates in the regulation of NO-mediated leishmanicidal activity. In L. donovani it was also shown that IL-12 is suppressed by modulation of expression and signaling of TLR2.

III. CUTANEOUS LEISHMANIASIS

It has been estimated that at least 16 species of Leishmania belonging to both Leishmania and Viannia subgenus are pathogenic to humans, causing CL. After infection, some individuals become resistant to infection (asymptomatic) although most part develops active lesions (symptomatic). Depending on the parasite species and host immune response, CL can be classified into localized CL (LCL), borderline disseminated CL (BDCL), mucocutaneous leishmaniasis (ML) and anergic diffuse leishmaniasis (ADL) (Fig. 2). However, the host immune competence determines the level of susceptibility or resistance to infection. Patients with LCL presenting moderated DTH with a preponderant response associated with CD4+ Th1 cell subsets, exhibit a resistant profile. This clinical form presents a good response to the classical treatment. Nevertheless, some clinical forms are not totally controlled by immune mechanisms and can present worse prognostics, as is the case of ML and ADL. The ML clinical form is mainly caused by L. braziliensis, but can also be generated by L. panamensis or L. peruviana. Patients frequently present a strong DTH response that is related to a hyper reactivity of IFN-γ and TNF-α – producing CD4+ T lymphocytes. Both these cytokines seem to exert a crucial role in the genesis of mucosal lesions, in addition to other host factors that also can be involved in lesion generation. ADL patients present negative DTH and leishmanial antigens hyporeactive Th1 cells associated with the expansion of CD4+ Th2 cells and skin accumulation of anti-inflammatory cytokines IL-4 and IL-10. Remarkable is the expansion of Th2 immune response associated with the progression and chronicity of cutaneous lesion that frequently is refractory to classical treatment, causing severe mutilation. Between the extreme clinical forms (ML and ADL) and the central form (LCL) of the leishmaniasis spectrum still is possible to characterize an intermediate form of the disease, the BDCL that can be caused by different Leishmania species. Patients with this clinical form present a faint or even negative DTH response and reduced activity of T lymphocytes. In cases caused by L. amazonensis, the Th1 response is inhibited and lesion spreading can take more than one year. However, in BDCL caused by L. braziliensis a strong CD4+ Th1 proliferation with high production of IFN-γ and TNF-α can occur, leading to mucosal involvement.

A. Interaction between innate and acquired immune response

In vitro studies made with New World Leishmania species showed that neutrophil response can be totally different from that triggered by the Old World species, and even contradictory. It was verified that L. major-infected neutrophils exhibit a delay in apoptosis when compared with non-infected cells. Since then it was postulated that L. major parasites can manipulate neutrophil functional activity. L. braziliensis induces the activation of neutrophils isolated from BALB/c mice but does not delay the apoptotic process. L. amazonensis or L. braziliensis-infected macrophages also induce neutrophil activation, leading the production of high levels of TNF-α and superoxide anion. In addition, NET are able to destroy extracellular L. amazonensis parasites. On the other side, the modulatory effect of neutrophils on L. amazonensis-infected macrophages was also investigated, and it was clearly associated with the neutrophil activation status. However, necrotic neutrophils activate macrophages promoting the infection reduction. In vivo studies also demonstrated the importance of neutrophils in controlling parasite spreading at the early phase of infection. Neutrophil-depleted BALB/c mice infected by L. amazonensis developed higher dermal lesions and parasite burden when compared with non-depleted mice.
associated with the increasing of IL-10, IL-17 and arginase⁴⁰, proteins related to lesion development. Interestingly, C57BL-6 mice, an animal model of higher resistance to L. major parasites, respond in a different fashion. In this particular case, neutrophil depletion has no detectable effect in dermal lesions and in L. amazonensis parasite load when compared with non-depleted infected mice.

In American CL, the role of DC has been analyzed and responses associated with resistance and susceptibility have been found not necessarily dependent on the infecting species of Leishmania. Murine DC exposed to L. braziliensis showed that uninfected cells (bystander) were able to upregulate markers of activation as well as IL-12 and TNF-α, having a possible positive impact on the infection outcome. On the other side L. braziliensis-infected DC failed to upregulate activation markers, affecting the interaction with T cells.⁴¹ According to Vargas-Inchaustegui et al. it is possible that bystander DC were able to activate and expand IFN-γ- and IL-17-producing CD4⁺ T lymphocyte subsets, leading to parasite elimination, while infected DC induce local pathology, since they present low capacity to migrate. In contrast, L. mexicana parasites cause inactivation of mitogen activated protein kinases in DC and reduce translocation of transcription factors associated with low expression of MHC and of co-stimulatory molecules, even after stimulation with lipopolysaccharide. In a model of chronic peritonitis induced by thioglycollate, the migratory property of DC was analyzed. In this case, L. amazonensis injected into the inflamed peritoneum of C57BL/6 mice was able to impair the migratory potential of DC when this exudate was transferred to healthy C57BL/6 mice, diminishing the induction of cellular immunity. The differentiation of human DC was also abrogated by L. amazonensis, reinforcing that the downregulation of surface molecules and signaling pathways or the delay in DC maturation is detrimental for infection in natural and experimental hosts. This can be another mechanism by which L. amazonensis can subvert the host immunity at its own favor.

In human biopsies, the accumulation of DC at the site of infection determines the gravity of the disease. In ADL cases, high densities of DC were found in the patient derrmis that in turn was not able to mount cellular immune responses. On the other hand, in biopsies of patients with LCL caused by L. amazonensis or L. braziliensis low densities of DC were found, and remarkably these patients had a good cell immune response⁴¹. It is conceivable that in ADL cases improperly stimulated DC stay anchored at the dermal site of infection, consequently mediating pathology. In contrast, DC in contact with L. braziliensis or LCL strains of L. amazonensis able to mature and migrate can induce cell immune response in lymphoid organs.

Recently, our group investigated the modulator potential of promastigote and amastigote forms of L. shawi in murine DC. Also in this case, not all DC become infected. In fact, when compared to macrophages, DC presented low infection index. However, the cytokine pattern generated by these cells is totally different after exposition to amastigotes or promastigotes. While DC exposed to promastigotes produce mainly IL-12, amastigote induce the release of TNF-α and of IL-10 (Fig. 3), suggesting that the amastigote form or its antigens are highly pathogenic, and probably this response will impair the interaction with T lymphocytes. Actually, DC exposed to L. shawi promastigotes direct CD4⁺ T lymphocytes to produce high levels of IL-10 and IL-12, suggesting the simultaneous induction of anti- and pro-inflammatory stimuli. Moreover, the interaction with CD8⁺ T lymphocytes also promotes an accentuated release of IL-10 and TNF-α associated with an exacerbated production of IL-12 (Fig. 4A), pointing out the important role of CD8⁺ T cells in protecting against L. shawi infection. In contrast, the interaction of amastigote-exposed DC with CD4⁺ T cells elicited a strong generation of IL-10 and TNF-α, but completely abrogated IL-12 production, which was not inhibited during the interaction of amastigote-infected DC and CD8⁺ T cells. In addition, high amounts of TNF-α were detected along with high levels of IL-10 (Fig. 4B). L. shawi parasites clearly modulate lymphocyte response as already confirmed by in vivo studies.⁴⁴ The cross talk established between APC and T lymphocyte subsets seems to be critical in guiding the immune response of the host and, consequently the infection outcome. Furthermore, the species of parasite involved, the DC activation status, interactions between APC and T lymphocytes, and the host genetic background will determine the outcome of leishmanial infection.

**Fig. 3.** Interaction of murine dendritic cells with promastigotes and amastigotes of L. shawi. DC were differentiated from bone marrow of BALB/c mice and infected with 10 promastigote or amastigote per DC. Twenty four hours later, supernatants were collected and levels of cytokines were quantified by ELISA. * and ***(p<0.05) indicate significant statistical differences when compared IL-10 and TNF-α produced by DC infected with promastigotes vs amastigotes, respectively.

**IV. CONCLUDING REMARKS**

Leishmania parasites warrant their own survival by evading and subverting host immune response early during infection. Immediately, after be introduced in the skin, the parasite evades the deleterious activity of the complement system and, even uses some of the complement factor to enable its recognition and speed up the phagocytosis, promoting a fast internalization by phagocytic cells. Neutrophils, a phagocytic cell that have oxidative and enzymatic mechanisms able to immediately
Fig. 4. Interaction of L. shawi infected dendritic cells with CD4+ and CD8+ T lymphocytes. DC differentiated from bone marrow of healthy BALB/c mice and infected with 10 promastigote (A) or amastigote (B) were co-cultured with CD4+ and CD8+ T lymphocytes for 24h. Supernatants were collected and levels of cytokines quantified by ELISA. *; ** and *** (p < 0.05) indicate significant statistical differences when compared IL-10, IL-12 and TNF-α produced by DC vs CD4+ or CD8+ T cells, respectively.

Despite data on complex human-parasite interaction be scarce, precluding a comprehensive view of the immune response and the enormous ability of these parasites to subvert the natural function of the diverse components of the immune system, a detailed understanding of host immune response at the early phase of infection and the comprehension of the mechanisms involved in the diverse degrees of sensitivity vs resistance evidenced by different individuals will further the knowledge, which is crucial for the control of this parasitic disease. Therefore, efforts should be made to clarify the relations established between the diverse parasites and specific genetic individuals, identify crucial targets able to direct the design of more efficient and affordable prophylactic and therapeutic tools aiming to reduce leishmaniasis incidence, thus improving human health and promoting a better way of life, special of the most vulnerable and exposed populations.

V. ACKNOWLEDGMENTS
None of the authors have any conflict of interest to declare

References


