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

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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

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I. INTRODUCTION

Leishmaniasis comprises a variety of clinical syndromes caused by different Leishmania species. Although considered a rare disease in Europe (affecting less than 1 person per 2,000 inhabitants. 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) 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 being 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* parasites can inhibit and modulate these pathways in order to survive. Complement activation leads to formation of opsonic 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 lysis. Therefore, the most infectious parasites can survive to first line of attack of host immune defense and be opsonized by iC3b 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 phagocytosis. Macrophages that internalize iC3b-opsonized parasites did not trigger respiratory burst and presented 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 undergoes 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 the production of chemotactic molecules (chemokines) and of chemokine receptors also occur. After parasite uptake, IL-12 producing DC subset drives the differentiation of naïve T cells into Th1 cells, releasing IFN-γ and TNF-α, which can induce the activation of cytotoxic cells and macrophages.

Parasites also have to survive to polymorphonuclear cells, such as neutrophils. Human neutrophils are attracted by complement proteins, chemokines, cytokines and, by *Leishmania* antigens followed by the affluence of macrophages that arrives into the site of infection in time to phagocyte the apoptotic-infected neutrophils. In the vertebrate host, macrophages are the final host cells of *Leishmania* and the responsible for parasite elimination and, by interacting with B and T lymphocytes, are also a key factor for the establishment of a bridge between the innate and acquired immunity, directing lymphocyte activation. After phagocytosis, the phagosomes containing the parasites merge with lysosomes containing hydrolases, originating phagolysosomes. Inside the phagolysosomes *Leishmania* has to be able to survive to the reactive oxygen species (ROS), proteolytic activity of lysosomal enzymes, osmotic stress and acid pH.

The main mechanisms used by macrophages to eliminate intracellular *Leishmania* include the NADPH system, through ROS and the production of nitric oxide (NO) by inducible nitric oxide synthase. Virulent promastigotes and amastigotes do not seem to stimulate ROS production, mostly due to their preferential interaction with CR1 and CR3 that are recognized as poor ROS promoters. Amastigotes show elevated enzymatic activity able to eliminate ROS using glutathione peroxidase, superoxide dismutase and catalase. *Leishmania* lipophosphoglycan (LPG) is a potent inhibitor of protein-kinase C, a strong inducer of ROS and an inhibitor of NO. In addition gp63 inactivates hydrolytic enzymes, preventing the occurrence of parasite degradation inside the phagolysosome. Although macrophages express reduced levels of class II molecules of major histocompatibility complex (MHCII) they are recognized as APC, leading to the activation of T lymphocytes. *Leishmania* also has several mechanisms able to
regulate T cell activation, like the suppression of MHCII, internalization and degradation of MHC molecules, inhibition of processing of parasite antigens and complexation with the MHC and, sequester of Leishmania antigens inside the endocytic compartments.

Macrophage migration inhibitory factor (MIF) is a widely expressed cytokine produced by phagocytic cells that promotes the release of chemokines and of pro-inflammatory cytokines. Several studies, mostly performed by L. major in rodent models indicate that MIF mediates host resistance by regulating ROS production. On the other hand, MIF favors host immune pathogenesis through an exacerbate release of pro-inflammatory cytokines. Additionally, the identification of an ortholog of mammalian MIF in L. major by Kamir et al, which can direct host immune response by inhibiting macrophage apoptosis, facilitating the persistence of the parasite and allowing parasite immune evasion.

Different leishmanial antigens activate human natural killer (NK) cells, triggering TNF-α, IFN-γ and IL-12 production. However, in vitro, the activation of NK cells seems to depend on IL-12 and IL-18. In vivo, L. major, L. donovani and L. infantum induce a fast and transient activation of NK cells, although without decisive effect on the infection outcome. However, the pro-inflammatory cytokines produced by NK cells seem to trigger macrophage activation. Furthermore, a close relationship between NK cells and DC seems to exist. NK cells promote DC maturation and DC can prime NK cells.

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 progress to a self-healing scenario.

Human disease caused 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 (DTH) or serological tests as immune markers of infection, identified two forms of symptomatic infection, classical and oligosymptomatic ZVL, and also a resistant asymptomatic form. Studies using DTH and indirect immunofluorescence assay (IFA) associated with the clinical status identified a wider immune spectrum of adult 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.
(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. Programed 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 L. infantum parasites can downregulate the production of chemokines and cytokines responsible for migration and cell communication.

Neutrophils are also able to eliminate invading parasites by secreting chromatin, 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.

Toll-like receptors (TLR) regulate intracellular mechanisms of inflammation, cell survival and cell proliferation. Upon signalization by parasite ligands, the activation of MAP kinases and of NF-kB can occur, leading to release of inflammatory mediators or, by the contrary, can interfere negatively in downstream signalization. The production and release of neutrophil elastase (NE) as a consequence of activation of macrophage TLR4 downstream pathway are 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 host susceptibility, in L. infantum infection this release is always triggered by the parasite.

The activation of the immune system to fight Leishmania infection is dependent of 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.

Fig. 2. American cutaneous leishmaniasis: clinical and immunological spectrum according to the species of Leishmania (adapted from Silveira et al.)

### III. Cutaneous Leishmaniasis

It has been estimated that at least 16 species of Leishmania belonging to both Leishmania and Viannia subgenera 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 moderate 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 hypo reactive 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.\textsuperscript{51}

A. Interaction between innate and acquired immune response

In vitro studies made with New World \textit{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 \textit{L. major}-infected neutrophils exhibit a delay in apoptosis when compared with non-infected cells. Since then it was postulated that \textit{L. major} parasites can manipulate neutrophil functional activity\textsuperscript{52}. \textit{L. braziliensis} induces the activation of neutrophils isolated from BALB/c mice but does not delay the apoptotic process\textsuperscript{53}. \textit{L. amazonensis} or \textit{L. braziliensis}-infected macrophages also induce neutrophil activation, leading the production of high levels of TNF-α and superoxide anion\textsuperscript{54,55}. In addition, NET are able to destroy extracellular \textit{L. amazonensis} parasites\textsuperscript{56}. On the other side, the modulatory effect of neutrophils on \textit{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\textsuperscript{54}. \textit{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 \textit{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 arginase\textsuperscript{57}, proteins related to lesion development. Interestingly, C57BL/6 mice, an animal model of higher resistance to \textit{L. major} parasites, respond in a different fashion. In this particular case, neutrophil depletion has no detectable effect in dermal lesions and in \textit{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 \textit{Leishmania}. Murine DC exposed to \textit{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, \textit{L. braziliensis}-infected DC failed to upregulate activation markers, affecting the interaction with T cells\textsuperscript{58}. According to Vargas-Inchaustegui et al\textsuperscript{59} 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, \textit{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, \textit{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\textsuperscript{60}, diminishing the induction of cellular immunity. The differentiation of human DC was also abrogated by \textit{L. amazonensis}\textsuperscript{61}, 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 \textit{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 dermis that in turn was not able to mount cellular immune responses. On the other hand, in biopsies of patients with LCL caused by \textit{L. amazonensis} or \textit{L. braziliensis} low densities of DC were found, and remarkably these patients had a good cell immune response\textsuperscript{69}. 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 \textit{L. braziliensis} or LCL strains of \textit{L. amazonensis} were able to mature, migrate and induce cell immune response in lymphoid organs.

Recently, our group investigated the modulator potential of promastigote and amastigote forms of \textit{L. shawi} in murine DC. Also in this case, not all DC became 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 \textit{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 \textit{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). \textit{L. shawi} parasites clearly modulate lymphocyte response as already confirmed by \textit{in vivo} studies\textsuperscript{62}. 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. According to the mouse genotype, \textit{L. major} infection can induce the differentiation of CD4+ Th1 cell subset, favoring the
None of the authors have any conflict of interest to declare

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