Review Article

Immunobiology of leishmaniasis

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Leishmaniasis is a parasitic disease caused by various species of *Leishmania*, a unicellular kinetoplastid protozoan flagellate. It manifests mainly in 3 clinical forms; visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL), of which VL is the most severe form of the disease. VL is lethal if untreated and spontaneous cure is extremely rare. Cutaneous leishmaniasis usually has milder course and often results into a self-healing of ulcers. Resolution of leishmanial infection is dependent on the coordinated interactions between components of cell mediated immune response, specifically the activation of targeted T-cell populations for appropriate cytokine production and activation of macrophages. In murine model, the development of Th1 response is associated with control of infection, and Th2 response is associated with disease progression. However, Th1 and Th2 dichotomy in the human system is not as distinct as in mice and the murine model does not strictly apply to human leishmaniasis. This review focuses the dichotomy of immune response against various clinical forms of the disease. An in-depth knowledge of sequences involved in the immune response to the parasite would help in designing prophylactic and therapeutic strategies against leishmaniasis.

Keywords: Immunobiology, Immunomodulation, Leishmaniasis, Macrophage, T-cell response

Leishmaniasis is one of the most diverse and complex of all vector borne diseases. It is caused by an obligate intracellular protozoan parasite belonging to the genus *Leishmania*. The disease, in different clinical forms, affects approximately 12 million people worldwide, with an increasing incidence of 1.5-2 million new cases diagnosed every year and 350 million people at risk. It manifests mainly in 3 clinical forms; visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL), of which VL is the most severe form of the disease, lethal if untreated, is caused by species of *Leishmania donovani* complex. A total of about 21 *Leishmania* spp. have been identified to be pathogenic to human. It is characterized by diversity and complexity, both in terms of geographical distribution and in the variety of clinical syndromes. These range from simple, self-healing skin ulcers to severe, life-threatening visceral disease. The disease is endemic in 88 countries on five continents. Of the 88 countries, 22 are in the New World and 66 in the Old World. More than 90% of the CL cases occur in Iran, Afghanistan, Syria, Saudi Arabia, Brazil, and Peru. VL is endemic in the tropical and sub-tropical regions of Africa, Asia, Southern Europe, South and Central America. India accounts for half of the total 500,000 VL or kala-azar infections that are recorded annually worldwide. In addition, leishmaniasis is spreading to several nonendemic areas of the world due to coinfection with human immunodeficiency virus (HIV). The parasites have a digenetic life cycle and exist in two distinct morphologies, the promastigote in sand fly vector, and the amastigote in mammalian host. The motile flagellated promastigotes exist, multiply and develop extracellularly in the alimentary tract of the blood sucking female sand fly vectors and are transmitted during the blood meal into mammalian host. Inside the mammalian hosts they infect macrophages of the reticuloendothelial tissue and differentiate into nonmotile amastigotes and multiply as such in the phagolysosomal vacuoles. Depending on the parasite species they either elicit cutaneous leishmaniasis (e.g. *L. major, L. tropica, L. mexicana, L. braziliensis*), mucocutaneous leishmaniasis (e.g. *L. braziliensis*) or visceral leishmaniasis (e.g. *L. donovani, L. infantum/chagasi*). Because there are many areas where different species and different forms of the disease overlap, detailed knowledge of the immune response and pathogenesis is extremely important to develop vaccines for the various forms of leishmaniasis. Not only the

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organisms of this genus have the ability to withstand, inhibit or circumvent the microbicidal activity of host macrophages, but under the appropriate circumstances, they can subvert the induction of both innate and adaptive immune responses. Although most of the informations on the immunologic mechanisms upon infection and protection from the Leishmania parasites are accumulated from the studies in mice, some critical findings of murine CL have been confirmed in humans in recent years. However, the immune responses to VL and pathogenesis of disease in human deviates considerably from the murine model. This review provides an insight into the immune mechanisms associated with leishmanial infection.

Primary host response against the parasite invasion

In order to develop a successful parasitic relationship with its host, the Leishmania must evade both the innate and adaptive immune responses. When Leishmania first enters the human body, it is in the promastigote form. Promastigotes are engulfed by macrophages but are resistant to proteolysis and degradation in the phagosome. Within the mammalian host, Leishmania resides as amastigotes in phagocytic cells such as macrophages, dendritic cells (DCs) and neutrophils. The complement protein C3b is one of the most potent immune opsonins. C3b binds to foreign material and promotes its uptake via C3b receptors on phagocytic cells. C3b binds to Leishmania parasite which results in uptake by the macrophage. Leishmania has a special surface glycoprotein, called gp63, which converts C3b into iC3b. From the parasitic's standpoint this conversion would favor phagocytic clearance rather than lytic clearance as Leishmania is very resistant to degradation once phagocytosed. Therefore, this conversion is crucial for Leishmania's survival. After being engulfed, the Leishmania must endure harsh conditions inside the phagosome like the oxidative burst used by the macrophage to destroy foreign material inside the phagosome. Macrophage plays a primary role in the host defense and regulation of immune responses upon activation. This process consists of an attack by superoxide and hydroxyl radicals on the parasite. Leishmania produces acid phosphatases on its surface which inhibit this burst. In addition to the oxidative burst, macrophages often attempt to degrade parasites with acidic enzymes. This occurs when lysosomes fuse with the phagosome. The Leishmania resists this attack through a proton pump present on the surface and allows its intracellular pH to remain close to neutral. Also, the protozoan molecule lipophosphoglycan (LPG) plays an active role by inhibiting lysosomal enzymes. The parasites perform a complex host-parasite interaction inside the severe environment of the phagolysosomes and eventually evade this immune defense mechanism.

Infection of Leishmania in human is characterized by the appearance of anti-leishmanial antibodies in the sera of the patients. In CL, usually they are present at low levels during the active phase of the disease. However, in some studies the presence of antibodies against L. braziliensis infection in the sera of infected patients has been critically monitored and utilized for the diagnosis and prognosis of the disease. In contrast, strong anti-leishmanial antibody titers are well documented in VL. The role of elevated anti-leishmanial antibodies in kala-azar patients towards protection or pathogenesis is still unclear. Critical analysis of Leishmania antigen-specific immunoglobulin (Ig) isotypes revealed elevated levels of IgG, IgM, IgE and IgG subclasses during disease. IgG not only fails to provide protection against this intracellular pathogen, but it actually contributes to disease progression. Passive administration of antileishmanial IgG resulted in larger lesions in BALB/c mice with greater amount of IL-10 production. This result can be correlated with the highly elevated titers of anti-leishmanial antibodies during the active phase of the disease and a consecutive fall in the antibody titer after a successful cure. The elevated antibody titers against promastigote or amastigote antigens, their fractions or recombinant antigens have been extensively exploited for specific serodiagnosis in last two decades.
Cell mediated immune responses

Immune response in experimental and human CL

In human and experimental leishmaniasis, immunity is predominantly mediated by T lymphocytes. T cells play a major role in generating specific and memory T-cell responses to intracellular parasitic infections and these have been extensively characterized in *Leishmania* infection. Th1 and Th2 cells can be distinguished by the cytokines they secrete: Th1 cells secrete activators of cell-mediated immunity such as IFN-γ, while Th2 cells secrete cytokines such as IL-4, which promote antibody responses. The Th1/Th2 paradigm of resistance/susceptibility to intracellular infection is largely based on investigations using *L. major*. Most strains of mice (C57BL/6, C3H, CBA) develop a self-limiting cutaneous disease when infected with *L. major*. In these mice, resolution of infection is mediated by Th1 cells that produce IFN-γ. IFN-γ induces the production of nitric oxide (NO) in phagocytic cells that harbor *L. major*, which leads to destruction of the parasite. T-cell differentiation either to Th1 or Th2-type effector cells depends chiefly on the priming during differentiation. IL-4 induces Th2 whereas IL-12 induces Th1 differentiation. Therefore, infection with *L. major* in these strains of mice resembles self-limiting CL in humans.

Upon infection with *L. major*, mice of the resistant phenotype clearly develop a dominant Th1 phenotype of immune response to the parasite antigens. By contrast, BALB/c mice develop a typical Th2 response. Targeted disruption of the IFN-γ gene in C57BL/6 mice causes these animals, which are otherwise resistant to infection with *L. major*, to become highly susceptible to these organisms. Moreover, IL-4-transgenic resistant C57BL/6 mice expressing low levels of this cytokine fail to clear the infection. In addition, targeted disruption of the IL-4 gene in BALB/c mice causes these animals, which are otherwise susceptible to infection with *L. major*, to become highly resistant to these organisms.

During early infection with *L. major*, both resistant and susceptible hosts have been shown to exhibit mixed Th1/Th2 responses of CD4+ cell population with IL-2, IL-4 and IL-13 production, while IFN-γ transcripts were variable in different strains of mice. Administration of antibody to CD4+ or IL-4 led to healing of infection, suggesting that an IL-4-producing CD4+ population plays a critical role in disease progression during the early stages of infection. The IL-4 induction in *Leishmania* infection was shown to be dependent on other T-cell factors such as IL-2. Administration of anti-IL-2 or anti-IL-2 receptor antibody ameliorate the *L. major* infection, indicating that IL-2 may also be a susceptibility factor for leishmaniasis.

Susceptibility and resistance to *Leishmania* infection in the mouse model are also associated with the emergence of a unique subset of T cells, namely the T regulatory cells (Treg) and with the levels of the cytokine, IL-10. IL-10 deficient BALB/c mice were relatively resistant to infection, indicating that endogenous IL-10 plays an important role in allowing disease progression in IL-10 sufficient mice. Treg cells (CD4+CD25+) suppress the activity of effector T-cell populations (CD4+CD25+) specific for self-antigens as well as foreign invaders such as *Leishmania* parasites through the production of IL-10. Interestingly, during infection of C57BL/6 mice with *L. major*, (CD4+CD25+) T cells accumulate in the leishmanial skin lesions, and these cells produce IL-10 upon *in vitro* stimulation with parasite antigens. IL-10 is also a potent inhibitor of IFN-γ production and has been shown to be a key cytokine that favors the persistence of the parasites in skin lesions. Therefore, Treg cells and IL-10 are important regulators of resistance/susceptibility to leishmaniasis. IL-10 is crucial for suppressing the healing response in mice with cutaneous lesions caused by *L. mexicana* and in preventing the clearance of *L. donovani* from the liver and spleen. Even in the *L. major*-BALB/c infection model, Th2 cell immune polarization appears to be superimposed on IL-10 mediated suppressive pathways to account for the hyper-susceptibility of this mouse strain, as BALB/c IL-4R-α-deficient mice are not fully resistant until IL-10 function is also impaired. It has been shown that IL-10 plays an essential role in *L. major* persistence in genetically
resistant C57BL/6 mice after spontaneous healing of their lesions (Fig. 2). They have demonstrated that a sterile cure was achieved in IL-10 deficient mice but not in IL-10 sufficient mice. IL-10-sufficient C57BL/6 mice treated transiently during the chronic phase with anti-IL-10 receptor antibodies achieved a sterile cure, suggesting that IL-10 was actively involved in preventing complete parasite elimination even in the presence of a Th1 response.

A role for T<sub>reg</sub> in the pathogenesis of *Leishmania* infection is not restricted to resistant strains. In susceptible BALB/c mice, cells that suppress *L. major* protective immunity have been shown to belong to an IL-4 and IL-10 producing population of cells with a regulatory T-cell phenotype that also inhibited colitis. In this susceptible strain, the removal of T<sub>reg</sub> transiently exacerbated the Th2 response but eventually led to a better control of the infection. Thus, the outcome of chronic infection by *L. major* was tightly controlled by the equilibrium between T<sub>reg</sub> and effector T cells (Fig. 2).

IL-12 and IFN-γ are the protective cytokines based on their ability to influence Th1 development *in vitro* in various systems. Normally, resistant mice depleted of IL-12 by genetic means or antibody neutralization become susceptible to *L. major*, while BALB/c mice treated with IL-12 develop a Th1 response and become resistant. Macrophages make very little IL-12 in response to *Leishmania* and infected macrophages show a decreased ability to make IL-12 in response to various stimuli. Several reports have shown that DCs are the source of IL-12 during early infection. Furthermore, the stimulation of IL-12 production by DCs probably requires more than one signal, and there are several host components that could contribute to the IL-12 response. For example, CD40–CD40L interactions enhance IL-12 production, and the mice lacking this
pathway are susceptible to CL. Other interactions between T cells and DCs may also contribute to IL-12 production in leishmaniasis. While IL-12 is the essential cytokine in the development of Th1 responses in leishmaniasis, under certain circumstances, other cytokines, such as IL-1α, migration-inhibitory factor (MIF), type 1 IFNs, IL-18 and tumor necrosis factor (TNF) also contribute to the development of resistance. Furthermore, IL-12 has been shown to be critical in promoting a Th1 response, IL-12 holds promise as an immunomodulatory agent. Indeed, IL-12 has been shown to be an efficacious adjuvant in a vaccine. IL-12 has been shown to be critical in promoting a Th1 response, IL-12 holds promise as an immunomodulatory agent. Indeed, IL-12 has been shown to be an efficacious adjuvant in a vaccine.

In human CL, a clear dichotomy in the T-cell response to invading Leishmania parasites, as is seen in mice, has not been demonstrated in humans. The cytokine response of peripheral T cells in patients with CL revealed mixed Th1 and Th2 immunity. In localized CL (L. braziliensis or L. major), Th1 cells predominate over Th2 cells. IL-4 was detected only in cases of diffuse MCL. Other investigators have clearly detected IL-13 and IL-4 in the skin after initial lesion development, suggesting that Th2 cytokines play an immunoregulatory role in early infections. However, cure of the infection was regularly associated with the production of IFN-γ only, while IL-10 was present in persisting lesions. In addition, treatment of non-healing CL with IFN-γ resulted in rapid and complete resolution of lesions.

Patients with cutaneous and mucosal leishmaniasis due to L. braziliensis infection have a strong T-cell response, characterized by a high lymphocyte proliferative response to Leishmania antigens and IFN-γ production. Although IFN-γ is produced during L. braziliensis infection, it is not clear why these patients develop disease. It is possible that these patients may have some abnormalities at the site of the lesion. Patient with chronic lesions shows a strong expression of proinflammatory cytokines such as TNF-α. Because the cytokines secreted in the early phases of infection in the experimental models of leishmaniasis are important to determine the progression or control of the infection, it may be expected that alterations during early infections in humans may account for parasite multiplication and clinical outcome of the disease. Many evidence shows that IFN-γ and TNF-α are important for the control of leishmaniasis. In human VL and DCL, there is evidence that the absence of IFN-γ allows parasite multiplication and progression from infection to disease. It is also expected that T-cell responses and monocyte functions are important in the control of L. braziliensis infection.
Immune response in experimental and human VL

Studies of infections with the visceralizing Leishmania species (L. donovani and L. infantum/chagasi) have underscored the fact that host responses to these parasites differ significantly from L. major infection. In rodent models, the Th1/Th2 paradigm is important in determining the outcome of murine L. major infection. This dichotomy is not as influential during murine L. donovani and L. chagasi disease, in which curative type1 responses are instead suppressed by IL-10 and TGF-β. L. chagasi directly affects its local environment by activating latent TGF-β, and both L. donovani and L. chagasi suppress host macrophage responses to IFN-γ. Studies of L. donovani infection in inbred strains of mice have shown that a major susceptibility gene Lsh influences the disease outcome of leishmanial infection. Protective immunity against L. donovani, as with species causing CL, is dependent on an IL-12-driven type1 response and IFN-γ production, which results in the induction of parasite killing by macrophages primarily via the production of reactive nitrogen and oxygen intermediates.

It is well documented that VL is characterized by suppression of CMI, which is proved from the unresponsiveness of the patients to the Leishmania skin test (LST) or Montenegro test. This test measures a DTH reaction to an intradermal injection of leishmanial antigens. Containment of the disease following a successful treatment is associated with a strong cell mediated DTH response. The cell mediated immune suppression is also evident from blastogenesis assay of the peripheral blood mononuclear cells (PBMCs) (lymphoproliferation) from untreated VL patients from Brazil, Africa and India. Reduction in proportion of the helper T cells and the immunosuppression is rapidly reversible with effective chemotherapy. As in human CL, no constant association between Th1 responses and resistance to disease with predominance of cells that produce IFN-γ has been identified in human VL. The levels of IFN-γ and IL-4 are elevated during active disease and decline significantly after cure. In active human visceral disease, PBMCs exhibit a poor proliferative response to parasite antigens and fail to generate IFN-γ in vitro. This lack of IFN-γ production by PBMCs seems to predict progression of the infection into fulminant VL. In contrast, lymphocytes from patients cured of disease demonstrate a vigorous proliferative response and readily release IFN-γ, IL-2 and IL-12 on stimulation with parasite antigens in vitro. Thus, both spontaneous and drug-induced healing is thought to be followed by strong protective immunity.

IL-12, known as a natural killer (NK) cell stimulating factor, cytotoxic lymphocytes maturation factor, and a central immunoregulation of the initiation and maintenance of the Th1 response, plays an important role in the induction of IFN-γ production by T and NK cells. Exogenous IL-12 was shown to induce IFN-γ production in VL-infected mice and by PBMCs from patients. It was also involved in regulating the host response to chemotherapy. In VL patients, IL-12 enhances Th1 responses and restores lymphocyte proliferative responses, IFN-γ production and cytokotoxic responses. IL-12 also decreases spontaneous or antigen induced PBMC apoptosis in VL patients. IL-12 plays a counter-regulatory effect against IL-10 in Leishmania infection, is proved from the observation that addition of recombinant IL-12 or neutralizing anti-IL-10 mAb could restore the IFN-γ production as well as the lymphoproliferative response in the Leishmania lysate stimulated PBMCs of active VL patients. Conversely, neutralizing anti-IL-12 or rIL10 inhibits the production of IFN-γ. IL-12 used in combination with Leishmania antigen restores proliferation of PBMC from VL patients more strongly than the use of anti-IL-4 or anti-IL-10 monoclonal antibodies or even of both monoclonals combined. IL-4 is considered to be the signature cytokine of Th-2 response. Although lymphocytes from patients with VL have a strong expression of mRNA for IL-4 and sera from VL patients have high IL-4 levels, there is no evidence that IL-4 is involved in the down-regulation of the Th1 type of response in human leishmaniasis. It has been shown that in vitro addition of mAb against IL-4 did not restore the lymphocyte proliferative response or IFN-γ production in L. chagasi-stimulated PBMC from VL patients. IL-4 also did not suppress lymphocyte proliferative response or IFN-γ production in subjects cured of leishmaniasis. Thus, the prominent role of IL-4 as the leading Th2 cytokine in murine leishmaniasis was not consistently seen in human leishmaniasis. Studies on regulatory T cells (CD4+CD25), which function through TGF-β and IL-10 production, could help to understand the role of TGF-β. However, it is evident that parasite survival is favored by the conversion of latent TGF-β of the host to active TGF-β by some parasite derived factors,
which help to create its immediate microenvironment to its own survival advantage.

IL-10 was initially characterized as a Th2 cytokine but later on it was proved to be a pleiotropic cytokine, secreted from different cell types including the macrophages. Experimental evidences indicate that IL-10 plays an important regulatory role in the progression of VL. IL-10 seems to represent the main macrophage-deactivating cytokine in contrast to IFN-γ, being present in many different clinical presentations of human leishmaniasis. IL-10 blunts several immunological responses mediated by lymphocytes from Leishmania-infected individuals. Patients with VL have increased expression of mRNA for IL-10 in bone marrow and lymph node cells and high levels of IL-10 in L. chagasi-stimulated PBMC supernatants. Moreover, the addition of monoclonal antibodies to anti-IL-10 restores the lymphocyte proliferative response and IFN-γ production in PBMC from VL patients. The fact that IL-10 abrogates the effect of IL-12 in inducing IFN-γ production in L. chagasi-stimulated PBMCs of VL patients strongly suggests that IL-10 is the major cytokine involved in the progression of Leishmania infection to visceral disease. IL-10 has been shown to block Th1 activation and consequently a cytotoxic response by down-regulating IL-12 and IFN-γ production. Additionally, because IL-10 also inhibits macrophage activation, it decreases the ability of these cells to kill Leishmania. Studies of tissue cytokine mRNA expression have revealed a role for IL-10 in down regulating CD4+ T-cell responses and the involvement of IL-10 in the disease pathology of L. donovani infections. However, active VL also finds to promote the clinical progression of AIDS and it was suggested that Leishmania parasites could be seen as potential co-factor in HIV-1 pathogenesis. Further, L. donovani promastigotes and LPG are proved to mediate CD4+ T cell activation induced HIV-1 replication. Leishmanial antigen induced TNF-α produced in the culture is important for the replication. The mutual interdependence as the agents for co-infection between these two pathogens is also reported. Co-infection in THP-1 macrophage cell line increases the multiplication of L. donovani amastigotes in the macrophages. It was shown that killed HIV preparation abrogate the proliferative response as well as IFN-γ production from the L. donovani antigen induced PBMCs of healthy individuals. Moreover, anti-IL-10 could not enhance IFN-γ production in HIV-VL co-infected patients as is generally found in only VL patients.

Immune responses in Leishmania-HIV co-infection

Among all types of leishmaniasis, VL is the most frequent potential opportunistic disease associated with HIV-1 since the mid 1980s. Although 90% of the reported cases are from southwestern Europe, incidences of co-infection are increasing in eastern Africa and the Indian subcontinent. VL is found to promote the clinical progression of AIDS and it was suggested that Leishmania parasites could be seen as potential co-factor in HIV-1 pathogenesis. Further, L. donovani promastigotes and LPG are proved to mediate CD4+ T cell activation induced HIV-1 replication. Leishmanial antigen induced TNF-α produced in the culture is important for the replication. The mutual interdependence as the agents for co-infection between these two pathogens is also reported. Co-infection in THP-1 macrophage cell line increases the multiplication of L. donovani amastigotes in the macrophages. It was shown that killed HIV preparation abrogate the proliferative response as well as IFN-γ production from the L. donovani antigen induced PBMCs of healthy individuals. Moreover, anti-IL-10 could not enhance IFN-γ production in HIV-VL co-infected patients as is generally found in only VL patients.

Immune response in Post Kala-azar Dermal Leishmaniasis (PKDL) patients

As a sequel to kala-azar, PKDL, appears in a dermatotropic form of L. donovani infection in >50 per cent patients in Sudan and 10-20 per cent patients in India. The immunopathogenesis of the disease is still not well understood. Although patients of PKDL
often bear the different forms of PKDL lesions for years and thus some workers consider these cases as reservoir for visceral leishmaniasis \(^{107,108}\), while others have found that VL and PKDL causing strains are different\(^{109}\). There are limited attempts to reveal the underlying immune mechanisms for the appearance of the disease\(^{108}\). Studies, which have so far been made, indicate that unlike VL there is positive cell mediated immune response against Leishmanial antigen in PKDL in terms of DTH (LST), lymphoproliferation and production of IFN-\(\gamma\) by the PBMCs\(^{71,72}\). PKDL patients manifest an aggravation of the disease with time in form of nodular lesions or erythematous plaque formation. Again, PKDL patients with early infection show better CMI than the chronic patients. These indicate that there are different domains of PKDL patients according to the immunopathogenesis of the disease. Though there is generalized prominence of Th1 in PKDL, as found from IFN-\(\gamma\) production, high levels of IL-10 in the lesions as well as plasma of the patients\(^{97}\) indicate the importance of Th2 response in chronic PKDL. Prevalence of the CD8\(^+\) cells over CD4\(^+\) cells in PKDL lesions of Indian patients\(^{110,111}\) suggest persistence of parasites as CD8\(^+\) cells might block the IL-2 production locally and in turn inhibit the IFN-\(\gamma\) production\(^{112}\) favoring disease.

**Conclusion**

To understand the nature of human infection with these parasites and to develop better chemotherapeutic and vaccine strategies, further in-depth studies focused on the immune modulation in subclinical and asymptomatic individuals are required. As there is tremendous ecological and genetic diversity among the different human populations exposed to the parasite, a conclusive understanding of the parameters of resistance vs. control in humans is difficult. Moreover, the immunological data available are still scarce. There is also an urgent need for a better experimental model mimicking human infections.

In contrast to the earlier ideas that antagonistic functions of IFN-\(\gamma\) and IL-4 determine the outcome of protection or pathogenesis of the disease, recent studies emphasize the importance of the balance of the two regulatory cytokines IL-12 and IL-10, critical for the regulation of the immune modulation during infection, pathogenesis and chemotherapy.

Macrophages are proposed primary host cells for *Leishmania* but the role of these cells has not been well characterized either in disease prevention or in progression independent of T cell. The effector functions of macrophages for *Leishmania* have always been described in a T-dependent manner. The fate of infected macrophages in pre- T-cell phase is not well known. Because T cells come later during infection, it is possible that the parasite modulates its host in terms of signaling or antigen presentation for its own benefit and induces factors that provide a disease-progressive environment and prime T cells for Th2 differentiation. It is also possible that parasites start modulating the macrophages at the time of entry and later on modulated parasitized macrophages interact with T cells and may induce IL-4 and disease-inducing factors from T cells that help in disease progression and parasite survival in a susceptible host. It is now known that IL-10 plays a role in disease progression but whether with IL-4 or before the IL-4 phase is not known. It is also known that *Leishmania*-parasitized macrophages produce IL-10 but not IL-4, suggesting the role of IL-10 before IL-4 in disease.
progression or in the susceptibility of the host. This suggests the crucial role of IL-10 in disease initiation independent of T cells and in disease progression later on in combination with IL-4.

Thus, the Th1/Th2 paradigm of resistance/susceptibility is an oversimplification of a far more complicated network of regulatory/counter-regulatory interactions. These will differ according to the Leishmania species being studied, the host organism used and the tissue site examined. Studies using these organisms are providing fascinating new insights into the basic immunological mechanisms controlling the outcome of infectious diseases in general, which will aid the future rational development of appropriate strategies for immune intervention or vaccination.

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