

Pro- and anti-inflammatory cytokines in cutaneous leishmaniasis: a review

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Cutaneous leishmaniasis (CL) is caused by different species of the genus *Leishmania*. Pro- and anti-inflammatory cytokines play different roles in resistance/susceptibility and the immunopathogenesis of *Leishmania* infection. The balance and dynamic changes in cytokines may control or predict clinical outcome. T helper 1 (Th1) inflammatory cytokines (especially interferon- γ , tumor necrosis factor- α and interleukin-12) are the crucial factors in the initiation of protective immunity against *L. major* infection, whereas T helper 2 cytokines including IL-5, IL-4, and IL-13 facilitate the persistence of parasites by downregulating the Th1 immune response. On the other hand, aggravation of inflammatory reactions leads to collateral tissue damage and formation of ulcer. For this reason, immunity system such as T regulatory cells produce regulatory cytokines such as transforming growth factor- β and IL-10 to inhibit possible injuries caused by increased inflammatory responses in infection site. In this article, we review the role of pro- and anti-inflammatory cytokines in the immunoprotection and immunopathology of CL.

Keywords: *Leishmania*, Cutaneous leishmaniasis, Immunoprotection, Immunopathology, Cytokine

Introduction

Cutaneous leishmaniasis (CL) is a significant health problem in large parts of the world, especially in under-developed countries.¹ At least 88 countries are endemic regions,¹ where about one-third of cases occur in each of three epidemiological regions, including the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia. Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru are the 10 countries with the highest incidence. It is estimated that about 0.7–1.2 million new cases of CL occur per year.¹ Also, the estimated global mean age-standardised disability-adjusted life years for CL was 0.58 per 100,000 people in 2013.² CL is caused by different species of the genus *Leishmania* (e.g. *L. major*, *L. tropica*, and *L. aethiopica* in old world and *L. amazonensis*, *L. mexicana*, and *L. braziliensis* in the new world).¹

Leishmania parasite passes its life cycle in two hosts: sand flies and mammalian hosts such as humans, dogs, and rodents. When an infected sand fly feeds on a mammalian host, *Leishmania* metacyclic promastigotes are injected into the skin. Then, the promastigotes are phagocytosed by phagocytic cells, such as macrophages, neutrophils, and dendritic cells (DCs). The promastigotes are able to survive in macrophages (final host cells) because of complex defense mechanisms and transform into amastigote forms (Fig. 1). The *Leishmania* parasites proliferate in tissue macrophages and spread to other macrophages depending

on various parasite and host factors. In CL, the infection is usually limited to the skin and lymphatic system, but it may influence on deeper tissues in diffuse CL or penetrate into the mucous membranes in MCL. The life cycle is completed when sand flies feed near the skin lesions and the amastigotes enter the midgut of the sand fly where they subsequently develop into promastigote forms.^{3–6}

There are many complexities in immunity against leishmaniasis. It is well documented that resistance to leishmaniasis is related to T helper 1 (Th1) development and production of pro-inflammatory cytokines (e.g. interleukin (IL)-12, IL-1, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and/or IL-2) that lead to activation of macrophages and parasite killing.^{7,8} Conversely, susceptibility to the infection is linked to T helper 2 (Th2) development and production of Th2 cytokines such as IL-4, IL-5, and/or IL-13 leading to parasite replication and persistence.^{8–10} However, several paradoxes remain about the role of immune responses in immunoprotection and immunopathology of CL. For example, although Th1 response and production of pro-inflammatory cytokines have pivotal roles for immunoprotection against CL,^{8,11} their excessive production may concomitantly lead to severe immunopathology in the disease.^{12–15} On the contrary, Th2 development is associated with parasite persistence in the site of infection^{8,11} but production of anti-inflammatory cytokines at lower levels will also mitigate inflammatory reactions and accelerate wound healing process.^{15–17} In addition, other T cells, such as Th17 cells by production of inflammatory cytokines (e.g. IL-22, IL-17 and/or IFN- γ)¹⁸ and T regulatory (Treg) cells

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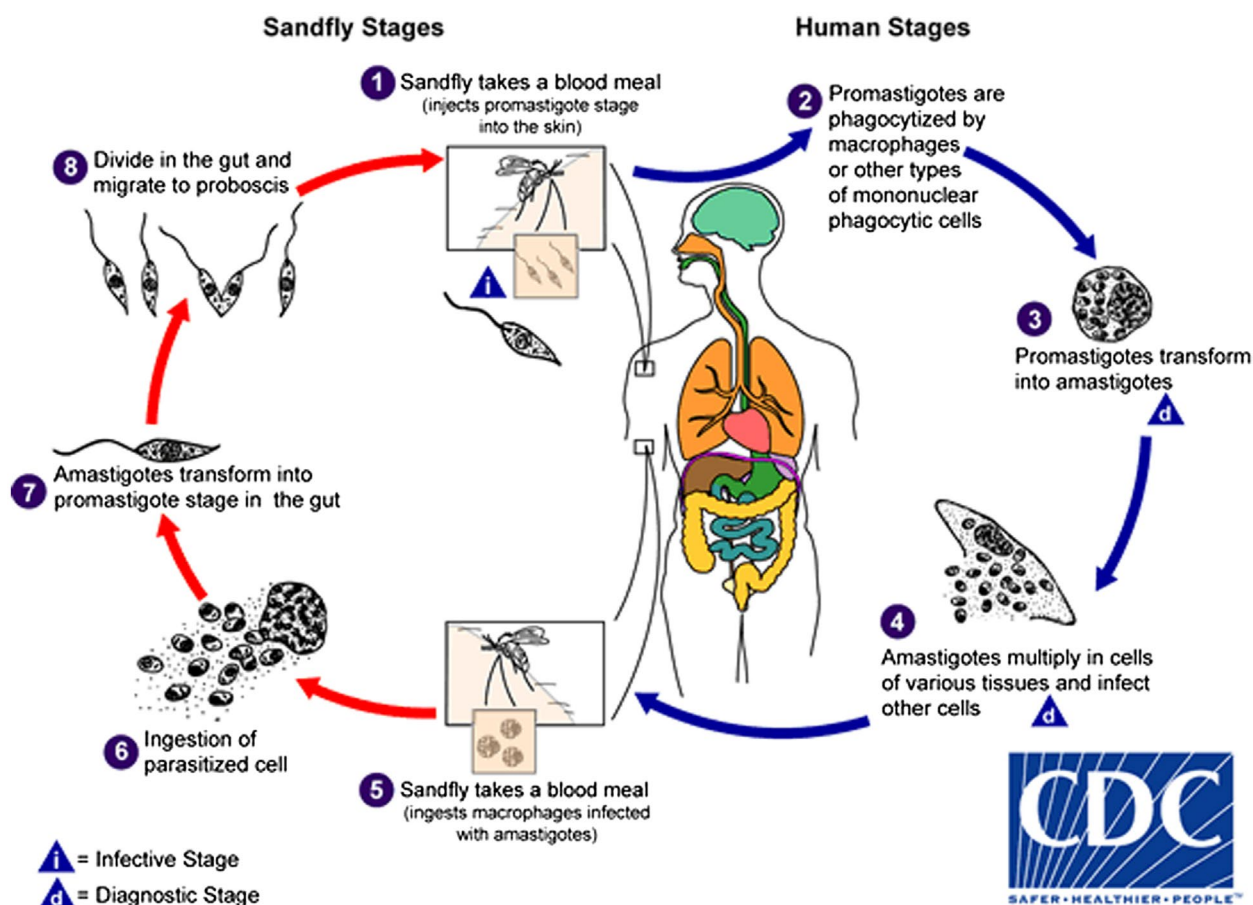


Figure 1 Leishmaniasis is transmitted by the bite of infected female phlebotomine sand flies. The sand flies inject the infective stage (i.e. promastigotes) from their proboscis during blood meals **1**. Promastigotes that reach the puncture wound are phagocytized by macrophages **2** and other types of mononuclear phagocytic cells. Promastigotes transform in these cells into the tissue stage of the parasite (i.e. amastigotes) **3**, which multiply by simple division and proceed to infect other mononuclear phagocytic cells **4**. Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. Sand flies become infected by ingesting infected cells during blood meals (**5**, **6**). In sand flies, amastigotes transform into promastigotes, develop in the gut **7** (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis **8**. Source: Centers for Disease Control and Prevention (CDC). <http://www.cdc.gov/dpdx/leishmaniasis/>.

Table 1 Resistant and susceptible mouse strains in leishmaniasis

Resistant mouse strains	Susceptible mouse strains
C57BL/6 ^{50,202}	BALB/c ^{50,202}
CBA ²⁰²	
C3H/He ²⁰²	

by production of regulatory cytokines (e.g. IL-10 and/or transforming growth factor (TGF)- β) contribute to disease progression or improvement depending on the *Leishmania* spp and also the genetic background of the host (Table 1).¹⁹ Hence, this review focuses on the role of pro- and anti-inflammatory cytokines in immunoprotection and immunopathology of CL.

Pro-inflammatory cytokines in CL *IFN- γ* and *TNF- α*

IFN- γ and TNF- α are two important pro-inflammatory cytokines involved in the immunoprotection and immunopathology of CL. IFN- γ is mainly secreted by Th1 CD4+

and CD8+ cytotoxic T lymphocytes, natural killer (NK) cells, and natural killer T (NKT) cells. These cytokines have essential roles in control of intracellular pathogens and tumor cells, but their increased production may lead to autoimmune diseases.²⁰ IFN- γ stimulates nitric oxide (NO) production in activated macrophages and inhibits intracellular parasite growth.²¹ Furthermore, IFN- γ promotes differentiation of CD4+ T cells to the Th1 subset and inhibits the development of Th2 and Th17 cells.²² It is observed that IFN- γ -deficient C57BL/6 mice are more susceptible to *Leishmania* infection than wild-type counterpart.²³ Compared with wild-type mice, *L. amazonensis* infection in IFN- γ -deficient C57BL/6 mice showed larger lesions, increased parasite burden, and development of Th2-type responses associated with IL-4 elevations than wild-type mice.²³

TNF- α is mostly produced by macrophages that play a crucial role in *Leishmania* clearance through increase in macrophage activity and NO synthesis.²⁴ This cytokine is able to promote Th1/IFN- γ responses against *L. major*

infection.²¹ TNF- α -deficient C57BL/6 mice infected with *L. major* showed fatal visceral infection despite the production of IFN- γ and IL-12 by macrophages.²⁵ Treatment of BALB/c mice with TNF- α decreased the parasite burden and lesion size in CL.²⁶ In contrast, neutralizing TNF- α receptor 1 led to non-healing lesions in resistant C57BL/6 mice following *L. major* infection.²⁷

Interestingly, it was observed that IFN- γ and TNF- α have synergistic killing effects against *L. major* infection through stimulation of macrophages to increased NO production.²⁸ Also in clinical studies, both IFN- γ and TNF- α have been detected in the lesions of CL patients.^{29–33} However, different studies demonstrated that upregulation of the pro-inflammatory cytokines (especially TNF- α and IFN- γ) are associated with increased tissue damage at the site of infection. In this regard, a positive correlation between lesion size with IFN- γ and TNF- α levels was observed in CL patients infected with *L. braziliensis*. Patients with greater lesions had higher levels of IFN- γ and TNF- α despite presence of IL-10 in the site of infection.^{31,34,35}

Levels of IFN- γ and TNF- α were lower in asymptomatic *L. braziliensis*-infected individuals than those in patients with typical signs of CL.³⁶ Indeed, patients with typical signs of the disease had excessive levels of IFN- γ and TNF- α with inflammatory reactions and skin ulcers at the site of infection.³⁶ In subclinical patients, the moderate production of IFN- γ and TNF- α was associated with control of parasite growth without induction of tissue destruction.³⁶ Th1 cells of ML patients have been reported to secrete a higher level of IFN- γ and TNF- α , and lower levels of IL-10 compared to Th1 cells of CL patients. In comparison to CL patients, lymphocytes isolated from ML patients exhibited a stronger proliferative response with higher secretion of these pro-inflammatory cytokines when stimulated with *Leishmania* antigen.^{37–39} Although IFN- γ and TNF- α production seems to be required for control of *Leishmania* infection, increased levels of these cytokines may lead to tissue destruction and development of progressive wounds.

IL-12

IL-12 acts as an essential cytokine for differentiation of Th1 cells in leishmaniasis.^{40,41} This cytokine is mainly produced by monocytes, macrophages, DCs, and B cells. IL-12 is involved in the development of Th1 response through IFN- γ production from NK and T cells.⁴² IL-12 stimulates also differentiation of naïve T cells into Th1 effectors and inhibits T cell apoptosis.^{43–46} The IL-12 family of cytokines, including IL-12, IL-23, and IL-27 share homology at the subunits, receptors, and signaling levels.^{47,48} Bioactive IL-12p70 is made up of two subunits, p35 and p40, which are essential for continued resistance to *L. major* infection.⁴⁹ Interestingly, the absence of each of these subunits promotes the development of Th2 response and increases susceptibility to infections such as

leishmaniasis.^{48,50} The IL-12p40 (also called IL-12/23p40) subunit is linked to IL-23p19 subunit to form IL-23. IL-27 is composed of IL-27p28 (p28) and Epstein–Barr virus-induced gene 3 (EBI3) subunits. IL-12 signals through the IL-12R β 1 and IL-12R β 2 subunits.⁵¹ Like IL-12, IL-23p40 subunit can bind to the IL-12R β 1, however IL-23p19 subunit cannot bind to IL-12R β 2 but has a second IL-23 receptor (IL-23R) subunit.^{51,52} However, in the JAK/STAT signalling pathway, IL-12 mainly activates STAT4 specific molecules, while IL-23 and IL-27 principally activate STAT3 and STAT1, respectively.⁵¹ IL-12 is an essential cytokine for stimulation of Th1 cells in leishmaniasis.^{40,41} BALB/c mice are susceptible mouse models to CL, and this susceptibility is related to the loss of genetic ability of IL-12 production. Hence, the immune responses fail to develop Th1 cells in *L. major*-infected BALB/c mice and in this situation IL-4-induced Th2 cells develop that resulted in progressive skin lesions with visceral invasion.⁴⁰ Conversely, genetically resistant mice lacking IL-12 developed Th2 response with high IL-4 and low IFN- γ levels along with progressive skin lesions similar to susceptible BALB/c mice following *L. major* infection.⁵⁰ IL-12 has been successfully used as an adjuvant for *L. major* vaccination.^{53–55} Moreover, immunotherapy with IL-12 led to resolution of *L. major* infection in BALB/c mice with concomitant reduction in parasite burden and lesion size, and increased IFN- γ and decreased IL-4 production.^{42,56} Notably, neutralization of IL-12 during primary infection with *L. major* led to deterioration with progressive lesions.⁴¹ It is of interest that neutralization of IFN- γ repealed the treatment effect of IL-12 and restored Th2 cytokine responses.⁵⁶ Therefore, IL-12 plays a crucial role in the shift of T cell into Th1 or Th2 immune responses in CL.

IL-2

IL-2 is a growth factor that is mainly synthesized by CD4+Th cells and also in smaller amounts by CD8+ T cells, NK cells and NKT cells.^{57,58} IL-2 signals affect various lymphocyte subsets during differentiation, immune responses, and homeostasis. This cytokine promotes immune responses by increase in proliferation, cytokine secretion, and cytolytic activity in CD4+, CD8+, and NK cells.^{59,60} For example, IL-2 stimulates the production of IFN- γ by Th1 cells and activates propagation of cytotoxic T cells via binding to IL-2 receptors on lymphocytes.⁶¹ By contrast, IL-2 promotes autoimmune diseases through the death of activated T cells due to IL-2 deprivation, initiation of pro-apoptotic pathways by increase in FasL expression on activated T cells and development of CD4+CD25+ Tregs.^{59,62–65}

Different studies have shown that IL-2 is involved in the protective immune response of CL.^{66–68} Together with IFN- γ , IL-2 facilitates Th1 response and macrophage activation for killing *Leishmania* parasite.²² It has been observed that the sera of CL patients with primary infection contain

higher concentrations of IFN- γ and IL-2 in comparison to uninfected individuals or those with secondary infection.⁶⁹ In humans, genetic mutations that lead to reduced IL-2 production are associated with exacerbated human CL.⁶⁸ Co-administration of recombinant IL-2/diphtheria toxin fusion protein (rIL-2/DTx) depletes Treg cells.⁷⁰ In this regard, Divanovic *et al.*⁷⁰ used rIL-2/DTx as adjunctive therapy for experimental *L. major* infection in a murine model. They observed that rIL-2/DTx therapy suppressed lesional Tregs, increased IFN- γ production, decreased parasite burden, and enhanced wound healing process. They also found an additive therapeutic effect when rIL-2/DTx combined with sodium stibogluconate (a choice drug for leishmaniasis treatment) that lead to a reduction in dose and duration of sodium stibogluconate therapy.⁷⁰ Conversely, IL-2 can also stimulate proliferation of Th2 cells through generation of IL-4.⁷¹ The IL-2 receptor is composed of multiple subunits and the common gamma chain is shared between IL-2 and IL-4.^{72,73} It is reported that neutralization of IL-2 in *L. major* infected BALB/c mice leads to decreased IL-4 and increased IFN- γ production in their lymph nodes.⁷⁴ Collectively, IL-2 seems to play as a bifunctional cytokine that may promote susceptibility or resistance to CL.

IL-1

The IL-1 family consists of two main agonistic proteins, including IL-1 α and IL-1 β that are involved in various immunopathologies and inflammatory disorders, as well as protective immune responses against infectious pathogens. IL-1 is an important pro-inflammatory cytokine that is mainly secreted by macrophages and like TNF- α act as 'alarm cytokine'.⁷⁵⁻⁷⁹ IL-1 is a critical regulator for early differentiation of Th17 cells and Th17-mediated autoimmunity. Also, IL-1 along with IL-6 and IL-23 regulates Th17 differentiation and maintains cytokine expression in effectors Th17 cells.⁸⁰ IL-1 can induce protective or pathogenic effects during *Leishmania* infection. For example, the production of IL-1 α in lymph nodes of *L. major*-infected BALB/c mice decreased three times in comparison to resistant C57BL/6 mice. Local treatment with IL-1 α significantly decreased lesion size and parasite burden in infected animals and led to enhancement of the Th1 response via high production of IFN- γ and low production of IL-4.⁷ *Leishmania* infection promotes Nod-like receptor protein 3 (NLRP3) inflammasome-derived IL-1 β productions that leads to host resistance to infection by NO production.⁸¹

Similar to IL-12, IL-1 acts as an adjuvant that supports the generation of IFN- γ -secreting T cells and helps in IgG2a production.⁸² Short-term treatment of *L. major*-infected C57BL/6 mice using IL-1 β during early phases of infection promotes Th1 response and protects against leishmaniasis.⁸³ In contrast, continuous treatment of *L. major*-infected C57BL/6 mice with IL-1 α induces Th2 response and exacerbates the disease outcome.⁸³ NLRP3

inflammasome-derived IL-1 β production is responsible for non-healing lesions in C57BL/6 mice infected with *L. major* Seidman strain. NLRP3 inflammasome promotes susceptibility to *L. major* infection by production of IL-18 and IL-1 β so that BALB/c mice lacking the inflammasome components NLRP3, ASC, or caspase 1 were resistant to *L. major* infection and produced high levels of IFN- γ and low levels of IL-4 and IL-5 leading to smaller footpad swelling and lower parasite burden in comparison to control mice.⁸⁴ IL-1 β also promotes pathology and the formation of exacerbated lesions in C57BL/6 mice infected with *L. major* through the development of Th17 cells and regulation of IL-17 levels.⁸⁵ Also, IL-1 induces inflammatory responses in *L. major* infected BALB/c mice that leads to progressive disease and lack of IL-1 genes delays development of the disease and induces more attenuated systemic inflammatory responses.⁸⁶ Another study showed that IL-1 signaling is dispensable for protection against CL in C57BL/6 mice.⁸⁷ Human studies have demonstrated that IL-1 can also contribute to disease progression by promoting TNF- α production.⁸⁸ Therefore, IL-1 β promotes differentiation of protective CD4⁺ T cells, while excessive production of IL-1 β during the chronic phase of infection leads to progression disease.

IL-18

IL-18 is a pleiotropic cytokine also named IFN- γ -inducing factor. IL-18 is secreted by different cells such as activated macrophages, DCs and Kupffer cells. IL-18 induces Th1 responses via IFN- γ production in collaboration with IL-12.^{89,90} Another study demonstrated that IL-18-deficient C57BL/6 mice show high susceptibility to *L. major* infection with decreased levels of IFN- γ and increased levels of IL-4 in comparison with wild-type mice.⁹¹ Monteforte *et al.*⁹² reported that IL-18^{-/-} C57BL/6 mice promotes Th1 responses in *L. major* infection. In this study, although IL-18^{-/-} C57BL/6 mice developed larger lesions during early phase of infection, disease resolved eventually in IL-18-deficient mice by production of IL-12 and IFN- γ but no IL-4 similar to IL-18^{+/+} mice. However, it seems that the genetic background and cytokine milieu influence on induction of Th1 or Th2 responses by IL-18.⁹³ Treatment of *L. major*-infected BALB/c mice with recombinant IL-18 promotes Th2 responses in the absence of IL-4 and leads to exacerbated disease in comparison with untreated animals.⁹³ In another study, NLRP3-dependent IL-18 production promotes Th2 responses during *L. major* infection so that neutralizing IL-18 reduces production of Th2 cytokines such as IL-4 and induces protection against *L. major* infection in BALB/c mice.⁹⁴ Overall, it seems that IL-18 promotes Th1 or Th2 responses during CL depending upon cytokine milieu and genetic background.

IL-15

IL-15 is a pleiotropic cytokine that plays the role in the homeostasis of the innate and adaptive immunity through

various mechanisms.⁹⁵ IL-15 is produced mainly by DCs, monocytes, macrophage, and epithelial cells.⁹⁶ This cytokine in collaboration with IL-12 facilitates IFN- γ and TNF- α production by NK and T cells.⁹⁷ IL-15 also enhances protective immune responses against intracellular pathogens.⁹⁸ Some studies also demonstrate that IL-15 is involved both in the development of Th1 responses by inducing IFN- γ production^{99–101} as well as Th2 responses by increase in IL-5 and IL-13 production.^{98,100,102} D'Agostino *et al.*¹⁰³ showed that similar to IFN- γ , IL-15 induces leishmanicidal activity in macrophages via both IL-12-dependent and IL-12-independent pathways.¹⁰³ In the recent study, endogenous IL-15 suppresses Th2 cytokines such as IL-4 in *L. infantum* infection without production of Th1 cytokines.¹⁰⁴

IL-8

IL-8 is a strong proinflammatory cytokine that plays an essential role in the recruitment and activation of neutrophils in the course of inflammation.¹⁰⁵ This cytokine is secreted by tissue-resident macrophages in response to *Leishmania* infections and plays a key role in the initial stages of infection or tissue damage.²² Monocytes isolated from *L. major*-infected individuals exhibited a high level of IL-8 expression.¹⁰⁶ IL-8 (a chemoattractant for neutrophils) and neutrophils are involved in early defense against *Leishmania* parasite in the site of infection.^{105,107} In leishmaniasis, neutrophils play different roles in stimulation of the immune response to infection. Neutrophils may kill the parasites or protect them depending on the parasite species and the host. For example, neutrophils can contribute to kill *L. amazonensis* and *L. braziliensis* promastigotes by neutrophil extracellular traps or by the activation of infected macrophages to kill parasites.^{108–111} Upon inoculation of *L. major* promastigotes, neutrophils are the first cells that migrate to the infected site.^{112,113} Two CXC chemokines, namely macrophage inflammatory protein-2 and KC (murine homologues of IL-8) are quickly produced by distinct cell types in the skin, that act as neutrophil chemoattractants and lead to the early neutrophil accumulation.¹⁰⁷ Infected neutrophils with *L. major* secrete high levels of IL-8 that lead to increased infiltration of neutrophils for phagocytosis of the parasite.^{114,115} Neutrophils increase the production of DCs by CC-chemokine ligand 3-dependent mechanism.¹¹⁶ *L. major*-infected neutrophils express apoptotic factors in their surface that promotes their preferential elimination by DCs and inhibits cross-presentation function in DCs. Consequently, reduction in DC activation leads to suppression of Th1 cell and CD8+ T cell function.^{117,118} Also, the capture of *Leishmania* infected neutrophils by macrophages can limit the activation of macrophages and leads to parasite survival.¹¹⁹ Collectively, IL-8 plays a significant role in death or survival of *Leishmania* parasites through the production of neutrophils.

IL-17

IL-17 is a highly inflammatory cytokine that is produced by Th17 cells and mediates tissue inflammation. IL-17 also induce different pro-inflammatory cytokines (such as IL-6 and TNF- α) and chemokines.¹²⁰ IL-1 β and IL-23 promote developments of Th17 cells. Also, TGF- β plus IL-6 differentiates naive T cells into Th17 cells. Furthermore, IL-6 leads to upregulation of IL-21 and IL-23, which promotes further Th17 development.^{121,122} IFN- γ suppresses differentiation of Th17 cells and IL-17 production by downregulation of TGF- β and IL-6 or IL-1 β and IL-23.^{123–126} Th2 cytokines such as IL-4 and IL-13 suppress IL-6 and TGF- β -induced differentiation of Th17 cells.^{123,124,127} IL-27 and IL-25 also regulate Th 17 cells by development of Th1 and Th2 responses.^{128–130} Also different studies demonstrate that IL-17 is involved in the immunopathology of CL.^{85,131} For example, lack of IL-10 in *L. major*-infected C57BL/6 mice induced severe immunopathology associated with elevated IL-17 and neutrophil production.⁸⁵ Lopez Kostka *et al.*¹³¹ observed that neutrophil-derived IL-17 promotes susceptibility to *L. major* infection in BALB/c mice. They found that IL-17-deficient BALB/c mice infected with *L. major* develop smaller cutaneous lesions with fewer parasite burden associated with a decreased number of neutrophils and decreased CXCL2-accumulation in the lesion site. Gonzalez-Lombana *et al.*⁸⁵ found that increased IL-17 production is responsible for immunopathology in IL-10-deficient C57BL/6 mice infected with *L. major* by infiltration of neutrophils at the site of infection. Bacellar *et al.*¹³² showed that lymphocytes of patients with mucosal leishmaniasis and CL produced a significantly higher level of IL-17 in comparison with uninfected control subjects. In contrast, several studies demonstrate that *Leishmania* vaccines in mouse and human models induce elevated IL-17 and IL-22 levels that play complementary roles with Th1 cytokines in protection against CL.^{19,133,134} Taken together, there are contradictory results about the role of IL-17 in pathogenesis and protection against leishmaniasis.

IL-22

IL-22 is secreted from Th cell subsets, including T helper 22 (Th22), Th17 and Th1 cells, as well as innate lymphocytes.¹³⁵ This cytokine has antimicrobial properties and plays a pivotal role in tissue repair. Although IL-22 is a beneficial cytokine for host, it is involved in many infectious and inflammatory disorders and can be pathogenic due to its inherent pro-inflammatory properties, especially when it is released together with other pro-inflammatory cytokines such as IL-17.¹³⁶ IL-22 plays a protective role against tissue damage during CL. For example, Gimblet *et al.*¹³⁷ reported that IL-22 deficient C57BL/6 mice infected with *L. major* develop more severe pathological changes with a higher parasite burden than wild-type mice.¹³⁷ In another study, IL-22 improves the efficacy of DNA vaccines against *L. major* in BALB/c mice^{138,139} so

that IL-22 plus a DNA vaccine encoding LACK antigen resulted in increased IFN- γ and decreased IL-4 levels than LACK gene alone.¹³⁹ However, conflicting results have been reported indicating an independence of IL-22 in host resistance of C57BL/6 mice to *L. major*.¹⁴⁰ Overall, although there are a few studies about the role of IL-22 in leishmaniasis, it seems that this cytokine has a protective role against *Leishmania* infection.

Anti-inflammatory cytokines in CL

IL-6

IL-6 is a pleiotropic cytokine that acts as both a pro-inflammatory and anti-inflammatory cytokine.¹⁴¹ IL-6 is produced by several cell types, including macrophages, DCs, and T cells.¹⁴¹ Also, this cytokine acts as a B-cell growth factor.¹⁴² IL-6 plus TGF- β stimulate the development of Th17 responses and produce IL-17 and IL-10 cytokines and lead to restrain pathogenic function of Th17 cells.¹²² Animal experimentations demonstrated that IL-6 promotes Th2 responses in CL.^{143,144} Moskowitz *et al.*¹⁴⁴ showed that IL-6-deficient C57BL/6 mice infected with *L. major* can control infection and promote strong Th1 responses as efficiently as wild-type C57BL/6 mice. Titus *et al.*¹⁴² reported that the production of both Th1 and T2 cytokines decreased in *L. major*-infected IL-6^{-/-} BALB/c mice but there were no significant difference between lesion size and parasite burden in IL-6-deficient and wild-type mice.¹⁴² Hatzigeorgiou *et al.*¹⁴⁵ found that pretreatment of macrophages with IL-6 suppressed IFN- γ and TNF- α production against *L. amazonensis* *in vitro*. Taken together, IL-6 is a susceptibility factor in CL.

IL-27

IL-27 is a pleiotropic cytokine produced by activated APCs such as macrophages and DCs.¹⁴⁶ IL-27 plays two-sided roles in immune responses. It acts as an inflammatory cytokine that initiates Th1-type responses, but also suppresses inflammatory T-cell responses.^{147,148} IL-27 inhibits production of pro-inflammatory cytokines, including IL-17 and IL-23 and promotes production of IL-10 from CD4⁺ T Cells.^{149,150} IL-27 initiates Th1 responses and induces protection against *Leishmania* infection so that IL-27R-deficient mice were susceptible to early *L. major* infection that associated with impaired Th1 response by decrease in IFN- γ production.¹⁵¹ Another study showed that IL-27 induces IFN- γ production and controls the infection only in the presence of IL-4.¹⁵² Therefore, IL-27 seems to play a bifunctional factor that may promote susceptibility or resistance to leishmaniasis.

IL-10

Although IL-10 is known to be a potent immunoregulatory cytokine, it is produced by different innate immunity cells (e.g. DCs, macrophages, mast cells, NK cells, eosinophils, and neutrophils) and adaptive immunity cells (e.g. Th1, Th2 and Th17 cell subsets, Treg cells, CD8⁺ T cells, and

B cells).^{153–158} In addition, IL-10 suppresses macrophage activation and maturation of DCs. IL-10 production by Th1 cells limits immune responses against intracellular parasite infections such as *L. major* and *Toxoplasma gondii*.¹⁵⁹ This cytokine is associated with the susceptibility to leishmaniasis and parasite persistence in infection site.¹⁶⁰ While infection of IL-10^{-/-} C57BL/6 Mice with *L. major* led to inhibit production of IL-10 by Treg cells during the chronic phase of CL that consequently lead to parasite clearance and wound healing.¹⁶¹ IL-10 and Treg cells limit the effective function of Th1 responses to control infection in the skin.¹⁶¹ Indeed, treatment of *L. major*-infected mice with anti-IL-10 receptor antibodies led to sterile cure and parasite clearance.¹⁶⁰ Castellano *et al.*¹⁶² reported that CL patients with active lesions had higher levels of Th1 and Th2 cytokines including IFN- γ , TNF- α , IL-12, IL-4, and IL-10 in comparison with cured patients, while cured patients had higher level of IFN- γ . Salhi *et al.*¹⁶³ reported that *L. braziliensis*-infected individuals with active lesions had polarized Th2 or mixed Th1/Th2 responses, both associated with increased IL-10 levels.¹⁶³ These results suggested that downmodulation of IL-10 and IL-4 and elevation of IFN- γ is associated with clinical cure in CL patients.¹⁶² Buxbaum and Scott¹⁶⁴ found that *L. Mexicana*-infected C57BL/6 wild-type mice had minimal immune response and chronic lesions, but 10^{-/-} mice resolved their lesions and had increase production of IFN- γ , NO and delayed-type hypersensitivity.¹⁶⁴ The quantitative IFN- γ /IL-10 ratio is important to the result of vaccination against CL. A low ratio has been found to result in vaccine failure whereas a high ratio provided vaccine success.¹⁶⁵ IL-10 production by Treg cells has been found to suppress the magnitude and quality of the Th1 response, while neutralization of IL-10 increased magnitude and quality of the Th1 response after vaccination.¹⁶⁶ Despite suppressive effects of IL-10 that leads to disease progression and parasite persistence, this cytokine is a vital immunoregulator that modulates immunopathology and tissue damage caused by excessive Th1 immune response and their inflammatory cytokines, especially IFN- γ in CL.¹⁶⁷

Based on cell surface markers or secreted cytokines, Treg cells are divided into two subsets: first, natural (n) Tregs that develop in thymus and express CD4, CD25 (IL-2 receptor α chain) and also Forkhead box protein 3 (Foxp3) (a specific marker for nTregs). nT reg (CD4⁺CD25⁺Treg) subset represents 5–10% of the adult peripheral CD4 T cells and are vital for self-tolerance and avoid autoimmune diseases.^{168–171} Second, acquired (a)/induced (i) Tregs develop from conventional T cells and are grouped two subsets: T regulatory1 (Tr1) and T-helper 3 (Th3). Tr1 cells do not express Foxp3¹⁷² and are able to produce high levels of IL-10 and TGF- β ,¹⁷³ while Th3 cells express Foxp3¹⁷⁴ and produce elevated levels of TGF- β .¹⁷⁵ Overall, Treg cells with high levels of Foxp3 (Foxp3^{high}) suppress the function of effector T cells (Th1 and Th2) and DCs by IL-10 production but promote differentiation

of Th17 cells by TGF- β production. Treg cells with low levels of Foxp3 (Foxp3^{low}) promote Th2 responses by IL-4 and IL-10 production. Treg cells lacking Foxp3 (Foxp3 null) may transform into different types of effectors T cells (Th1, Th2, and Th17).¹⁷⁰

During infection with *L. major*, CD4+CD25+ T cells accumulate in the dermis and suppress function of CD4+CD25- effectors T cells to eliminate the parasite from infection site by both IL-10-dependent and IL-10-independent mechanisms. CD4+CD25+ T cells are responsible for the persistence of *L. major* in healed lesions that leads to concomitant immunity and host resistance to reinfection.¹⁵³ In another study, increased level of IL-10 in CD4+CD25-Foxp3-Th1 cells is responsible for development of nonhealing lesions following *L. major* infection.¹⁷⁶

Taken together, IL-10 acts as a double-edged sword that suppresses cellular immune response and production of inflammatory cytokines (IFN- γ and TNF- α) that lead to parasite persistence in the infection site. On the other hand, IL-10 inhibits an exacerbated immunopathology and tissue damage following increased production of inflammatory cytokines and plays a central role in the regulation of tissue remodeling during wound healing.¹⁷⁷

IL-4

IL-4 plays an important role in the differentiation of Th0 cells into Th2 cells.¹⁷⁸ IL-4 is mainly produced by Th2, mast cells, basophils, and activated eosinophils.¹⁷⁹⁻¹⁸¹ IL-4 production drives upregulation of arginase and polyamine biosynthesis that inhibits leishmanicidal activity of macrophages and prolonged survival of parasites.¹⁰ IL-4 limits the generation of Th1 cytokines through downregulation IL-12 production.¹⁸² Furthermore, IL-4 downregulates the production of chemokines that recruit Th1-type cells to the infection site.¹⁸³ Pro-inflammatory cytokines such as IFN- γ stimulate M1 macrophages that lead to NOS2 activation, NO release, and parasite death, while Th2 cytokines such as IL-4 and IL-13 stimulate M2 macrophages to induce arginase activity which result in parasite survival and inhibition of inflammation by counteracting the effects of NOS2 activation and nitric. Indeed, the balance between classically activated macrophage (M1) and alternatively activated macrophage (M2) regulates inflammatory responses and leads to homeostasis in immunity system and wound healing.¹⁸⁴⁻¹⁸⁷ Also, IL-4 suppresses IL-6 and TGF- β -induced differentiation of Th17 cells that leads to inhibition of immunopathology caused by IL-17.^{123,124,127} Studies using IL-4 transgenic and knockout mice have shown an important role of IL-4 in susceptibility to *Leishmania* infection. Kopf et al.¹⁸⁸ found that IL-4-deficient BALB/c mice are resistant to *L. major* infection. Also, they observed that IL-4 transgenic C57BL/6 mice were more susceptible to *L. major* infection in comparison to wild-type mice.¹⁸⁸ Radwanska et al.¹⁸⁹ showed that deletion of IL-4R α on CD4 T cells lead to resistance of BALB/c mice to *L. major* infection. Sadick

et al.¹⁹⁰ found that neutralization of IL-4 by anti-IL-4 mAb inhibits development of Th2 response in *L. major*-infected BALB/c mice so that IFN- γ mRNA expression increased fourfold in the lymph nodes of infected mice. They also found that neutralization of IL-4 led to complete cure in 85% of infected mice and attenuation of infection in 100% of animals.¹⁹⁰

Heinzel et al.¹⁹¹ found that IL-4 mRNA was expressed only in BALB/c mice infected with *L. major* but not in infected C57BL/6. However, IFN- γ mRNA increased in draining nodes and spleen of C57BL/6 mice than that in BALB/c mice except at 4 and 6 weeks of infection, when splenic IFN- γ levels were transiently comparable. In this study, neutralization of IL-4 by anti-IL-4 mAb led to disease healing by a reduction in serum IgE, lesion size, and parasite burden in infected BALB/c mice.¹⁹¹ Another study showed although both IL-4 and IL-4R α -deficient BALB/c mice were more resistant to *L. major* infection in comparison to wild-type mice, IL-4R α ^{-/-} mice efficiently controlled infection by reduction of Th2 responses, while IL-4^{-/-} mice partially controlled the infection.¹⁹² In contrast, Noben-Trauth et al.¹⁹³ showed that disruption of the IL-4 gene in *L. major*-infected BALB/c mice did not promote polarization of Th1 response and had no effect on wound healing and parasite clearance. Although IL-4 plays a critical role in susceptibility to *Leishmania* infection, several contributing factors help in its susceptibility. For example, IL-4^{-/-} and IL-13^{-/-} *L. mexicana*-infected BALB/c mice revealed that IL-4 plays a pivotal role in initiation of lesion development, but IL-13 plays a crucial role in development of chronic and non-healing infection.¹⁹⁴ Failure of IL-12 production is another contributing factor that leads to susceptibility to *L. major* infection.¹⁹⁵ Also study in IL-4-deficient BALB/c mice demonstrated that different parasite isolates, and differences in the age of the mice and in the arginase activity are other influencing factors of susceptibility to *L. major* infection of IL-4^{-/-} BALB/c mice.^{10,196} Therefore, IL-4 is an important cytokine in susceptibility to *Leishmania* infection.

IL-13

IL-13 shares signaling pathway with IL-4.¹⁹⁷ Like IL-4, IL-13 is produced by Th2, mast cells, and basophils.¹⁸¹ IL-13 decreases the inflammatory responses via downregulation of pro-inflammatory cytokines such as IL-1, IL-6, TNF- α , and IL-12.^{198,199} IL-13 inhibits the production of IL-12 by macrophages and limits *L. major* killing.^{200,201} Therefore, upregulation of IL-13 in transgenic animals delays the onset of a Th1 response subsequent to *L. major* infection.²⁰² IL-13 can limit NO production in human mesangial cells through downregulation of iNOS.²⁰³ Subsequently, NO suppression decreases the parasiticidal activity of macrophage.¹⁹⁹ IL-13 makes specific T cells unresponsive to IL-12 by downregulation of the IL-12R β 2 chain.²⁰⁴ As observed by Bourreau et al.²⁰⁴ IL-13 was the predominant Th2 cytokine in

peripheral blood mononuclear cells of *L. guyanensis*-infected patients, while the absence of IL-12R β 2 chain in lesions indicated the pivotal role of IL-13 in susceptibility to CL caused by *L. guyanensis*.²⁰⁴ Matthews et al.²⁰² found that IL-13 is a susceptible factor for *L. major* infection, while IL-13-deficient BALB/c mice are resistant to the infection. Overexpression of IL-13 in C57BL/6 mice leads to suppression of IFN- γ and IL-12 expression and increases susceptibility to *L. major* infection, even in the absence of IL-4 expression.²⁰² Additionally, transgenic mice expressing IL-13 failed to control *L. major* infection and showed 1000-fold higher parasite load than wild-type C57BL/6 mice. In contrast, Sosa et al.²⁰⁵ showed that IL-13-deficient C57BL/6 mice were susceptible to infection and indicated progressive and non-healing wounds as the wild-type mice infected to *L. mexicana*, but IL-4⁻/IL-13⁻ mice were highly resistant with lower parasite burden and higher levels of IL-12 and IFN- γ than wild-type and IL-13⁻ mice. The study shows that IL-13 is not a major susceptible factor to *L. mexicana* but IL-4 is a dominant cytokine for pathogenesis of cutaneous *L. mexicana* infection. Indeed, IL-4 may compensate lack of IL-13 and promotes susceptibility to *L. mexicana* in C57BL/6 mice and led to pathogenesis of *L. mexicana* infection. Alexander et al.¹⁹⁴ using *L. mexicana*-infected-IL-4/IL-13⁻ mice demonstrated that IL-13 is a crucial factor to maintaining non-healing forms of CL in chronic phase but IL-4 plays a crucial role in primary lesion formation. Collectively, IL-13 plays a vital in susceptibility to leishmaniasis, especially in promoting chronic phase of disease.

TGF- β

TGF- β is a pleiotropic growth factor with significant anti-inflammatory and immunosuppressive properties and plays central roles in homeostasis of immune system.²⁰⁶ TGF- β is produced by different cells, including CD4⁺ T cells (Tregs), monocytes, neutrophils, and DCs.^{207–210} TGF- β cytokine and Treg cells are essential for control of immune responses against foreign pathogens, the maintenance of homeostasis, and promoting immune tolerance.^{170,208,211,212} TGF- β suppresses both adaptive immune response and the innate immune response by inhibiting the function of inflammatory cells and promoting the function of Treg cells.^{213–215} Also, TGF- β suppresses differentiation of T cells to Th1 and Th2 subsets.^{216,217} In addition, TGF- β suppresses generation of activated T cells by inhibiting production of IL-2 and IL-1.^{218,219} TGF- β differentiates CD4⁺CD25⁻ naïve T cells to iTreg cells (CD4⁺CD25⁺ Tregs) in peripheral lymphoid organs and other tissues.²²⁰ Although nTregs does not require TGF- β for their differentiation in the thymus, TGF- β is essential for survival and function of nTregs.^{221–223} nTregs and iTregs (Tr1 and Th3 cells) produce high levels of TGF- β .^{169,170,172,173,175} Therefore, Tregs produce TGF- β and provide a positive feedback loop.

TGF- β is an immunoregulatory cytokine that inhibits Th1 responses against *Leishmania* parasite by down-regulation of IFN- γ , inactivation of macrophages, and inhibition of IL-2R stimulation.²²⁴ TGF- β enhances susceptibility to *Leishmania* infection by suppression of NO, TNF and IFN- γ production.²²⁵ This cytokine exacerbates infection due to *L. amazonensis* and *L. braziliensis* via stimulation of production of Th2 cytokines such as IL-10. TGF- β modulates differentiation of T cells into Th1 cells via down regulation of T-bet, an independent mechanism of down regulation of IL-12 receptor β 2 chain expression leading to decrease in IFN- γ production and inhibits parasite clearance.²²⁶ Barral et al.²²⁷ reported that the addition of recombinant TGF- β to murine or human macrophages increased the parasite load *in vitro*.²²⁷ Similar studies showed that *in vivo* immunotherapy with TGF- β for resistant mice changed their immune response and led to overexpression of IL-10 in draining lymph nodes, whereas treatment of susceptible animals with anti-TGF- β mAb led to the decrease in IL-4 expression and increase in IFN- γ expression in draining lymph nodes.^{228,229} Hence, TGF- β acts as an infection promoting factor in CL.^{228,229} Another study²³⁰ showed that local inoculation of anti-TGF- β mAb into the *Leishmania* lesion led to decrease in parasite burden and more rapid healing of wound without alteration in IL-4 and IFN- γ production. Immunohistochemical test showed that anti-TGF- β treatment increased NO production within parasitized lesions.²³⁰ This study suggested that TGF- β may act as an important regulatory cytokine during chronic stages of CL that inhibits NO production in macrophages.²³⁰ In addition, this study expressed that during the lack of TGF- β , even with dominant Th2-type responses, relatively low levels of IFN- γ are sufficient to activate macrophages for parasite killing within parasitized lesions.²³⁰ Also, several studies have reported that IL-10 and TGF- β expression increase in long lasting lesions than acute lesions in CL.^{231–233} TGF- β modulates lymphocyte proliferation and production of inflammatory cytokines as it limits increased inflammatory reactions that are responsible for tissue damage.^{234,235} Moreover, TGF- β plus IL-6 promote the differentiation of Th17 cells from naïve T cells.^{236,237} In contrast, increased production of TGF- β and IL-6 restimulates activated Th17 cells that have resulted in IL-10 production to control immunopathology caused by Th17 cells (a self-regulating mechanism).¹²² TGF- β is a crucial immunoregulatory cytokine that limits inflammatory reactions by downregulating inflammatory cytokines in leishmaniasis.

Conclusion

Cytokines play vital roles in cell propagation and differentiation toward defense against pathogens. However, the balances of pro- and anti-inflammatory cytokines are needed to prevent immunopathological disorders. In leishmaniasis, protective immunity depends predominantly on a Th1 response and production of pro-inflammatory

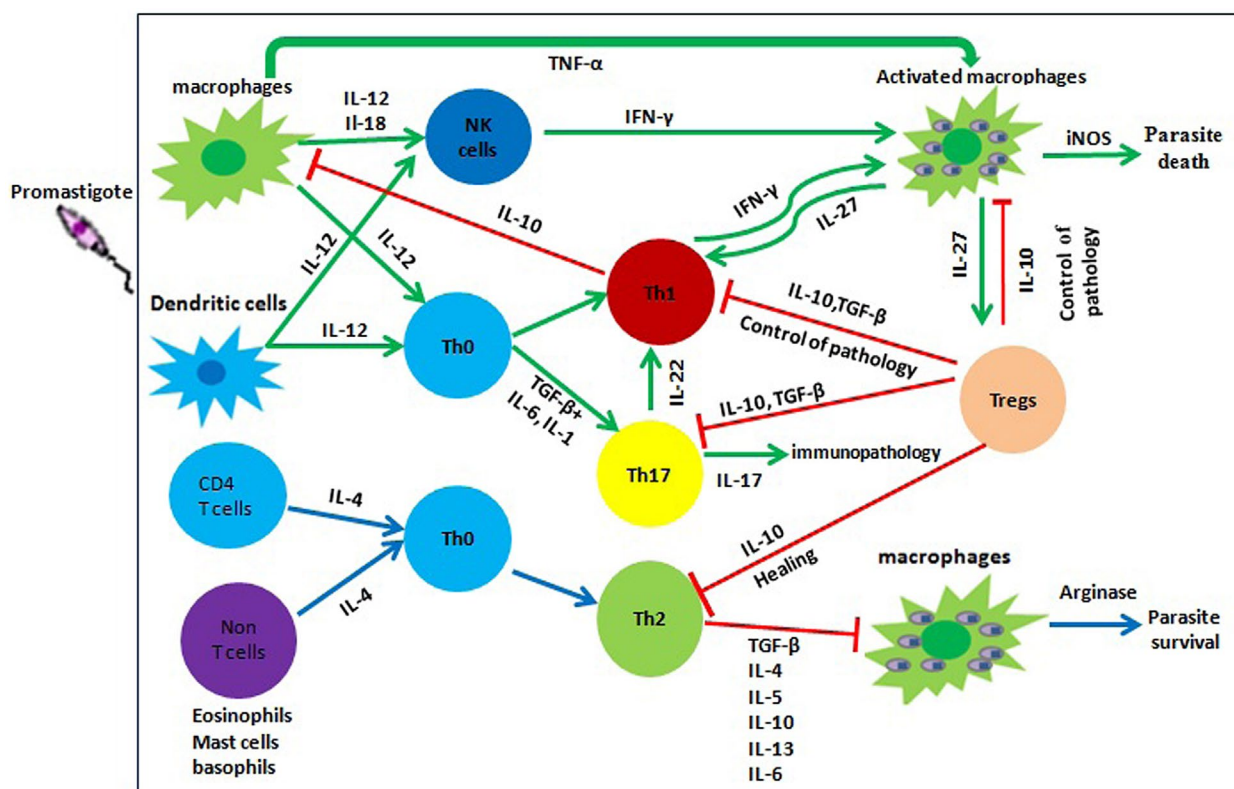


Figure 2 Immunological pathways of against cutaneous leishmaniasis. This figure shows different immunological pathways in CL that all depend on the differentiation of CD4+ T cell subsets into Th1, Th2, Treg, and Th17. Following parasite entry, APCs (macrophages and dendritic cells) are stimulated to produce pro-inflammatory cytokines such as IL-12. These cytokines promote Th1 differentiation and IFN- γ production lead to activation of macrophages and parasite killing by NO production. Conversely, anti-inflammatory cytokines promote differentiation of Th0 toward Th2 that inhibit macrophage activity and lead to parasite survival. Overproduction of inflammatory cytokines results in severe immunopathology and non-healing infection. TGF- β and IL-27 cytokines secreted by macrophages or DCs stimulate Treg cells to produce IL-10 that act back on the macrophages and DCs to reduce the release of inflammatory mediators, forming a negative feedback loop and the balance of pro- and anti-inflammatory cytokines controls pathology and tissue destruction.

cytokines like TNF- α , IL-12, IFN- γ . A less controlled inflammatory response and contributing cytokines are responsible for immunopathology and tissue damage in CL.^{30,237} In addition, secreted cytokines of Th17 cells promote the pathogenesis and lesion development in leishmaniasis^{85,131,132} although recent studies demonstrate that Th17 cytokines induce protection against the disease as well.^{19,138,139} Th2 cytokines such as IL-13 and IL-4 promote disease progression. On the other hand, IL-4 and IL-13 suppress immunopathology caused by Th17 cells.^{123,124,127} Regulatory cytokines such as IL-10 and TGF- β counteract the immunopathology caused by pro-inflammatory cytokines but are also responsible for maintenance of parasites in infection site.³⁹ Therefore, the cytokines act like a double-edged sword that may induce protection against leishmaniasis or implicate in the pathogenesis of CL (Figure 2). A balance between immune responses control the infection and prevent inflammatory reactions that leads to wound healing in CL. Overall, immune mechanisms involved in leishmaniasis are complex because the exact role of some cytokines remains unclear until now. Also cytokines act as a network because different cytokines have synergistic or antagonistic effects during an immunological reaction against CL. Comprehensive understanding

of the immunity mechanisms can help researchers find new therapeutic strategies and development of effective vaccines.

Conflict of interest

Authors disclose that there is no conflict of interest.

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