Cutaneous leishmaniasis: immune responses in protection and pathogenesis

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Abstract | Cutaneous leishmaniasis is a major public health problem and causes a range of diseases from self-healing infections to chronic disfiguring disease. Currently, there is no vaccine for leishmaniasis, and drug therapy is often ineffective. Since the discovery of $CD4^+$ T helper 1 (T_H1) cells and T_H2 cells 30 years ago, studies of cutaneous leishmaniasis in mice have answered basic immunological questions concerning the development and maintenance of $CD4^+$ T cell subsets. However, new strategies for controlling the human disease have not been forthcoming. Nevertheless, advances in our knowledge of the cells that participate in protection against *Leishmania* infection and the cells that mediate increased pathology have highlighted new approaches for vaccine development and immunotherapy. In this Review, we discuss the early events associated with infection, the $CD4^+$ T cells that mediate protective immunity and the pathological role that $CD8^+$ T cells can have in cutaneous leishmaniasis.

Delayed-type hypersensitivity
(DTH). An inflammatory response that develops 48–72 h after injection of antigen into the skin. DTH indicates that an individual has a population of T cells that make interferon-γ and recognize that antigen.

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Cutaneous leishmaniasis — which is caused by several protozoal parasites of the genus *Leishmania* — is endemic to South and Central America, Northern Africa, the Middle East and parts of Asia, and an estimated 1 million new cases arise each year[1]. Of particular interest to immunologists is the wide range of clinical manifestations associated with this disease, which, similar to tuberculosis and leprosy, is dictated largely by the type and magnitude of the immune response of the host. As in most infections, the immune response to cutaneous leishmaniasis depends on many host factors, as well as on the differences between the infecting *Leishmania* spp. Experimental infections in mice also exhibit a spectrum of clinical presentations depending on the mouse strain and the infecting parasite species or strain used (TABLE 1).

The immunological spectrum observed in patients with leishmaniasis ranges from individuals with a strong T cell response, characterized by delayed-type hypersensitivity (DTH) and high levels of interferon-γ (IFNγ), to individuals who lack a DTH response but may have high levels of antibodies[2]. Because *Leishmania* spp. are killed by IFNγ-activated macrophages and are not neutralized by antibodies, individuals with a strong DTH have few parasites in their lesions, whereas those with only a humoral response are unable to control the parasite load[2,3]. As expected, patients without a T cell response exhibit a severe disease called diffuse cutaneous leishmaniasis. At the other end of the spectrum, patients with an exaggerated immune response also develop a severe disease phenotype known as mucosal leishmaniasis, which is driven by immunopathology. Between these extremes are patients who develop lesions that may self-heal or become chronic, with intermediate levels of T cell and antibody responses[4] (FIG. 1).

The differential development of T helper 1 (T_H1)- and T_H2-type responses was initially thought to translate directly to the spectrum of clinical presentations seen in patients. This reasoning was based on findings that $CD4^+$ T_H1 cells mediate resistance in *Leishmania major*-infected mice whereas $CD4^+$ T_H2 cells promote susceptibility[5,6]. However, advances in our understanding of the disease in both humans and mice indicate that a more complex cellular response dictates the outcome of infection. In particular, substantial advances have been made in our understanding of both protective and pathological immune responses to leishmanial infection. These advances should ultimately influence the development of vaccines and immunotherapies for leishmaniasis. In this Review, we discuss these advances and, where possible, link findings in mouse models to human disease.

Early immune responses to *Leishmania*

Several *Leishmania* spp. cause cutaneous leishmaniasis, and each species has individual characteristics. However, they share a similar life cycle in which a sand fly transmits a flagellated form of the parasite, called a promastigote, to mammalian hosts, including humans,

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dogs and rodents[7]. Once the promastigotes are injected into the skin via the bite of a sand fly, they enter several types of phagocytic cells. Within the phagolysosome of macrophages, promastigotes transform to a round non-flagellated replicative form called an amastigote. The life cycle is complete when sand flies ingest amastigotes while feeding on a host, and the amastigotes subsequently transform to promastigotes and replicate within the sand fly. Most experimental infections involve injecting promastigotes into the skin with a needle; however, during a natural infection, additional factors present in the sand fly saliva are introduced in the skin that influence early immune responses[8]. Hence, the biological significance of studies investigating the early response to infection without considering the conditions present during natural infection, such as the inoculation site, number of parasites and the components present during the sand fly bite, should be carefully interpreted[9,10].

Although macrophages are the primary host cell for *Leishmania* parasites, monocytes, dendritic cells (DCs) and neutrophils that are recruited to the infection site can become infected and have important and distinct roles in shaping the immune response to infection.

The role of neutrophils. Neutrophils are rapidly recruited to the site of a *Leishmania* infection[11], but their role here is complicated; they may kill the parasites or protect them depending on the parasite species and the host. For example, *Leishmania amazonensis* promastigotes are killed by neutrophil extracellular traps (NETs)[12,13] (FIG. 2a); however, salivary proteins from the sand fly can protect the parasites against neutrophil-mediated death[14]. Thus, it remains unclear whether NETs have a protective role *in vivo*. Neutrophils can also contribute to the control of *Leishmania braziliensis* and *L. amazonensis* by interacting with infected macrophages[15,16] (FIG. 2a). By contrast, uptake of apoptotic neutrophils by macrophages and DCs after *L. major* infection can limit the activation of macrophages and DCs, leading to better parasite survival[17,18]. However, this process may not occur with every *Leishmania* spp. because apoptosis was not observed following *Leishmania mexicana* infections[19]. Neutrophils also promote increased CC-chemokine ligand 3 (CCL3)-dependent recruitment of DCs[20], and the expression of apoptotic markers on neutrophils promotes their preferential phagocytosis by DCs[21]. The consequent decrease in DC activation reduces the ensuing T_H1-type response and inhibits cross-presentation for $CD8^+$ T cell activation[21,22] (FIG. 2b).

Studies of *Leishmania* infection in the absence of neutrophils suggest that the role of neutrophils depends on the genetic background of the host. For example, neutrophil-depleted C57BL/6 mice exhibit a normal course of infection with *L. major*, whereas neutrophil depletion in BALB/c mice blocks the early characteristic interleukin-4 (IL-4) response and thereby inhibits the development of the non-protective T_H2-type response[23]. However, evaluating the *in vivo* role of neutrophils is complicated because the monoclonal antibody (mAb) that is most frequently used to deplete neutrophils, RB6-8C5, recognizes both LY6G (which is expressed on neutrophils) and LY6C (which is expressed on other cells, including monocytes). Studies using the more specific mAb 1A8 and the use of neutropaenic Genista mice[24] will need to be performed to help resolve this issue. Strikingly, Genista mice are resistant to infection with parasites that normally cause non-healing lesions, such as *L. mexicana* and the Seidman strain of *L. major*[19,25], which suggests that neutrophils may have

Table 1 | **Human and mouse disease caused by *Leishmania* spp. that are frequently used in experimental studies**

Leishmania spp.	Human disease	Mouse disease				Refs
		C57BL/6 mice		BALB/c mice		
		Type of disease	Dominant immune response	Type of disease	Dominant immune response	
Leishmania major	Self-healing or chronic cutaneous leishmaniasis usually caused by a single skin lesion	Self-healing	T_H1	Chronic	T_H2	5,6
Leishmania major Seidman strain	Chronic cutaneous leishmaniasis	Chronic	T_H1	Chronic	T_H2	138
Leishmania amazonensis	Self-healing or chronic cutaneous leishmaniasis usually caused by a single skin lesion, and diffuse cutaneous leishmaniasis	Chronic	T_H1 and T_H2	Chronic	T_H2	139–141
Leishmania mexicana	Healing or chronic cutaneous leishmaniasis usually caused by a single skin lesion, and diffuse cutaneous leishmaniasis	Chronic	T_H1 and T_H2	Chronic	T_H2	139,142, 143
Leishmania braziliensis	Healing or chronic cutaneous leishmaniasis usually caused by a single skin lesion, and mucosal leishmaniasis	Self-healing	T_H1	Self-healing	T_H1	76

T_H, T helper.

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a primarily detrimental role. However, further studies using different parasite species in different genetic backgrounds will be required to obtain a clear picture of their *in vivo* role in cutaneous leishmaniasis.

The role of DCs and inflammatory monocytes. Inflammatory monocytes and DCs are also recruited to the site of infection, and over the first few days become the dominant cells infected with *Leishmania* parasites[21] (FIG. 2c). Even within the first few hours of infection, some DCs and monocytes are infected with the parasites[26,27]. The early recruitment of inflammatory monocytes is dependent on CCL2, which is produced by cells within the infection site following activation by platelet-derived growth factor[27]. The consequence of monocyte infection is markedly different from infection of macrophages; monocytes exhibit a strong respiratory burst upon infection, leading to early parasite control, whereas macrophages need to be activated by IFNγ to kill the parasites[27] (FIG. 2c). C57BL/6 mice lacking CC-chemokine receptor 2 (CCR2) develop a non-healing *L. major* infection, which is characterized by an increased and sustained recruitment of neutrophils and the development of a $CD4^+$ T_H2-type response[28]. Furthermore, in neutropaenic Genista mice, increased resistance to infection correlated with the recruitment of inflammatory monocytes[19]. Taken together, current data suggest a protective role for monocytes in *Leishmania* infection, although more *in vivo* studies are needed to confirm this role. Thus, although neutrophils may have a dual role during infection, inflammatory monocytes seem to be important in controlling the infection.

Innate mechanisms of Leishmania killing. The two major mechanisms responsible for controlling *Leishmania* parasites are the production of reactive oxygen species (ROS), generated by the respiratory burst that occurs during phagocytosis, and nitric oxide (NO), generated by inducible NO synthase (iNOS) following activation of cells by IFNγ. Although *Leishmania* parasites are sensitive to ROS, the respiratory burst that occurs in non-activated macrophages following infection is insufficient to kill the parasites[29], which could be due to the parasites inhibiting ROS generation in phagolysosomes[30]. However, IFNγ enhances the respiratory burst in macrophages, leading to better parasite killing[31]. By contrast, both human and mouse monocytes produce high levels of ROS and can mediate ROS-dependent killing of *Leishmania* without prior activation[27,31]. ROS production may be particularly important before the development of the adaptive immune response for all *Leishmania* spp., but it is not absolutely required as mice deficient in components of the NADPH complex, which is required to generate ROS, can still control disease[32]. This effect is probably due to the important role of NO in mouse models of *Leishmania* infection.

IFNγ and tumour necrosis factor (TNF) act synergistically to promote optimal activation of macrophages to eliminate *Leishmania* parasites by inducing iNOS[33,34]. As NO can diffuse across cell membranes, it can mediate killing of both intracellular parasites within the NO-producing cell and those in bystander cells[35]. In mice, NO is considered essential for controlling *Leishmania*, as iNOS-deficient mice are susceptible to *L. major* infection even though they develop a greater T_H1-type response compared with wild-type mice[36]. However, the role of NO in humans is less clear. Although *in vitro* blockade of NO can affect parasite growth in human macrophages in some studies, NO cannot be measured in human cell cultures[37]. Although iNOS expression has been detected in lesions from patients with cutaneous leishmaniasis[38], there was no change in the expression of the human gene encoding iNOS (*NOS2*) in lesions of patients with cutaneous leishmaniasis compared with normal skin[31]. Thus, although NO is the main mediator of killing *Leishmania* in mice, the relative roles of ROS and NO for *Leishmania* control in humans remain unclear.

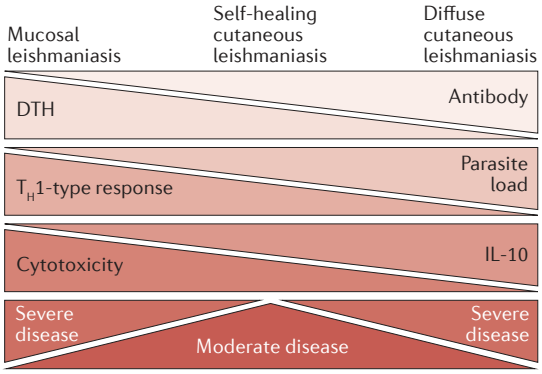

Figure 1 | **Spectrum of disease in human cutaneous and mucosal leishmaniasis.** Mucosal and diffuse cutaneous leishmaniasis are severe forms of disease that fall on opposite ends of the immunological spectrum. The spectrum ranges from high levels of cell-mediated immunity to high levels of antibody. Although all clinical forms require T helper 1 (T_H1)-type responses to cure the disease, an exacerbated T_H1-type response and an increased number of $CD8^+$ cytotoxic T cells are associated with increased disease severity. The consequence of an extremely exaggerated cellular response is the development of mucosal leishmaniasis, in which parasites metastasize to the nasopharyngeal mucosa and cause disfiguring lesions. By contrast, patients at the other end of the spectrum have high parasite numbers within the lesions, which is a consequence of low levels of T_H1 cytokines. This form of the disease, termed diffuse cutaneous leishmaniasis, is also associated with high antibody titres. In addition, patients with diffuse cutaneous leishmaniasis produce high levels of the regulatory cytokine interleukin-10 (IL-10), whereas patients with mucosal leishmaniasis have low levels of IL-10. DTH, delayed-type hypersensitivity.

Adaptive immunity to *Leishmania*

Early adaptive immune responses. The early immune response is important in determining whether a *Leishmania* infection in the skin will be self-healing or chronic. Experimental *L. major* infections in various mouse strains have been used to identify the factors promoting the differential development of T_H1 and T_H2 cells. Some mouse strains develop $CD4^+$

Respiratory burst
The rapid release of reactive oxygen species from immune cells during phagocytosis.

CC-chemokine receptor 2
(CCR2). A receptor that binds monocyte chemoattractant protein (CCL2) and is involved in monocyte migration from the bone marrow to inflammatory sites.

Reactive oxygen species
(ROS). Chemically reactive molecules that contain oxygen, and include superoxide anions, hydroxyl radicals and hydrogen peroxide.

Nitric oxide
(NO). A free radical that is a gas that performs several biological functions and is involved in killing pathogens by macrophages.

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IL-12
A heterodimeric cytokine containing an IL-12p35 and an IL-12p40 chain that stimulates the production of interferon-γ from cells. IL-12 is crucial for the differentiation of $CD4^+$ T helper 1 cells.

T_H1 cell-mediated resistance following infection with *L. major*, whereas other mouse strains develop a $CD4^+$ T_H2-type response and are extremely susceptible to infection (TABLE 1). IL-12 is essential for the development of protective $CD4^+$ T_H1 cells, as determined by a combination of antibody treatments and knockout mice[39,40]. By contrast, IL-4 promotes T_H2 cell development and susceptibility in mice[41], but the degree to which $CD4^+$ T_H2 cells mediate susceptibility in human leishmaniasis is less clear.

DCs initiate the antigen-specific immune response to *Leishmania* and are the main source of IL-12 (REF. 42). Some DCs that prime naive T cells are resident in the lymph node[43], but most DCs are derived from inflammatory monocytes that are recruited to the cutaneous lesion and subsequently differentiate into monocyte-derived DCs that migrate to the draining lymph node (DLN)[44] (FIG. 2c). Before the development of T_H1 cells, IFNγ is primarily produced by natural killer (NK) cells within the DLN[45], which reside in close association with DCs. Once

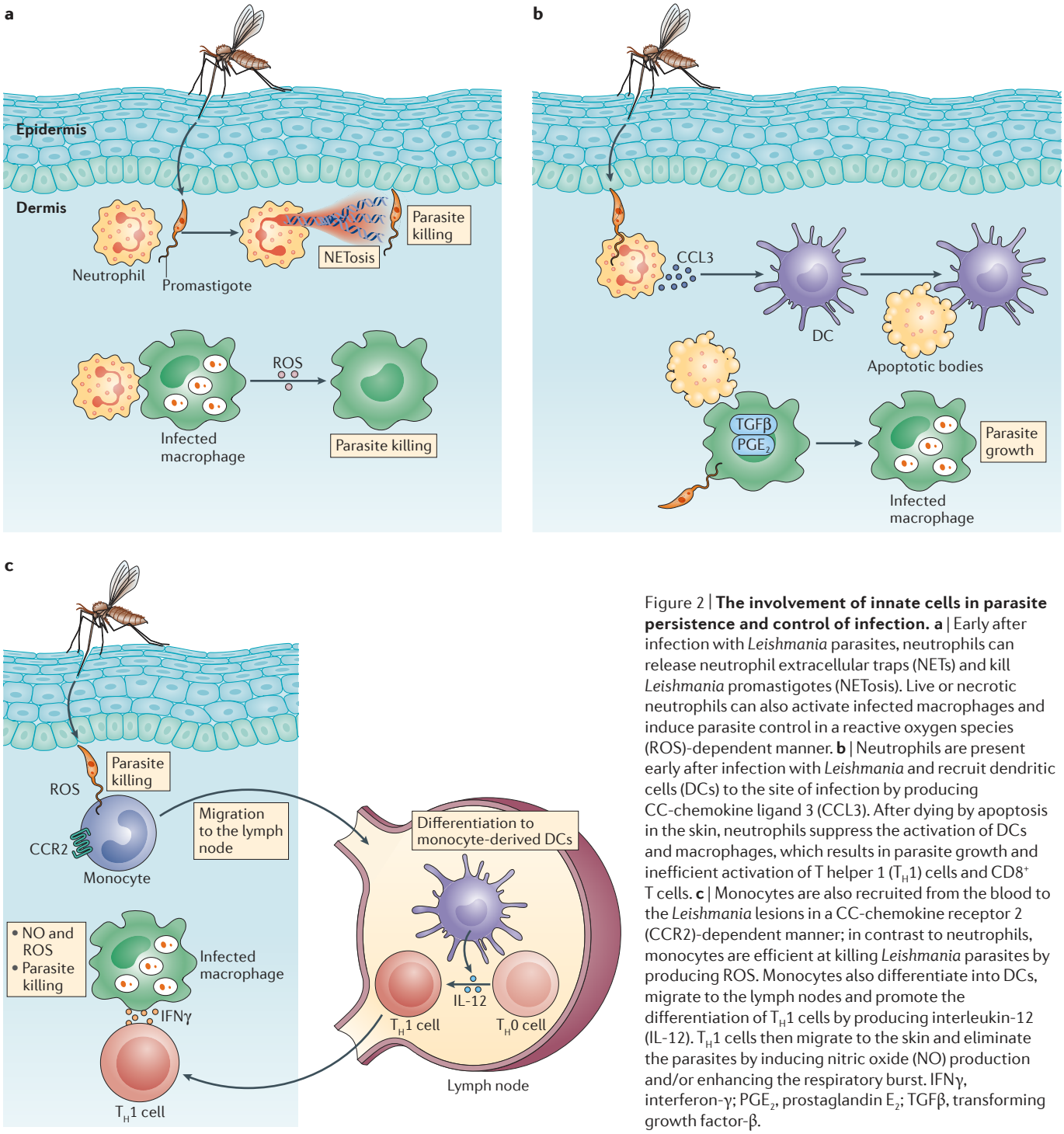

Figure 2 | **The involvement of innate cells in parasite persistence and control of infection. a** | Early after infection with *Leishmania* parasites, neutrophils can release neutrophil extracellular traps (NETs) and kill *Leishmania* promastigotes (NETosis). Live or necrotic neutrophils can also activate infected macrophages and induce parasite control in a reactive oxygen species (ROS)-dependent manner. **b** | Neutrophils are present early after infection with *Leishmania* and recruit dendritic cells (DCs) to the site of infection by producing CC-chemokine ligand 3 (CCL3). After dying by apoptosis in the skin, neutrophils suppress the activation of DCs and macrophages, which results in parasite growth and inefficient activation of T helper 1 (T_H1) cells and $CD8^+$ T cells. **c** | Monocytes are also recruited from the blood to the *Leishmania* lesions in a CC-chemokine receptor 2 (CCR2)-dependent manner; in contrast to neutrophils, monocytes are efficient at killing *Leishmania* parasites by producing ROS. Monocytes also differentiate into DCs, migrate to the lymph nodes and promote the differentiation of T_H1 cells by producing interleukin-12 (IL-12). T_H1 cells then migrate to the skin and eliminate the parasites by inducing nitric oxide (NO) production and/or enhancing the respiratory burst. IFNγ, interferon-γ; PGE_2, prostaglandin E_2; TGFβ, transforming growth factor-β.

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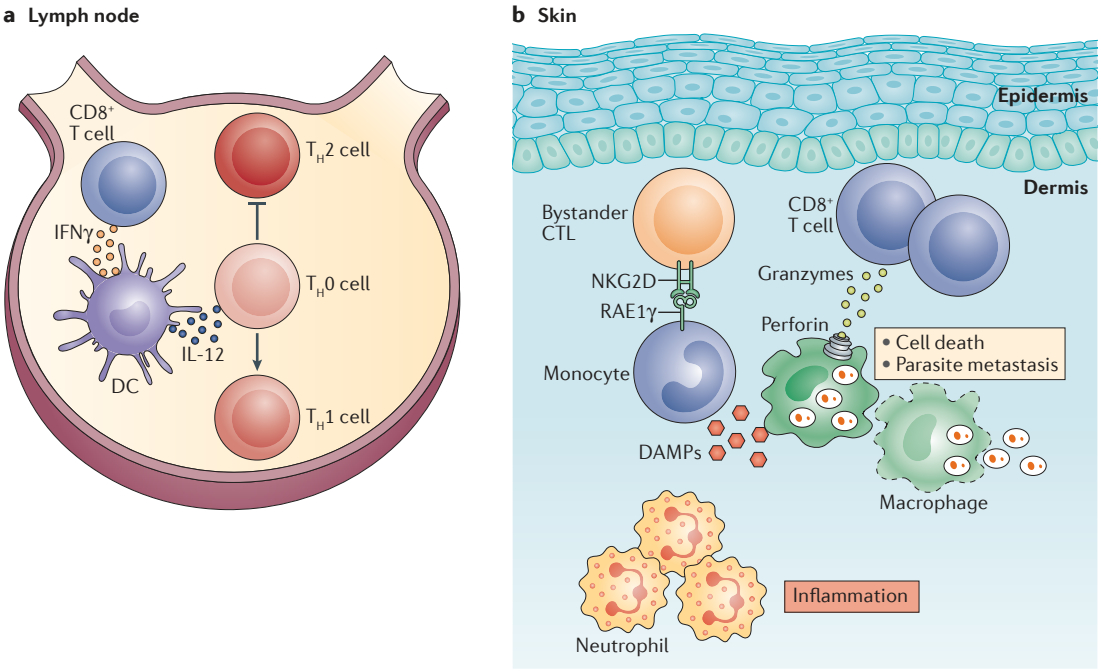

Figure 3 | **The dual role of CD8⁺ T cells in leishmaniasis. a** | During the priming of T helper 1 (T_H1) cells, CD8⁺ T cells produce interferon-γ (IFNγ) in the lymph nodes and activate dendritic cells (DCs) to produce the interleukin-12 (IL-12) necessary for T_H1 cell differentiation and T_H2 cell suppression. Not depicted are natural killer cells that can also provide the initial IFNγ production necessary for T_H1 cell differentiation. **b** | In the skin, parasite-specific and bystander cytotoxic T lymphocytes (CTLs) are present. Bystander CD8⁺ T cells recognize signals — retinoic acid early transcript 1γ (RAE1γ) in the mouse and MHC class I polypeptide-related sequence A (MICA) and MICB in humans — that are present on the surface of innate cells such as monocytes. CD8⁺ T cells induce target cell death in an natural killer group 2, member D (NKG2D)-dependent manner. CD8⁺ T cells that recognize *Leishmania* antigen promote granule-mediated cytotoxicity in the skin and induce target cell death. Dead cells release parasites and damage-associated molecular patterns (DAMPS), which leads to spread of the parasite and severe inflammation.

activated by *L. major* infection, NK cells are recruited to the paracortex where they produce IFNγ[46], which enhances the production of IL-12 by DCs. Transforming growth factor β (TGFβ) regulates the NK cell response by reducing IFNγ production[47]. Interestingly, CD8⁺ T cells can also shape the early adaptive immune response to leishmaniasis by producing IFNγ in lymph nodes, but whether CD8⁺ T cells are required for this immune response depends on the magnitude of the initial infection[48]. For example, C57BL/6 mice develop a T_H1-type response and lesions heal in the absence of CD8⁺ T cells following a high infectious *L. major* dose, whereas CD8⁺ T cells producing IFNγ are required to promote CD4⁺ T_H1 cell development after a low infectious dose[48,49] (FIG. 3a).

T cell-mediated immunity. As mentioned above, CD4⁺ T_H1 cells are essential for controlling *Leishmania*, and following infection these cells are recruited to the cutaneous lesions where they produce IFNγ to activate macrophages. Intravital imaging studies have demonstrated that CD4⁺ T cells are not evenly distributed in *Leishmania* lesions and T cells do not interact with all infected cells[50]. However, the produced IFNγ has a long-range effect, enabling NO production by infected cells that are at least 80 μm away[51]. In addition to CD4⁺ T cells, a poorly understood population of double-negative T cells — which do not express CD4 or CD8 but do express CD3 and the αβ T cell receptor — is also expanded in patients with cutaneous leishmaniasis[52]. A similar double-negative T cell population exists in *L. major*-infected mice[53]. These cells are phenotypically distinct from classical CD4⁺ T cells as they have an innate cell-like gene expression profile, but, similar to CD4⁺ T cells, they proliferate and produce IFNγ upon MHC class II antigen recognition of *Leishmania* and thereby contribute to immunity[53].

The resolution of a primary infection with *Leishmania* leads to long-lasting immunity to reinfection that is mediated primarily by CD4⁺ T cells[54]. However, a low number of parasites remain following lesion resolution due to an IL-10-mediated downregulation of the immune response[55]. These persistent parasites maintain a population of *Leishmania*-specific effector CD4⁺ T cells that can respond immediately upon re-challenge. Some of these circulating T cells have recently been characterized as short-lived CD4⁺LY6C⁺Tbet^{hi} T cells that upon re-challenge migrate to the challenge site and promote parasite killing[56]. In addition to these short-lived effector T cells, *Leishmania*-specific T cells with an effector memory T (T_{EM}) cell phenotype exist (BOX 1), but it is currently unclear whether they survive in the absence of persistent parasites[57]. However, *Leishmania* infection

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Box 1 | **CD4⁺ T cell subsets**

Effector T cells. A subset of short-lived T cells that circulate in the blood and can enter tissues. Identified as $CD44^+$, $CD62L^{low}$, interleukin-7 receptor $(IL\text{-}7R)^{+/-}$ and $LY6C^+$.

Effector memory T cells (T_{EM} cells). A subset of long-lived T cells that circulate in the blood and can enter tissues. Identified as $CD44^+$, $CD62L^{low}$ and $IL\text{-}7R^+$.

Central memory T cells (T_{CM} cells). Long-lived T cells that circulate in the blood and can enter secondary lymphoid organs (lymph nodes). Identified as $CD44^+$, $CD62L^{hi}$ and CC chemokine receptor 7 $(CCR7)^+$. Upon secondary stimulation, T_{CM} cells differentiate into effector T cells.

Tissue-resident memory T cells (T_{RM} cells). T cells that enter the tissues and remain there. Identified as $CD44^+$, $CD62L^{low}$, and probably $CD69^+$, $CCR7^-$, and in the skin, P-selectin ligand^{hi} and E-selectin ligand^{hi}.

can induce a population of long-lived central memory T (T_{CM}) cells, which have been identified in mice infected with non-persistent attenuated *L. major* parasites[58]. In contrast to effector T cells or T_{EM} cells, $CD4^+$ T_{CM} cells migrate to the DLN where they proliferate and differentiate into effector T cells, which subsequently migrate to the lesion site. Thus, T_{CM} cells provide a pool of *Leishmania*-reactive T cells that can become effector cells and protect mice upon adoptive transfer, although with delayed kinetics compared with the transfer of effector T cells[56,58].

Mice that are immune to *Leishmania* infection contain a population of circulating effector T cells and T_{CM} cells (BOX 1) that contribute to immunity, but transfer of either of these populations, individually or combined, to a naive mouse does not provide the same level of protection seen in an immune mouse[57–59]. Although this effect may be due to an insufficient number of cells transferred, the identification of tissue-resident memory T (T_{RM}) cells residing in the gut, brain, lung and skin[60], suggested that T_{RM} cells may also contribute to protection in leishmaniasis. In support of this idea, a population of $CD4^+$ T_{RM} cells have been identified at sites distant from the primary lesion in *L. major* immune mice[61]. Grafting immune skin onto naive mice revealed that T_{RM} cells are maintained for at least 4 weeks in the absence of persistent parasites, and the presence of T_{RM} cells enhances the ability of circulating effector cells to mediate protection. How these T_{RM} cells are generated in leishmaniasis, how they are maintained in the skin, how they enhance immunity, and whether they can be generated following vaccination are important questions that remain to be addressed.

Following resolution of a primary infection in mice, a population of $CD8^+$ T cells is retained that contributes to immunity following reinfection or challenge after vaccination[62–67]. These $CD8^+$ T cells have not been characterized in depth, and whether they are effector T cells that are maintained due to the presence of persistent parasites, or also include bona fide memory T cells, is not known. Although $CD8^+$ T cells may contribute to protection in experimental vaccines for cutaneous leishmaniasis[64], as discussed below, $CD8^+$ T cells also have a pathogenic role in cutaneous leishmaniasis, which suggests that they might be suboptimal targets for vaccine strategies (BOX 2).

Immune responses driving pathogenesis

Multiple pathways can contribute to disease severity following infection with *Leishmania*, and the type of immune response that develops is crucial in determining disease outcome (that is, self-healing or chronic disease) (FIG. 1). The virulence factors that contribute to the differential outcome of infection with different *Leishmania* spp. or strains are still poorly defined. However, it was recently demonstrated that a double-stranded RNA virus present in some *Leishmania* isolates might contribute to more severe disease in cutaneous leishmaniasis[68]. Although this *Leishmania* virus was first identified in the late 1980s[69], its biological importance has been only recently recognized. By comparing *Leishmania guyanensis* strains that harbour different levels of this virus, it was demonstrated that higher viral loads are associated with the induction of a pro-inflammatory response marked by increased production of CXC-chemokine ligand 10 (CXCL10), TNF, IL-6 and IFNβ[68]. The importance of these findings was recently demonstrated in a study showing that the presence of the virus in *Leishmania* isolates from infected patients could predict treatment failure, symptomatic relapse and development of mucosal leishmaniasis[70–72]. However, the presence of this RNA virus is limited to specific regions in South America; thus, this RNA virus is only one of the virulence factors that promotes severe disease, because parasite metastasis and treatment failure still occurs in areas where the RNA virus infection is not observed[73,74].

Limited control of parasite replication. Most *Leishmania*-infected BALB/c mice develop progressive lesions with increased parasite replication, with some exceptions[75–78] (TABLE 1). The severity of the lesion in these mice is partly dependent on the development of a $CD4^+$ T_H2-type response, as lesions resolve following treatment with an IL-4-specific mAb[79]. However, IL-10-deficient BALB/c mice can also resolve a *L. major* infection, suggesting that IL-10 promotes disease in susceptible mice[80]. Indeed, even in *L. major*-resistant strains, such as C57BL/6, control of *L. major* infection can take weeks, and a low number of parasites persist after the lesion resolves. This protracted parasite control and persistence following *L. major* infection is largely due to the production of IL-10, which can be produced by myeloid cells, regulatory T (T_{reg}) cells and conventional T cells[55,81,82].

High levels of IL-4 are not observed in patients with severe diffuse cutaneous leishmaniasis, suggesting that $CD4^+$ T_H2-type responses may be less important for disease progression in humans. Instead, other factors contribute to the lack of an appropriate immune response against *L. amazonensis* and *L. mexicana*, which cause diffuse cutaneous leishmaniasis. Indeed, C57BL/6 mice that normally self-heal following infection with *L. major* fail to resolve an infection with *L. mexicana* or *L. amazonensis* parasites due to a defective priming of T_H1-type responses[83]. This lack of disease resolution may be due to an enhanced IL-10 production, leading to inadequate DC activation and IL-12 production[84,85]. In addition, recent work has shown that the level of arginase I — which is essential for parasite replication — and other suppressive factors, such as prostaglandin E_2 and TGFβ,

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Box 2 | ***Leishmania* vaccines**

Although several strategies have been pursued to induce protection against *Leishmania* infection, there is currently no effective vaccine for either cutaneous or visceral leishmaniasis. The most successful way to prevent leishmaniasis is infection with live parasites; this procedure, called leishmanization[130], was used for centuries to protect against disfiguring lesions on exposed parts of the body. Although usually effective, leishmanization can be associated with loss of parasite virulence, difficulty in standardization and, most importantly, the development of non-healing lesions. In addition, because the parasites are never cleared, individuals are at risk of recurrent infections if they become immunocompromised. Hence, this approach is not used today. Nevertheless, the success of leishmanization provided support for the idea that a vaccine is possible for leishmaniasis. However, the gap from a live vaccine to more traditional vaccines has turned out to be much greater than initially thought.

Attempts to vaccinate with whole killed parasites, attenuated live parasites, parasite proteins, subunit recombinant vaccines, vectored vaccines and DNA vaccines have had limited success[131]. Despite demonstrations of safety, multiple phase III vaccine trials with killed whole parasites were unsuccessful[132]. This lack of success is partially due to the inability to generate long-term cell-mediated immunity by traditional vaccines and adjuvants. With advancements in the understanding of innate immune responses, newer adjuvants are being developed that may overcome this problem[133,134]. Another issue that has delayed the development of a leishmaniasis vaccine is the lack of an immunodominant antigen recognized by $CD4^+$ T cells. However, it was recently discovered that the *Leishmania* protein glycosomal phosphoenolpyruvate carboxykinase is an immunodominant antigen recognized by $CD4^+$ T cells and is conserved in many different *Leishmania* parasites. Importantly, immunization with this protein provided significant protection against both cutaneous and visceral leishmaniasis in animal models[135]. As *Leishmania* is transmitted by sand flies, several studies have also investigated the potential role of sand fly salivary proteins in vaccines[136]. For example, vaccination with a sand fly salivary protein induced significant protection against sand fly transmission of *Leishmania major* in rhesus macaques[137]. This result suggests that incorporating sand fly salivary proteins in a vaccine may promote better protection.

These data highlight the continued efforts being made towards developing a leishmanial vaccine. We now have an increased understanding of the memory T cells to target[56,61], new adjuvants in development[133,134] and identified an immunodominant antigen shared by many *Leishmania* spp. Together with novel approaches for vaccine design[137], including a better understanding of the role of the sand fly in challenge experiments[128] and the potential role of sand fly proteins as part of a vaccine[136], a leishmanial vaccine remains an achievable goal.

are increased in plasma and skin biopsies from patients with diffuse cutaneous leishmaniasis[86]. However, how certain *Leishmania* spp. enhance disease by dampening the immune response, whereas others do so by exacerbating immune responses, remains unclear.

The role of T_H cell responses. Although T_H1-type responses are required to control *Leishmania* infection, the T_H1 cytokines TNF and IFNγ have also been implicated in its pathogenesis. Similar to other infections, TNF has a dual role in the outcome of infection. TNF is a cofactor for macrophage activation, and TNF receptor-deficient mice are more susceptible to *L. major* infections[87,88]. However, high levels of TNF are associated with more severe disease and lesion chronicity in patients with cutaneous leishmaniasis[89]. In support of a causative role, clinical trials revealed that a combination of antiparasitic drugs and TNF inhibitors leads to better outcomes in patients[90]. Similar to TNF, high levels of IFNγ are seen in patients with more severe disease, such as in mucosal leishmaniasis[3]. However, whether IFNγ exacerbates pathology directly is not known.

Perforin
A calcium-sensitive membranolytic protein that is found in cytoplasmic granules of cytotoxic T lymphocytes and natural killer cells.

$CD4^+$ T_H17 cells protect against certain bacteria and fungi, and are major players in mediating the immunopathology associated with autoimmune diseases. BALB/c mice have high levels of IL-17 after infection with *L. major*, and IL-17 deficiency promotes better control of disease[91]. Mimicking the low levels of IL-10 observed in patients' lesions[92], blocking IL-10 signalling in mice increases IL-17 production and causes more severe disease following infection with high doses of *L. major*, which is reversed by neutralizing IL-17 (REF. 93). Similarly, IL-17 levels correlate with the inflammatory response in the skin of patients with cutaneous and mucosal leishmaniasis[94,95]. Most of the human-based work studying the pathogenesis induced by IL-17 has been performed in patients with *L. braziliensis* infection, and the role that IL-17 has in cutaneous leishmaniasis caused by other *Leishmania* spp. is unexplored.

The role of cytotoxic $CD8^+$ T cells in pathogenesis. Cytotoxicity was first associated with disease severity in patients with *L. amazonensis* infection in the late 1990s. Studies showed that peripheral blood cells from patients with mucosal leishmaniasis exhibited higher cytolytic capacity than those from healthy controls and patients with cutaneous leishmaniasis[96]. In *L. braziliensis*-infected patients, as disease progresses from early (non-ulcerated) lesions to late (ulcerated) lesions the ratio between $CD4^+$ and $CD8^+$ T cells changes, and more $CD8^+$ T cells are found in patients with ulcerated lesions[97]. In contrast to $CD4^+$ T cells that express IFNγ, $CD8^+$ T cells in lesions have a cytotoxic profile marked by granzyme expression[92,98,99]. Genome-wide transcriptional profiling of lesions from *L. braziliensis*-infected patients has confirmed that cytotoxicity is a major signature of *L. braziliensis* lesions[100]. In addition, the expression of genes associated with cytolytic function and genes involved in skin barrier function were negatively correlated, suggesting that cytotoxicity and loss of skin integrity occur together in *L. braziliensis* disease in humans[100].

The observation that $CD8^+$ T cells in the skin correlate with disease severity in patients was unexpected because $CD8^+$ T cells can promote resistance in mice[49]. However, *Leishmania* infection of recombination-activating gene (*Rag*)-deficient mice that have been reconstituted with $CD8^+$ T cells leads to both severe non-healing primary and metastatic lesions, which are unrelated to the parasite burden[49,101]. This increased pathology is due to the cytolytic activity of the $CD8^+$ T cells in the skin, because $CD8^+$ T cells lacking perforin are not pathogenic in this model[101]. The cytolytic activity of $CD8^+$ T cells during *Leishmania* infection has also been visualized by spinning disc confocal microscopy[101] (see Supplementary information S1 (movie)). These findings show that cytolytic $CD8^+$ T cells are pathogenic when a large number is recruited to *Leishmania* lesions. Furthermore, previous infections with pathogens known to induce a large $CD8^+$ T cell response (such as lymphocytic choriomeningitis virus or *Listeria monocytogenes*) were associated with increased lesion development following subsequent challenge

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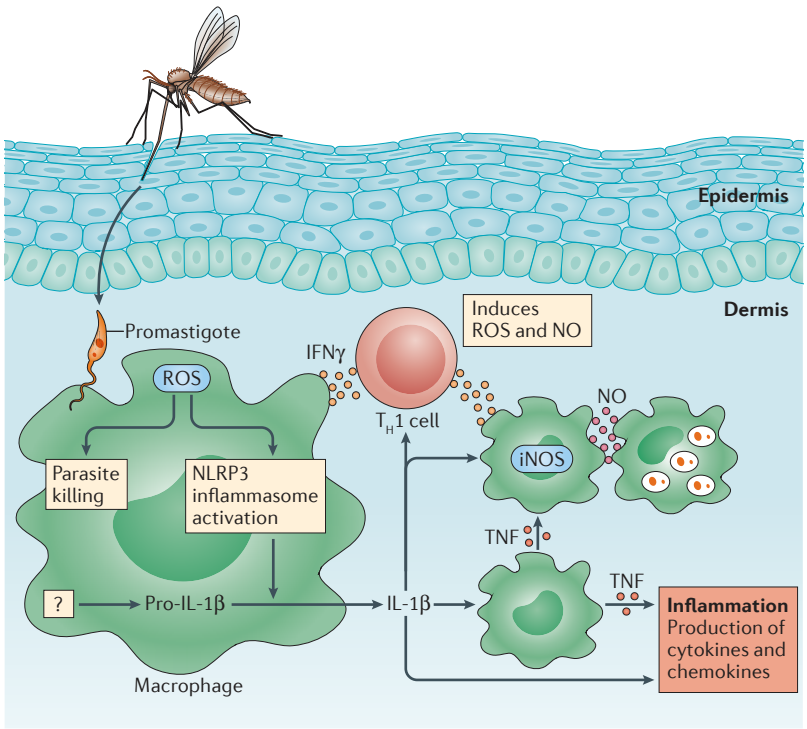

Figure 4 | **IL-1β and TNF can be protective or pathogenic in cutaneous leishmaniasis.** Phagocytosis of *Leishmania* parasites by innate cells leads to the production of reactive oxygen species (ROS). ROS can induce parasite elimination as well as activate the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome. The factor (or factors) inducing pro-interleukin-1β (IL-1β) production in the skin is currently unknown. Nevertheless, pro-IL-1β is processed by the inflammasome in the skin and its mature form can function in several ways during *Leishmania* infection. IL-1β is important in T helper 1 (T_H1) cell expansion by promoting IL-12 production. Also, IL-1β induces nitric oxide (NO) activation either directly, by activating macrophages, or indirectly, by promoting T_H1-type responses and interferon-γ (IFNγ) production. IL-1β can also induce tumour necrosis factor (TNF), which can be protective by synergizing with IFNγ and thus increasing inducible NO synthase (iNOS) production in innate cells. NO can promote parasite control in the iNOS-expressing cell, but it can also diffuse through tissue and act on neighbouring cells. In contrast to their protective roles, IL-1β and TNF can also enhance the production of several chemokines and cytokines and promote the expression of adhesion molecules, leading to the amplified recruitment of cells from the blood. This enhanced inflammation results in tissue destruction and disease severity.

with *L. major*[102]. Notably, bystander CD8⁺ T cells that express the NKG2D-activating receptor lysed NKG2D ligand-expressing cells in the lesions. In this model, inflammation is dependent on CD8⁺ T cells inducing cell death in an NKG2D-dependent manner. Consistent with the ability of bystander CD8⁺ T cells to contribute to the immune response within *Leishmania* lesions, *Toxoplasma*-specific CD8⁺ T cells have been identified in lesions of *L. braziliensis*-infected patients[103]. As humans have been exposed to a variety of pathogens that might leave an expanded pool of memory CD8⁺ T cells, these results uncover an additional factor that may influence the development of immunopathology in human cutaneous leishmaniasis.

How CD8⁺ T cells can have both protective and pathological roles is currently unclear. It seems most likely that this dual role depends on whether the CD8⁺ T cells are cytolytic or produce IFNγ, and further study is needed to determine why CD8⁺ T cells appear to be preferentially cytotoxic in the skin during leishmaniasis (FIG. 3a,b). Furthermore, mouse cytotoxic T cells do not seem to kill *Leishmania*, which could be due to the absence of granulysin in mouse CD8⁺ T cells[104]. Finally, although it is also unclear how cytolysis drives pathology, transcriptional analysis of lesions suggests that it may be due to activation of the inflammasome by dead cells, which leads to the production of pro-inflammatory IL-1β[100].

Inflammasome activation and IL-1β. Similar to TNF, IL-1β can lead to protective or pathogenic effects during *Leishmania* infection (FIG. 4). On the one hand, short-term treatment with IL-1β at the beginning of *L. major* infection in C57BL/6 mice provides protection[105], and the absence of IL-1β in *L. amazonensis*-infected mice leads to exacerbated disease[106]. On the other hand, continuous IL-1β treatment of *L. major*-infected mice leads to more severe disease[105]. IL-1β also exacerbates lesions in *L. major*-infected BALB/c mice[107,108], and can promote pathology in C57BL/6 mice by inducing the development of T_H17 cells[93]. Furthermore, IL-1β was recently shown to be responsible for the disease severity in C57BL/6 mice after infection with the non-healing *L. major* Seidman strain[25]. Only a few studies have investigated the role of IL-1 in *Leishmania*-infected patients and these studies have indicated that IL-1 also contributes to disease in humans. For example, during *L. mexicana* infection, IL-1β expression correlates with disease severity[109], and *IL1B* mRNA levels positively correlate with the expression of cytolytic genes associated with pathology in *L. braziliensis*-infected patients[100]. IL-1 can also enhance inflammation by promoting TNF production[110]. Hence, at the T cell-priming phase of infection, IL-1β may enhance the differentiation of protective CD4⁺ T cells, whereas excessive production of IL-1β during the chronic phase of infection is detrimental to the host.

IL-1β activation is primarily accomplished by caspase 1-mediated cleavage following inflammasome activation. In addition to IL-1β, the inflammasome pathway is a transcriptional signature of *L. braziliensis* infection in humans[100]. In mice, the inflammasome has been implicated in either protection or pathogenesis of leishmaniasis depending on the mouse model and the parasite species used. On the one hand, IL-1β processing by the inflammasome appears to promote NO production in *L. amazonensis*-infected mice, although not sufficiently for the mice to heal[106]. On the other hand, pathology induced by infection with the non-healing *L. major* Seidman strain is dependent on the inflammasome, as mice deficient in inflammasome components display increased control of the infection[25]. IL-18 is also processed by the inflammasome, and it can also be either protective or pathogenic in *L. major* infection depending on the mouse genetic background. In C57BL/6 mice, IL-18 can synergize with IL-12 and promote T_H1-type responses, whereas in BALB/c mice IL-18 enhances T_H2 cell development by inducing the production of

NKG2D
(Natural killer group 2, member D). A protein expressed on the surface of activated natural killer and CD8⁺ T cells that binds to self-ligands that are induced following stress, development of malignancy and infection. Interactions between NKG2D and its ligands can induce lysis of the NKG2D ligand-expressing cell.

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IL-4 (REFS 111–113). In *L. major*-infected BALB/c mice, inflammasome deficiency reduces lesion sizes due to a defect in IL-18 production[111]. These results raise the question of how parasites contribute to inflammasome activation. Studies published to date indicate that *Leishmania* may not activate the inflammasome directly and in certain cases may even inhibit the inflammasome[114]. *Leishmania* parasites are poor inducers of IL-1β alone, but do promote IL-1β when macrophages are also stimulated with lipopolysaccharide[25,106]. The induction of ROS after parasite phagocytosis through C-type lectin receptors may indirectly induce inflammasome activation in both mice and human macrophages[115]. Hence, although inflammasome activation and maturation of IL-1β certainly plays a part in *Leishmania* infection, how the inflammasome is activated in cutaneous leishmaniasis is less clear.

Regulation of the immune response

The role of T_{reg} cells and IL-10. T_{reg} cells have been observed in lesions from *Leishmania*-infected patients, and these purified T_{reg} cells can be suppressive *in vitro*[116,117]. However, some studies have found that T_{reg} cell function is impaired in chronic cutaneous leishmaniasis caused by *Leishmania panamensis* or *L. braziliensis*[118]. Although there is still much to be learned regarding the role of T_{reg} cells in humans, their role in mice has been explored in several studies. T_{reg} cells from lesions of *L. major*-infected C57BL/6 mice respond to *L. major* antigen and accumulate rapidly in a CCR5-dependent manner at the site of infection and suppress $CD4^+$ T cell activity, which favours parasite persistence[55,119,120]. Moreover, depletion of T_{reg} cells results in sterile immunity; consequently, mice lose their normal resistance to reinfection with *L. major*[55]. T_{reg} cells are important both during primary infection with *L. major* and in secondary infections, because induction of T_{reg} cells can render otherwise immune mice susceptible to infection[121] or reactivate a secondary infection[122]. However, a different role for T_{reg} cells is seen following infection with New World species of *Leishmania*. For example, transfer of T_{reg} cells from an infected mouse to a naive mouse immediately before infection with *L. amazonensis* reduces lesion development[123], which suggests that T_{reg} cells control immunopathological responses. In addition, T_{reg} cells inhibit disease progression in *L. panamensis* infections by downregulating pathological responses and by reducing the parasite load[124]. These findings demonstrate the difficulty of making generalized statements about the role of T_{reg} cells in regulating cutaneous leishmaniasis, as the immune response is probably influenced by both the parasite species and host genetics.

Although T_{reg} cells function in both an IL-10-dependent and -independent manner[55], most studies have focused on IL-10 because *L. major*-infected *Il10*$^{-/-}$ mice can control parasite replication[80,122]. Other important sources of IL-10 in mice are conventional T_H1 cells[81] and macrophages exposed to IgG-coated *L. major* amastigotes[80]. In *L. braziliensis*-infected patients, IL-10 can be produced by both T_{reg} cells and other cells such as circulating monocytes[125,126]. Regardless of the source, all of the studies indicate that IL-10 is an important regulator of immunity in leishmaniasis.

Conclusion and future perspectives

Here, we have summarized recent advances in our understanding of the immune response to cutaneous leishmaniasis and, when possible, integrated our knowledge from mouse models to human disease. We have learned a great deal about the immune system from studies using mouse models of cutaneous leishmaniasis; however, those advances have yet to substantively change treatment for this disease or lead to an effective vaccine. New therapies for cutaneous leishmaniasis are urgently needed because most of the drugs currently used to treat patients are either toxic or expensive, and may require several rounds of treatment. Moreover, the treatments have high failure rates, possibly because they only target the parasite, which may not alleviate the immunopathological responses that drive disease in many forms of cutaneous leishmaniasis. Thus, in addition to developing new drugs to target the parasite, research efforts should focus on testing immunotherapies that could reduce the severity of pathology seen in cutaneous leishmaniasis. Several of the drugs being developed for other chronic inflammatory diseases, such as those that inhibit TNF, IL-1 or cytotoxicity, might be useful for such therapy and could be used in combination with antiparasitic drugs. Furthermore, recent work has demonstrated the influence of the skin microbiome in the pathology induced by *Leishmania*[127] and, although a full understanding of how skin commensals alter disease is in its infancy, there is strong evidence to indicate that the microbiota present in the skin affects several diseases, one of which is likely to be cutaneous leishmaniasis. As our knowledge grows in this area, it is possible that we will be able to incorporate that information into new treatments.

A long-term goal for *Leishmania* research is to develop an effective vaccine, which has so far been unsuccessful (BOX 2). The development of mouse models that better mimic the wide spectrum of human cutaneous leishmaniasis is important, as well as the development of sand fly challenge models that better mimic natural infection[128]. Currently, the best immune protection requires persistent parasites, which is a clear hurdle for vaccine development. Furthermore, whether we need a vaccine that provides complete protection is an important decision because it is currently unlikely that any vaccine will provide sterile immunity. Thus, the main goal of vaccine development in leishmaniasis might be to reduce the time of healing and avoid the most severe clinical forms of the disease. Important discoveries of protective antigens in leishmaniasis and the development of newer and better adjuvants will continue, but the key issue that remains is whether vaccination can induce long-term memory in leishmaniasis. Advances in our understanding of memory T cells in general[129], and the discovery that T_{CM} and T_{RM} cells can provide longer term protection in leishmaniasis[58,61], might form the basis for future vaccine strategies in which longer-lived T cells are targeted that can lessen the development and severity of cutaneous leishmaniasis.

Inflammasome
An innate immune sensor that recognizes pathogens and self molecules released during tissue damage. It is a molecular complex of several proteins; once assembled, the inflammasome processes pro-interleukin-1 (pro-IL-1) and pro-IL-18 to their active forms.

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