Chapter

Protective and Pathogenic Immune Responses to Cutaneous Leishmaniasis

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Abstract

Leishmania (Kinetoplastida: Trypanosomatidae) parasites are known to cause a broad spectrum of clinical diseases in humans, collectively known as the leishmaniases. Cutaneous leishmaniasis is the most common clinical presentation with varying degrees of severity largely driven by host immune responses, specifically the interplay between innate and adaptive immune response. The establishment of a T lymphocyte driven cell-mediated immune response, leading to activated phagocytic cells, leading to *Leishmania* parasite killing and control of infection. Alternatively, the *Leishmania* parasite manipulates the host immune system, enabling parasite proliferation and clinical disease. Here we review how the cumulative interactions of different aspects of the host immune response determines disease outcome, severity, and immunity to re-infection.

Keywords: *Leishmania*, innate immunity, adaptive immunity, cytokine, T-cell response, immunopathology

1. Introduction

The leishmaniases are a diverse group of vector-borne diseases resulting from infection with parasites of the genus *Leishmania* (*L.*) (Kinetoplastida: Trypanosomatidae). More than 20 species of *Leishmania* parasites are considered public health threats with the *Leishmania* (*Leishmania*) and *Leishmania* (*Viannia*) subgenera encompassing the medically important human pathogenic *Leishmania* parasites (reviewed in [1]). Leishmaniasis is acquired through the bite of an infected phlebotomine sandfly, with the genera *Phlebotomus* (Old World; OW) and *Lutzomyia* (New World; NW) responsible for human transmission. The *Leishmania* life-cycle (**Figure 1**) is complex as the parasites alternate between a flagellated promastigote form within the insect vector (reviewed in [2]) and an intracellular amastigote form that resides within phagolysosomes of mammalian phagocytic cells (reviewed in [3]). Clinical manifestations of infection with *L.* (*Leishmania*) and *L.* (*Viannia*) species vary from spontaneous self-healing localized lesions (cutaneous leishmaniasis; CL) to life-threatening systemic multi-organ disease (visceral leishmaniasis; VL, also known as kala-azar). Nearly all *Leishmania* parasites can cause CL of varying severity ranging from sub-clinical (also referred to as asymptomatic;

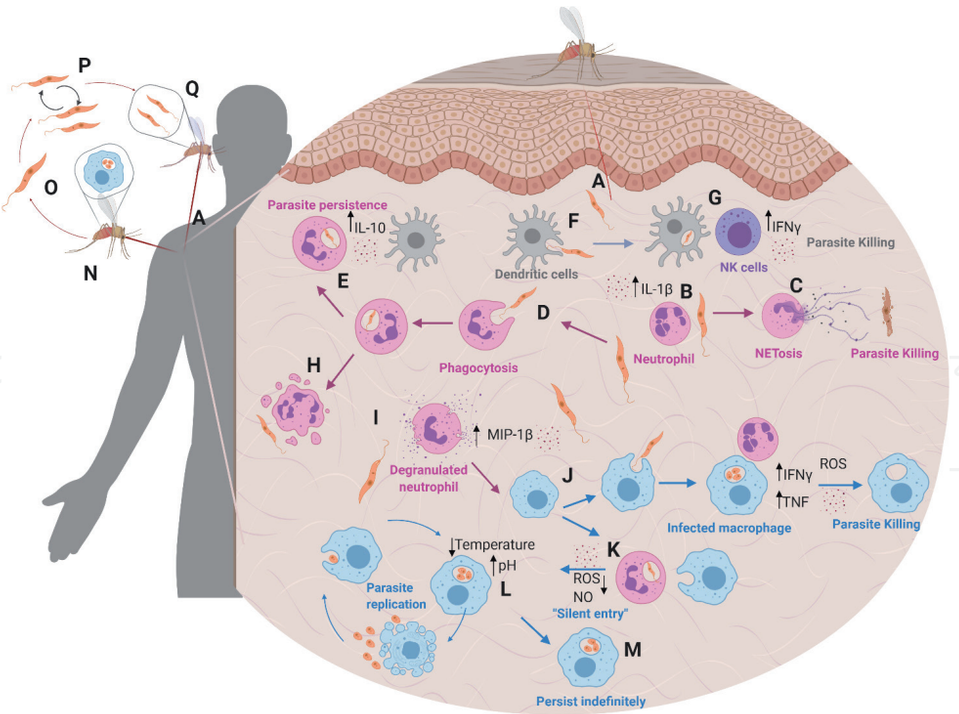

Figure 1.
The development of Leishmania parasites and their interaction with cells of the immune system. (A) During blood feeding, promastigotes are injected into the skin. (B) Neutrophils are the first phagocytic cells to arrive at the site of inoculation and play several roles. They arrive rapidly and release interleukin-1β (IL-1β), which is triggered by sandfly gut microbiota and promotes phagocytosis. (C) Neutrophils release neutrophil extracellular traps (NETs) and kill promastigotes through NETosis. (D) Neutrophils phagocytose promastigotes and (E) infected neutrophils interact with dendritic cells (DCs) inducing IL-10 which favors parasite survival. (F) DCs also phagocytose promastigotes and (G) interact with natural killer cells, resulting in the production of IFNγ. (H) Leishmania can escape apoptotic neutrophils. (I) Neutrophils degranulate and release mediators, such as macrophage inflammatory protein (MIP-1β), which recruits monocytes and macrophages. (J) Macrophages phagocytose promastigotes and neutrophils can then activate infected macrophages to induce intracellular parasite killing by releasing reactive oxygen species (ROS). (K) Apoptotic infected-neutrophils are engulfed by macrophages providing a silent entry for the parasite by downregulating ROS and nitric oxide (NO). (L) Within the macrophage, the promastigotes undergo significant biochemical and metabolic changes by transforming into their intracellular amastigote form to proliferate and infect more cells and/or (M) persist indefinitely. The life cycle is continued when (N) a female phlebotomine sandfly ingests a blood meal containing Leishmania infected phagocytes. (O) Within the vector, the amastigotes develop into the promastigote stage, (P) replicate and undergo further development (not shown here) (Q) concluding in a migration to the stomodeal valve to enable transmission to a mammalian host. Created with BioRender.com

reviewed in [4]) and self-resolving lesions to persistent chronic infections that result in severe tissue destruction and disfigurement (**Table 1**) [1].

The interaction between the parasite and the host immune response is complex and varied leading to a range of possible different disease outcomes. While the species of *Leishmania* parasite plays a large role in determining disease manifestations, host immunity and genetics largely influence the severity of infection. The classic T helper 1/T helper 2 (T_H1/T_H2) model has been applied for many years to explain the disease severity and outcome, with $CD4^+$ T_H1 cells mediating resistance to *Leishmania* and $CD4^+$ T_H2 cells promoting host susceptibility [12]. However, this assumption is based primarily on an experimental *Leishmania* (*L.*) *major* model of infection in congenic mouse strains, which are not entirely relevant to human infections. The model fails to explain the different immune responses and clinical presentations observed in the range of CL phenotypes caused by the various *Leishmania* species. Similar to the immunological spectrum observed in humans, the combination of mouse strain (reviewed in [13]), mode of challenge [14], infectious dose [15] and infecting parasite species or strain (reviewed in [16]), influences clinical presentation. With a focus on innate and adaptive immunity and subsequent immunopathology, here we describe

Clinical form	*Leishmania* parasite	Clinical manifestations in humans	References
Old world cutaneous leishmaniasis			
LCL	**Subgenus** *Leishmania*	Characterized by a single localized skin lesion that develops over a period of weeks to months at the site of the phlebotomine sandfly bite. Erythema first appears before developing into a papule. This further advances into a nodule, which progressively becomes ulcerated with a well-demarcated, raised border. Depending on the infective parasite, LCL may present in various forms (see below). Following resolution of disease, permanent scarring is common	[1, 5–9]
	Leishmania aethiopica	Rather than having a classic ulcer, patients present with crusty lesions with a patchy distribution, local oedema, and color changes often persisting for several years	
	Leishmania major	Multiple ulcero-crusted nodules and wet sores; necrosis and severe inflammation	
	Leishmania infantum *Leishmania donovani*	Manifests as papules and nodules with minimal ulceration that recovers slowly. More commonly causes systemic infection	
	Leishmania tropica	Dry ulcerating lesions, frequently presenting in multiple sites which may persist for several years	
MCL	*Leishmania aethiopica*	Mucosal lesions present simultaneously with lesions on the skin; primarily on the skin with spread to mucosa afterwards	
DCL	*Leishmania aethiopica*	Chronic and progressive condition affecting large areas of the skin with multiple nodules across the skin that often lack ulceration. Parasites grow uncontrollably in lesions and lesion growth can persist for decades	
New world cutaneous leishmaniasis, collectively grouped as American tegumentary leishmaniasis (ATL)			
LCL	**Subgenus** *Viannia* *Leishmania braziliensis* *Leishmania guyanensis* *Leishmania panamensis* **Subgenus** *Leishmania* *Leishmania mexicana* *Leishmania amazonensis*	Presents with severe, ulcerating lesions that may later manifest as MCL (see below). Characterized by single or multiple ulcerated lesions with elevated borders. The self-healing time of lesions can range from a few months (*L. mexicana*) to several years (e.g., *L. braziliensis*).	[1, 10]
MCL	*Leishmania braziliensis* *Leishmania guyanensis* *Leishmania panamensis*	Healed LCL can progress to destruction of the mucosa affecting predominately the nasopharyngeal mucosa (90% have had a previous history of CL). Characterized by the destruction of tissues of the nasal septum, lips, and palate. The excessive immune response seen with MCL has been attributed to the presence of *Leishmania* double-stranded RNA (dsRNA) virus (LVR), which is unique to the NW *L.* (*Viannia*) subgenus [11]	
DCL	**Subgenus** *Leishmania* *Leishmania amazonensis* *Leishmania mexicana*	Multiple non-healing cutaneous lesions, erythematous nodules and papules with various types of eruptions. DCL manifests as multiple widespread papules and non-ulcerating nodules with large numbers of viable parasites	
DsCL	*Leishmania braziliensis*	Characterized by multiple pleomorphic lesions in two or more non-contiguous areas of the body. Lymphatic spread is common for *L. braziliensis* DsCL is characterized by various lesions located on the body with few detectable parasites	

**Abbreviations: NW, New World; OW, Old World; LCL, localized cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; DsCL, disseminated cutaneous leishmaniasis.*

Table 1.
Clinical manifestations of cutaneous leishmaniasis caused by medically important Old World and New World Leishmania parasites.[*]

the key immune responses induced by cutaneous *Leishmania* infection. We further discuss the coordination between innate and adaptive immune responses in parasite control and how persistent parasites play an important role in protective immunity.

2. The innate immune system in *Leishmania* infection and disease

The innate immune response is the host's first line of defense against invading pathogens and consists of physical (e.g., skin), chemical (e.g., nitric oxide and reactive oxygen species), soluble factors (e.g., complement, chemokines and cytokines) and cellular defenses (e.g., neutrophils and macrophages), all of which play a vital role in determining the course of infection.

2.1 Complement activation

Inoculated *Leishmania* promastigotes rapidly interact with the host's complement system. All three complement pathways (alternative, classical and lectin) are involved to varying degrees in *Leishmania* parasite killing and result in the activation of complement (C) protein C3 convertase cleaving C3 to generate C3b (**Figure 2**; reviewed in [17]). C3b facilitates the deposition of the C5b-C9 membrane attack complex (MAC) onto the surface of culture-derived stationary phase *Leishmania* promastigotes (a stage predominately found in the sandfly midgut), resulting in lysis of the parasite and subsequent uptake by phagocytic cells [17, 18]. C3b also acts as an opsonin, promoting direct phagocytosis and destruction by immune cells. *In vitro* experiments demonstrated killing of up to 90% of culture-derived procyclics *Leishmania* promastigotes (including *L. donovani*, *L. amazonensis*, *L. infantum* and *L. major* species) via complement-mediated lysis within the first few minutes of serum contact [19]. The remaining resistant parasites used the surface bound C3b to enter immune cells and cause infection. Contrary to culture-derived procyclics promastigotes, metacyclic promastigotes (the infective stage that is deposited into the skin by blood-feeding phlebotomine sandflies) are able to subvert phagocytosis to promote their survival and mediate host pathogenesis [17–19]. The glycocalyx component, known as lipophosphoglycan (LPG), and metalloproteinase glycoprotein 63 (GP63), is distinct to the surface of the infective metacyclic promastigotes, preventing the formation of MAC and complement lysis by cleaving the C3b into an inactive form of C3b (iC3b) [18, 20, 21], thereby subverting the complement system. The MAC can also be physically inhibited by elongated LPG on the surface of metacyclic promastigotes [17]. Moreover, iC3b serves as an opsonin that facilitates the parasite's uptake by binding to complement receptor 1 (CR1) and CR3 on macrophages and neutrophils. Binding via CR3 inhibits the production of interleukin 12 (IL-12) and oxidative burst, which provides safe parasite entry into macrophages [22].

2.2 Pattern recognition receptors on innate immune cells

Pathogen recognition receptors (PRRs) expressed on innate immune cells are critical for recognizing invading pathogens via pathogen-associated molecular patterns (PAMPs) and initiating the host immune response (**Figure 3**). Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are the most studied PRRs in leishmaniasis and play a dual role in promoting protection or resistance depending on the infecting *Leishmania* species, which receptor the parasite interacts with first and the model used [23–25].

Both TLR2 and TLR4 (extracellular receptors) are found on the surface of host macrophages and neutrophils and recognize *Leishmania* promastigote LPG and GP63

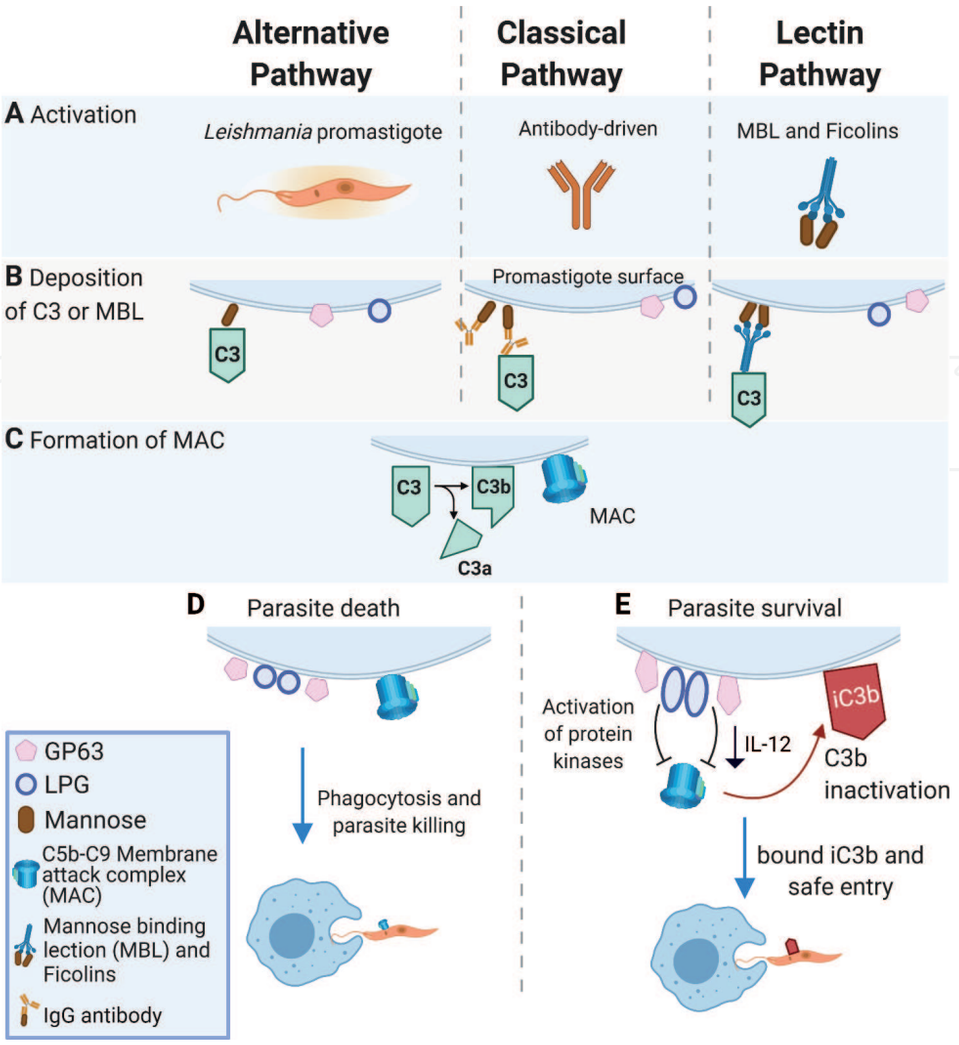

Figure 2.
Activation of complement by Leishmania parasites. (A) All three complement pathways are activated by the Leishmania parasite. (B) The alternative pathway is activated directly by the Leishmania parasite and is considered to be the main complement pathway involved in Leishmania clearance. The classical pathway is antibody-driven, while the lectin pathway is activated by the binding of mannose-binding lectin and ficolin on the parasite [16]. (C) Following activation of all pathways, the complement protein C3 convertase cleaves C3 to generate C3b. C3b facilitates the deposition of the C5b-C9 membrane attack complex (MAC) onto the surface of the Leishmania parasite, (D) ultimately resulting in uptake by neutrophils and macrophages following lysis of the parasite. (E) However, the lipophosphoglycan (LPG) metalloproteinase glycoprotein (GP63) on the parasite's surface inhibits MAC formation through its virulence factor, such as activating protein kinase and inducing interleukin-12 (IL-12) [16]. LPG and GP63 resist complement lysis by cleaving the C3b into inactive C3b (iC3b) to inhibit MAC convertase leading to safe entry into host cells and protection from complement-mediated attack. Created with Biorender.

[23–25] and *Leishmania* amastigote LPG (*L. major* specific) and proteophosphoglycan (PPG), which are expressed on the amastigote and promastigote surface [26, 27].

TLRs are activated and use the adaptor protein myeloid differentiation primary response 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF) for signal transduction. The MyD88 adapter was shown to be required for the clearance of *L. major* infection in C57BL/6 mice, with MyD88-null C57BL/6 mice showing a greater susceptibility to infection than WT mice [23]. Furthermore knocking out TLR2 in C57BL/6 mice [$TLR2^{-/-}$] resulted in mice displaying higher resistance to *Leishmania* (*V.*) *braziliensis* infection compared to WT and this resistance was associated with increased enhanced IFN-γ production [24]. Similarly, C57BL/6 $TLR2^{-/-}$ mice infected with *Leishmania* (*L.*) *amazonensis* showed a reduced parasite burden compared to infected WT C57BL/6 mice [25]. It has been proposed that LPG on the surface of *Leishmania* promastigotes may explain why TLRs promote

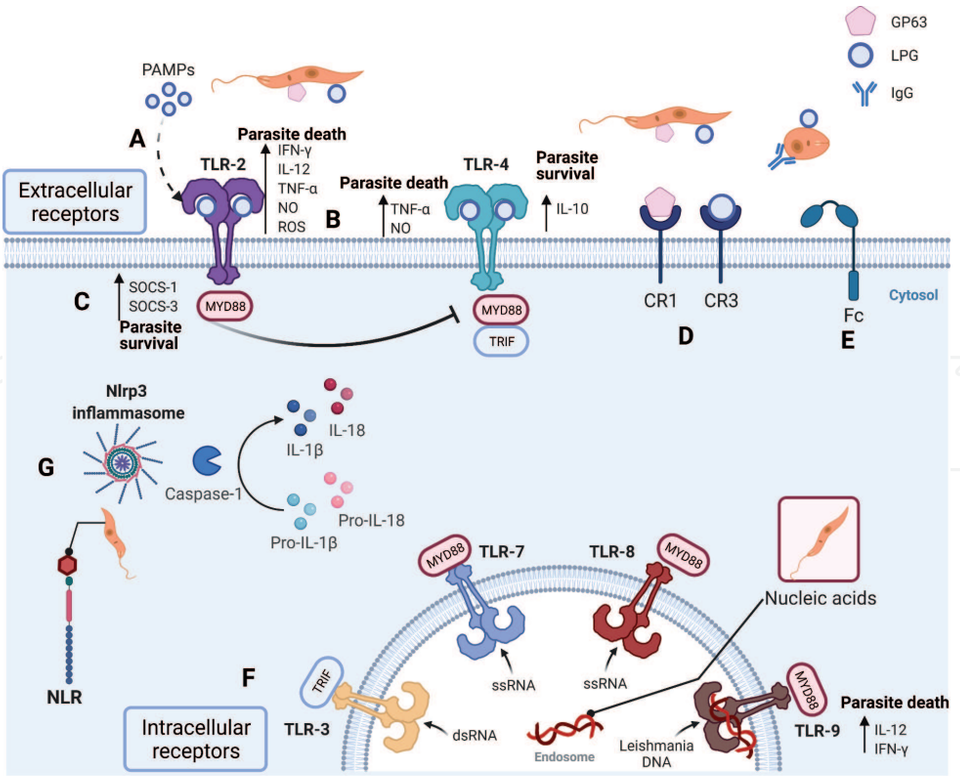

Figure 3.
Macrophage recognition of Leishmania parasites. Toll-like receptors (TLR) are categorized as extracellular receptors (TLR2 and TLR 4) and intracellular receptors (TLR3, TLR7, TLR8 and TLR9). TLRs are activated and use the adaptor proteins (myeloid differentiation primary response 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF)) for signal transduction, which is important for Leishmania clearance. TLR2, TLR4, TLR7, TLR8 and TLR9 use MyD88, TLR3 uses TRIF and TLR4 uses both MyD88 and TRIF. (A) On the macrophage surface, TLR2 and TLR4 recognize lipophosphoglycan (LPG) molecules found on the surface of Leishmania promastigotes and amastigotes (L. major). (B) Upon recognition of Leishmania, macrophages release cytokines and nitric oxide (NO) that promote either parasite death or survival. (C) TLR2 activation by LPG can also induce the release of suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3, which inhibits TLR4 signaling. (D) Complement receptors 1 (CR1) and CR3 are also categorized as extracellular receptors and can recognize LPG and metalloproteinase glycoprotein 63 (GP63) both expressed on the promastigote surface. (E) Fc receptors, located on the extracellular surface of macrophages, can also recognize immunoglobulin G (IgG) on the surface of amastigotes. (F) Intracellular TLRs recognize Leishmania RNA (TLR3, TLR7 and TLR8) and DNA (TLR 9). In the cytoplasm, (G) the NLRP3 inflammasome activates caspase-1, which cleaves pro-interleukin-1β (IL-1β) and pro-IL-18 to generate mature IL-1β and IL-18. Created with Biorender.com.

both protection and resistance, as the density and diversity of surface polysaccharide extensions to the LPG molecules varies between *Leishmania* species and between their morphological stages [24]. Similarly, TLR4 has a dual role that depends on the time of stimulation [28]. When TLR4 on mouse macrophages is primed *in vitro* with interferon-γ (IFNγ) prior to *L. major* infection, host protective TNF-α and NO are induced, promoting parasite killing. However, when IFNγ is added at the time of infection without sufficient priming time, macrophages increase IL-10 production, favoring parasite persistence [28, 29]. Interestingly, *ex vivo* studies using human monocytes from CL patients revealed that infection with *L. braziliensis* up-regulated TLR2 and TLR4 expression on inflammatory monocytes subsets [30, 31]. Moreover, a correlation with detrimental outcomes of CL was linked to the TLR up-regulation and production of TNF-α and IL-10 in infected monocytes [31]. These results using monocytes from human CL patients infected with *L. braziliensis* suggest that TLR2 and TLR4 expression triggers an inflammatory response and pathology.

TLR3, TLR7 and TLR9 are intracellular receptors recognizing *Leishmania* parasites in the endosomes of macrophages and are activated by *Leishmania* nucleic acids [17]. TLR9 is the most studied intracellular receptor and is associated with disease

outcome having an important role in the early events of lesion development and parasite burden. A direct correlation was seen between TLR9 expression and lesion size in mice infected with *L. braziliensis* [32, 33]. Similarly *ex vivo* human monocytes from CL patients presenting with larger lesion size, were found to express higher levels of TLR9 [33]. Little is still known about the role of TLR3 in CL. TLR3 promotes immune protection against *L.* (*Leishmania*) *donovani* (visceral *Leishmania* species) through the production of TNF-α and NO [34]. Recent studies identified TLR7 as having an essential role in early *L. major* infection control by neutrophils. In $TLR7^{-}/^{-}$ C57BL/6 mice infection with *L. major* leads to long-term exacerbation of CL [35].

In contrast to TLRs, NLRs are cytoplasmic pattern recognition receptors. The NLRP3 inflammasome is a major regulator of IL-1β and IL-18 in *Leishmania* infection [36]. Similar to TLRs, the involvement and role of NLRs is dependent on the infecting *Leishmania* species. In murine models, activation of the inflammasome and IL-1β production have been shown to be associated with a protective role in parasite control during infection with *L. amazonensis* and *L. braziliensis* [37–39]. In contrast, they have no involvement in resistance to *L. major* infection. Moreover, the NLRP3 inflammasome promotes the development of TH2 cells resulting in non-healing lesions during *L. major* infection in BALB/c mice [40].

2.3 Innate cellular immunity

The recruitment and activation of innate immune cells are critical for the killing of invading pathogens by phagocytosis. However, these cells can also facilitate the survival of *Leishmania* parasites (**Figure 1**). *Leishmania* has evolved mechanisms to subvert host killing by modulating the response of specific immune cells. Macrophages and monocytes are the primary host cell for *Leishmania* parasites; however, a variety of immune cells are recruited to the inoculation site and play critical roles in determining the course of infection and disease outcome.

2.3.1 Neutrophils

Neutrophils are the first phagocytic cells to arrive at the site of the phlebotomine sandfly bite [41]. These cells are capable of clearing *Leishmania* parasites early in infection through phagocytosis and via the production of an array of microbicidal factors that target *Leishmania* parasites (recently reviewed in [42]). Neutrophils release neutrophil extracellular traps (NETs) to capture and kill *Leishmania* promastigotes through a cell death mechanism (NETosis) [43]. Infected neutrophils degranulate and secrete inflammatory mediators, such as the chemokine macrophage inflammatory protein 1β (MIP-1β) and CC-chemokine ligend-3 (CCL3), aiding in the migration of macrophages, and recruitment of monocytes and dendritic cells [44, 45]. Under normal circumstances, compromised neutrophils undergo spontaneous apoptosis, however prevention of neutrophil apoptosis is an important mechanism that *Leishmania* uses to subvert death [41, 44]. For example, infected apoptotic neutrophils can act as silent vectors by providing a safe entry for *Leishmania* promastigotes into macrophages without triggering mechanisms to kill *Leishmania* [44, 46]. This silent entry into macrophages has been likened to the Trojan horse scenario [41, 47], as the promastigotes suppress neutrophil apoptosis until macrophages arrive at the site of infection and then downregulate the microbicidal responses (ROS and NO) [44, 48]. Infected neutrophils are engulfed by macrophages allowing promastigotes to transform into amastigotes and proliferate. *L. major* is able to delay neutrophil apoptosis for up to two days by inducing the secretion of the anti-apoptotic cytokines IL-8 and granulocyte macrophage colony-stimulating factor (GM-CSF) [48]. Infected neutrophils undergoing apoptosis have

also been reported to release higher levels of MIP-1β to attract macrophages to the site of infection thereby ensuring a safe entry for the parasite [44].

The ability of neutrophils to promote parasite killing or parasite survival [35, 49] appears to be *Leishmania* species-specific, impacted by the route of infection [35, 50], and influenced by the genetic background of the host [41, 44, 49, 51–53]. Studies investigating the role of neutrophils in the development of CL utilized two mouse models namely the susceptible (BALB/c) and resistant (C57BL/6) mice and found differences in the number of neutrophils recruited at the site of *L. major* inoculation. Interestingly, only lesions of susceptible mice demonstrated a sustained presence of neutrophils and this was associated with early IL-4 activation and the development of a T_H2 response [51]. These observations suggest that in susceptible BALB/c mice the early events of the immune response are important in initiating a subsequent T_H differentiation following infection with *L. major*.

In vitro studies with human neutrophils suggest that they play either protective or pathogenic roles depending on the infecting *Leishmania* species. A study comparing neutrophils from CL and healthy subjects, which were then infected with *L. braziliensis ex vivo*, observed that neutrophils from CL patients produced more ROS and higher levels of the chemokines CXCL8 and CXCL9 which are both associated with the recruitment of neutrophils and T_H1-type cells [54]. Neutrophils from both groups were equally competent to phagocytose *L. braziliensis*, however the cells from CL patients exhibited a pro-inflammatory profile necessary for parasite clearance [54]. The protective role of neutrophils depends on the infecting *Leishmania* species. *In vitro* infection of human neutrophils with *L. amazonensis* resulted in neutrophil production of ROS and leukotriene B4 (an inflammatory mediator) leading to neutrophil degranulation and the killing of *L. amazonensis* [55, 56]. In contrast, human neutrophils infected with *L. major* have been shown to contribute to pathogenesis through the secretion of high levels of MIP-1β, which attracts macrophages to the site of infection. These macrophages then engulf apoptotic infected-neutrophils, thereby providing a silent and safe parasite transmission into macrophages [44].

2.3.2 Macrophages and monocytes

Macrophages and monocytes are recruited to the inoculation site by degranulating, infected neutrophils releasing inflammatory mediators, such as MIP-1β and CCL2 [44, 57]. These cells become infected either by phagocytosing apoptotic *Leishmania*-infected neutrophils, by free *Leishmania* promastigotes that have escaped neutrophils, or by amastigotes that have previously ruptured their host cell [41]. Cells of the monocyte lineage are the main host cells of *Leishmania* parasites and once inside, *Leishmania* promastigotes differentiate into amastigotes, where they survive and replicate.

Both macrophages and monocytes are efficient in controlling *Leishmania* in the early stages of infection (reviewed in [3]). During phagocytosis, these cells release ROS, through a mechanism known as the respiratory burst, which kills *Leishmania* rapidly leading to early parasite control [30]. These cells also produce NO, which is generated by inducible NO synthase (iNOS) [58]. NO diffuses across cell membranes to initiate parasite killing within both the NO-producing cells and bystander cells [58]. For macrophages to release ROS that is sufficient in parasite killing, the cells need to first be activated by IFNγ and TNF-α, which enhance the respiratory burst [59]. Though non-activated macrophages will still release ROS through the respiratory burst following infection, it is insufficient to kill *Leishmania*. In a mouse model, the respiratory burst and subsequent release of ROS that occurs in *Leishmania*-infected macrophages were found to be insufficient to kill the parasites if the host cell was not previously activated by IFNγ [59]. During infection, the main producers of IFNγ are CD4+ T_H1 cells. Prior to the differentiation and activation of CD4+ T_H1 cells, natural killer (NK) cells are the

primary producers of IFNγ [60]. In contrast, *in vitro studies* with human and mouse monocytes infected with *Leishmania* species showed competence in parasite killing through the secretion of ROS and without the need for prior activation [30, 47, 59].

The majority of studies investigating the role of NO have used rodent models, where NO is considered necessary to control *Leishmania* [58, 61, 62], however it is not yet clear if NO is required for *Leishmania* control in humans as activated human macrophages have not been shown to produce NO upon *Leishmania* infection [59, 63], It has been suggested that inhibiting NO promotes *Leishmania* infection in phagocytes [63]. Similar, the exact role of ROS in human *Leishmania* infection is yet to be elucidated, although it is believed that the production of ROS is an important mechanism in eradicating *Leishmania* parasites throughout the course of disease [59].

2.3.3 Dendritic cells

DCs play an important role as a bridge between the innate and adaptive immune systems (reviewed in [64]). In addition to phagocytosing *Leishmania* parasites and infected apoptotic neutrophils [45], DCs are important in the maintenance of immunity and in rapid stimulation of the adaptive immune response during the early stage of infection. DCs present *Leishmania*-specific antigen to naïve T cells and promoting their differentiation. The migration of DCs to the lymph node (where they activate T cells) is vital to establish an efficient adaptive immune response. *Leishmania* has evolved strategies to inhibit interaction between DCs and T cells by reducing DC migration [64, 65]. It was demonstrated that *Leishmania* was capable of blocking CCR2 (expressed on DC surface) thereby impairing the cells' ability to migrate, however the mechanisms used by the parasite remain elusive [65].

3. Adaptive immune system in *Leishmania* infection and disease

Following the involvement of innate immune cells in targeting *Leishmania* parasites and antigen presentation, immune cells of the adaptive immune system are activated to induce a *Leishmania*-specific response. The adaptive immune system plays a pivotal role in *Leishmania* infection through the interplay between T cell-mediated and antibody-mediated immune responses and the induction of immune memory. The complexity of these immune responses, which facilitate the resolution of CL is also reflected by the various phenotypes of clinical CL presentations observed in individuals [66]. On one end of the immune spectrum, a strong T cell response is observed. Although the high levels of IFNγ lead to parasite control, an exacerbated T helper (T_H) type-1 response and increased number of CD8+ cytotoxic T cells may also lead to the development of MCL. In contrast, the other end of the immune spectrum is characterized by a high level of *Leishmania*-specific antibodies and a limited T cell-mediated response. Individuals have an uncontrolled parasite load (as parasites are not neutralized by antibodies), which is a consequence of low levels of T_H1 cytokines and this results in DCL manifestations [16, 67, 68]. An intermediate level of both T cell and antibody responses will lead to a form of CL that will normally self-heal over time.

3.1 $CD4^+$ T cells

The generation of *Leishmania*-specific $CD4^+$ T cells is required for protective immunity, and they play a major role in shaping the adaptive immune response. $CD4^+$ T_H cells are essential in determining disease outcome by driving the differentiation and activation of different $CD4^+$ T_H cell subsets through the production of cytokines, which either mediate host protection or promote disease pathogenesis (**Figure 4**).

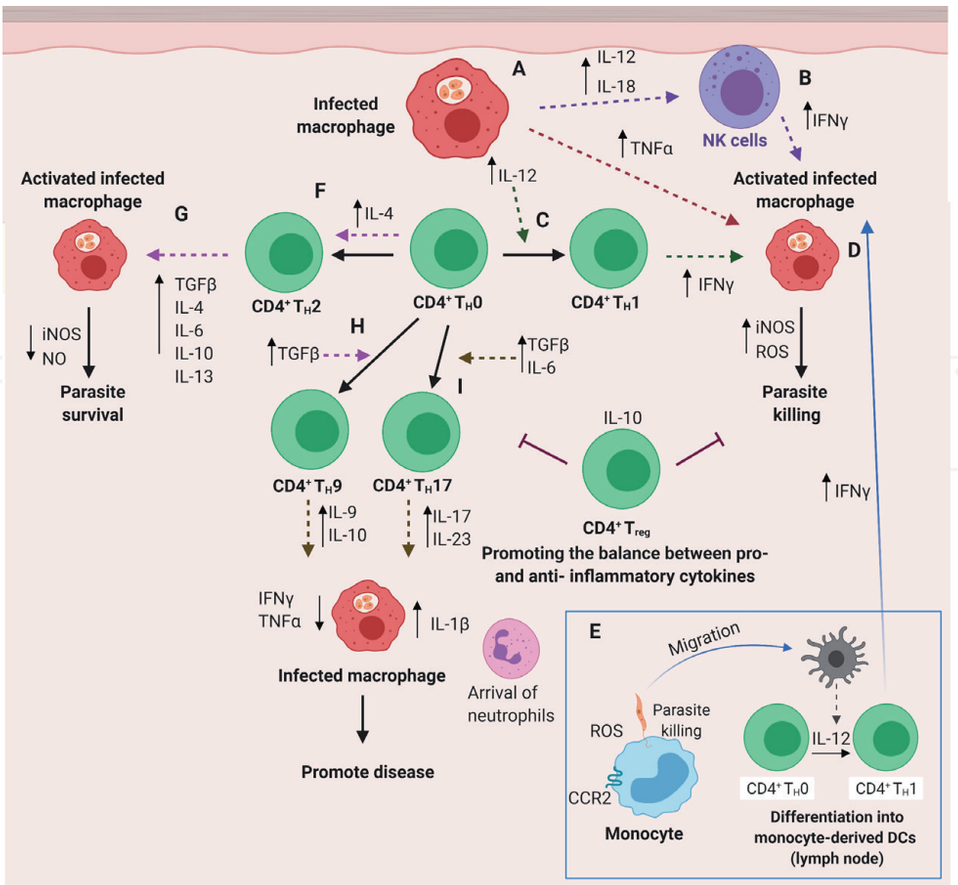

Figure 4.
Interaction between immune cells and Leishmania parasites. (A) Infected macrophages produce pro-inflammatory cytokines such as interleukin-12 (IL-12), IL-18 and tumor necrosis factor-α (TNF-α). These cytokines (B) recruit natural killer (NK) cells to the infection site and (C) promote CD4+ TH cell differentiation into $CD4^+$ T_H1. (B) NK cells and (C) $CD4^+$ T_H1 cells activate infected macrophages by producing interferon-γ (IFNγ). (D) Activated infected macrophages then release reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS), which results in parasite killing. (E) Infected monocytes kill Leishmania parasites through the release of ROS and migrate to the lymph node. Here they promote $CD4^+$ T_H1 differentiation by producing IL-12. CD4+ TH1 migrates to the skin where they (D) activate infected macrophages. In contrast (F) $CD4^+$ T_H cells produce IL-4 (an anti-inflammatory cytokine) which drives the differentiation of CD4+ TH2 cells. (G) Secretion of anti-inflammatory cytokines (such as transforming growth factor-β; TGFβ) by $CD4^+$ T_H2 suppresses the production of iNOS and NO by macrophages leading to parasite survival. (H) TGFβ drives the differentiation into $CD4^+$ T_H9 cells, which downregulates the release of IFNγ and TNF-α from infected macrophages, thereby promoting disease. (I) TGFβ and IL-6 drives differentiation into $CD4^+$ T_H17 cells that stimulates the secretion of IL-1β and infiltration of neutrophils that are believed to aggravate the disease. Adapted from [69]. Created with Biorender.com

Previously, it was widely believed that the induction of either a $CD4^+$ T_H1 or T_H2 response determined the outcome of infection i.e., induction of protection versus pathology. Subsequent studies have shown that there are a multitude of factors that contribute to the outcome of *Leishmania* infection, thus the T_H1/T_H2 model oversimplifies a complex interaction between host and parasite. Factors such as the genetic background of the model (or host) and the *Leishmania* parasite (species and strain) studied, contribute to differential disease outcomes. It is acknowledged that several $CD4^+$ T cell subsets are implicated in disease outcome, such as $CD4^+$ regulatory T (T_{reg}) cells, $CD4^+$ T helper populations (T_H1, T_H2, T_H9 and T_H17 effector) and T follicular helper (TF_H) cells [58, 70–72].

Cytokines produced by $CD4^+$ T cell subsets and other infected immune cells are generally classified as pro-inflammatory or anti-inflammatory and have been shown to be differentially associated with disease protection or progression, respectively (**Table 2**). Their role in activating and recruiting immune cells to the infection site shapes the adaptive immune response.

Immune mediators	Cell association/expressed by	General function	Role in cutaneous leishmaniasis
IL-1	• Secreted by epithelial cells, endothelial cells, activated macrophages, DCs, neutrophils and lymphocytes	• Pro-inflammatory cytokine • Critical regulator for early differentiation of T_H17 cells • Supports the generation of IFNγ secreting T cells (similar to IL-12) • Prolonged high levels of IL-1α induces T_H2 differentiation and increases pathology severity • IL-1β promotes (with IL-23) development of T_H17 cells	• Maintains cytokine secretions in T_H17 effector cells (together with IL-6 and IL-23) • Can be both protective by secretion of IL-1α and promotion of TNF-α production, and pathogenic during *Leishmania* infection • Secretion of IL-1α mediates disease resolution, reduction in parasite burden and enhancement of T_H1 response (via higher secretion of IFNγ and lower production of IL-4) • Continuous treatment with IL-1α in *L. major* infected C57BL/6 mice induced T_H2 responses and promoted disease susceptibility [69] • IL-1β treatment during early phases in *L. major* infected C57BL/6 mice mediates protection by promoting T_H1 responses [69] • Conversely, during the chronic phase, IL-1β can contribute to pathogenesis and worsen clinical symptoms of CL in *L. major* infected C57BL/6 mice through development of T_H17 cells and regulation of IL-17 levels [69] • IL-1β and IL-1α drive pathogenesis in *L. major* infected BALB/c mice. It was shown that IL-1α deficient and IL-1β deficient mice were resistant to infection and presented delayed nodule development and death [73]
IL-2	• $CD4^+$ T_H1 cells secrete IL-2 which promotes proliferation of T cells • Secreted in smaller amounts by $CD8^+$ T cells, NK cells and NKT cells [74]	• Pro-inflammatory and growth factor cytokine • Plays a dual role that may promote susceptibility to infection (by limiting secretion of IL-12 via T_H cells) and can also mediate resistance • Promotes immune responses by increasing proliferation and cytokine secretion (IFNγ by T_H1 cells), cytolytic activity ($CD4^+$, $CD8^+$ and NK cells; binding via IL-2 receptors on lymphocytes) • Can stimulate proliferation of T_H2 cells through generation of IL-4	• Involved in the protective immune response against CL and facilitates (along with IFNγ), macrophage activation and a T_H1 response and for parasite killing • Reduced IL-2 production has been associated with aggravated human CL [75]

Immune mediators	Cell association/expressed by	General function	Role in cutaneous leishmaniasis
IL-4	• Secreted by activated T cells, T_H2 cells and T_{FH} cells	• A signature anti-inflammatory cytokine of the T_H2-type immune response • Activates T_H2 cell differentiation from naïve $CD4^+$ T cells and production of T_H2-associated cytokines (IL-5, IL-10 and IL-13) • Powerful inhibitor of IFNγ-producing $CD4^+$ T cells and suppressor of T_H1 cells and pro-inflammatory cytokines	• Associated with non-healing forms of CL in mice (similar to IL-13) [76] • Induces T_H2 responses in *L. major* infected mice [77] • High levels of IL-4 in early stage of infection lead to the secretion of IL-12 by DCs and subsequent T_H1 proliferation [76] • Functions as a powerful inhibitor of IFNγ-producing $CD4^+$ T cells and suppressor of protective T_H1 immune responses
IL-6	• Secreted by T_H2 cells, macrophages, fibroblasts and endothelial cells	• Can act as a pro-inflammatory and anti-inflammatory cytokine • Together with TGFβ, IL-6 can stimulate production of T_H17 cells to secrete IL-17 and IL-10	• IL-6 deficient ($^{-/-}$) BALB/c mice showed no difference in pathology (parasite burden, lesion burden) when infected with *L. major* in comparison to BALB/c wild type (WT) mice. However, IL-$6^{-/-}$ mice did produce lower levels of T_H1 and T_H2 cytokines [78]
IL-8	• Secreted by tissue-resident macrophages in response to *Leishmania* infection	• Monocyte-derived neutrophil chemotactic factor; an activating cytokine • Plays a role in the initial recruitment and activation of neutrophils	• *L. major* infected neutrophils secrete high levels of IL-8 that leads to increased infiltration of neutrophils for parasite phagocytosis [79]
IL-10	• Secreted by Regulatory T (T_{reg}) cells, T_H2 and T_H9 cells, DCs, activated macrophages, NK cells and neutrophils	• Anti-inflammatory cytokine • Suppresses activity of T_H1 cells, NK cells and macrophages • Down-regulates expression of IFNγ, IL-2, IL-3 and TNF-α	• Important regulator of immunity in CL • Associated with CL susceptibility. High levels of IL-10 are strongly associated with non-healing forms of disease [16] • The absence of IL-10 in murine models is associated with the control of parasite replication and resolution of cutaneous infection. IL-$10^{-/-}$ mice express higher levels of IFNγ and produce more nitric oxide (NO) than IL-$10^{+/+}$ mice [80]
IL-12	• Secreted by monocytes, macrophages, dendritic cells (DCs) and B lymphocytes	• Pro-inflammatory cytokine • Activates T helper type 1 (T_H1) differentiation; stimulates differentiation of naïve T cells into T_H1 effectors; inhibits T cell apoptosis • Together with IL-15, this cytokine facilitates IFNγ and TNF-α secretion by natural killer (NK) and T cells	• The absence of the IL-12, IL-23, and IL-27 promotes the development of a T_H2 response and increases susceptibility to *Leishmania* infection [81]

Immune mediators	Cell association/expressed by	General function	Role in cutaneous leishmaniasis
IL-13	• Secreted by T_H2 cells and NK cells	• Anti-inflammatory cytokine • Activates the differentiation of naïve T_H0 cells into T_H2 cells	• High levels are associated with chronic CL • BALB/c IL-$13^{-/-}$ mice were able to control *L. major* infection (production of T_H1 responses and effectively control parasite growth), whereas C57BL/6 mice became susceptible to disease pathology due to the increased T_H2 responses [82].
IL-17	• Secreted by T_H17 cells, DCs	• Pro-inflammatory cytokine and mediates tissue inflammation • IL-17 can both mediate protection and susceptibility • Stimulates secretion of cytokines and chemokines (e.g., TNF-α, IL-1β, CXCL1 and CXCL10)	• Increased levels of IL-17 (together with IL-23) and rapid neutrophil infiltration are associated with aggravated CL and ML diseases [83] • Increased IL-17-dependent neutrophil recruitment into lesions has been shown to significantly promote disease outcome (*L. major* infected BALB/c mice) [84] • BALB/c mice infected with L. major shows high levels of IL-17 in contrast to IL-$17^{-/-}$ BALB/c mice despite typical T_H2 development (reduction in recruitment of neutrophils in lesional tissue and CXCL2 levels in infected skin) [84]
IL-18	• Secreted by activated macrophages and DCs, $CD8^+$ memory T cells, neutrophils	• Pro-inflammatory cytokine • An IFNγ inducing factor (induces T_H1 responses via IFNγ production with IL-12) • Plays a role in early control of CL caused by *L. major*, but not critical for the development of protective T_H1 responses or resolution of infection	• IL-$18^{-/-}$ C57BL/6 mice had increased susceptibility to *L. major* infection in the early phase of infection but were able to resolve the infection similar to IL-$18^{+/+}$ mice due to an increased level of IL-12 and IFNγ secretion [85]
IL-22	• Secreted by T_H17, T_H1 cells and NKT cells	• Critical role in tissue repair during CL • Strengthens epithelial barrier functions; involved in tissue homeostasis, tissue repair and wound healing • Induces keratinocyte proliferation and hyperplasia resulting in thickening of the epidermis	• *L. major* infected IL-$22^{-/-}$ C57BL/6 mice developed increased pathology in contrast to WT mice due to deficient wound healing of keratinocytes in the absence of IL-22 [86] • IL-22 is associated with pathogenesis when secreted with cytokines such as IL-17 [70]
IL-27	• Secreted by macrophages and DCs	• Anti-inflammatory cytokine and pro-inflammatory • T_H17 cell suppressor • Promotes differentiation and production of IL-10 producing T_{reg} cells	• Promotes the differentiation and expansion of T_{reg} cells (main producers of IL-10) and suppresses T_H17 cells • IL-$27^{-/-}$ WSX-1 mice developed severe *L. major* infection, which correlated with the increased levels of IL-17 $CD4^+$ T_H17 cells, reduced levels of IL-10 and increased in IL-4 [87]

Immune mediators	Cell association/expressed by	General function	Role in cutaneous leishmaniasis
IFNγ	• Secreted by $CD4^+$ T_H1 cells; $CD8^+$ T_H1 cells, NK cells, and NKT cells	• Pro-inflammatory cytokine (involved in protection and pathology of CL) [88] • Stimulates iNOS expression and activity in infected cells, which promotes parasite killing • Stimulates NO secretion in activated macrophages and inhibits amastigote growth • Promotes differentiation of naïve $CD4^+$ T_H cells into T_H1 cells and inhibits the development of T_H2 and T_H16 cells	• Compared to WT mice, C57BL/6, IFNγ $^{-/-}$ mice were more susceptible to *L. amazonensis* infection with large lesions, increased parasite burden and development of T_H2-type responses associated with increased IL-4 [89] • High levels of IFNγ can be detrimental and found in patients with MCL [7]
TNF-α	• Mostly produced by macrophages • Secreted by T_H1 cells, T_{FH} cells	• Pro-inflammatory cytokine (involved in protection and pathology of CL) • Plays a vital role in *Leishmania* clearance through increasing macrophage activity and NO synthesis	• Promotes T_H1/IFNγ responses against *L. major* infection • TNF-α $^{-/-}$ C57BL/6 mice infected with *L. major* manifested as fatal disease, a strong protective T_H1 response [90] • High levels of TNFα can promote disease pathogenesis leading to lesion chronicity [91]

−/−, deficient; DCs, dendritic cells; IL, interleukin; IFN, Interferon; MIP, macrophage inflammatory protein; NK, natural killer; NKT, natural killer T cells; NO, nitric oxide; TFH, T follicular helper cells; TGF, transforming growth factor; TH, T helper cell; TNF, tumor necrosis factor; Treg, T regulatory cell; WT, wild type.

Table 2.
Selection of cytokines and their role in cutaneous leishmaniasis.

It is recognized that the development of $CD4^+$ T_H1 immune responses promotes host protection against CL and is associated with the production of pro-inflammatory cytokines (such as IFNγ and IL-12). $CD4^+$ T_H1 cells are key producers of IFNγ, which has been shown in resistant and susceptible mouse models to be vital in controlling *L. major* parasites [92, 93]. In human and mice, the production of IFNγ activates infected macrophages to enhance the respiratory burst (as discussed above), which eliminates parasites residing and replicating within the phagolysosome, as explained earlier [59].

In contrast to the protective role of $CD4^+$ T_H1 cells, susceptibility to *Leishmania* infection and CL progression is influenced by the induction of an IL-4-driven T_H2-type immune response as well as the production of the anti-inflammatory cytokines, IL-10, IL-13 and TGFβ [94]. Rodent studies have shown that IL-4-secreting $CD4^+$ T_H2 cells and IL-10 secreting T_{reg} cells promote parasite growth and disease susceptibility [95]. For example, the $CD4^+$ T_H2-secreting cytokines, IL-4 [96] and IL-10 [97], was identified as having important roles in BALB/c mice' susceptibility to infection. In the absence of IL-4 or IL-10, BALB/c mice, were able to control parasite growth and resolve lesions resulting in a protective $CD4^+$ T_H1 response. Likewise, IL-10 likewise plays a role in disease self-healing C57BL/6 mice. When lacking IL-10, C57BL/6 mice exhibited a faster lesion healing time compared to WT [98]. The roles of IL-4 and IL-10 in promoting susceptibility in human patients with CL are less clear, although elevated IL-10 has been linked to uncontrolled parasite growth in VL [99].

Some cytokines are also considered to have a dual role in relation to disease outcome [100]. The production of the $CD4^+$ T_H1 cytokines IFNγ and TNF-α is critical in controlling *Leishmania* infection, however an aggravated production of these two cytokines have been affiliated with severe disease with lesion chronicity [91].

3.2 $CD8^+$ T cells

The role of $CD8^+$ T cells in *Leishmania* infection is still poorly understood. They have both a protective and a pathological role depending on whether the cells are producers of cytokines or are acting as cytolytic T cells, respectively (reviewed in [101]). The contribution and effectiveness of $CD8^+$ T cells in relation to parasite control is determined by the *Leishmania* species and experimental model (infective dose and host genetics).

RAG knockout (KO) mice (deficient in both B and T cells) developed lesions at a slower rate (*L. major* infection) compared to WT mice or not at all (*L. braziliensis* and *L. amazonensis* infection) [102–104]. When reconstituted with $CD8^+$ T cells, RAG KO mice developed severe pathology with lesions [102, 103]. In BALB/c mice infected with *L. braziliensis*, depletion of $CD8^+$ T cells resulted in reduced lesion size despite having a similar level of parasites in the skin compared with control mice [103].

Mimicking a natural low-dose infection with *L. major*, studies revealed that $CD8^+$ T cells play a role in protection, associated with high production of IFNγ, which activates macrophages leading to parasite control [102, 105]. Furthermore, IFNγ stimulates DCs to produce IL-12 which promote the development and differentiation of $CD4^+$ T_H1 cells. This suggests that $CD8^+$ T cells are important in skewing towards T_H1 response through the production of IFNγ and in eliminating the majority of parasites before lesion development. The role that $CD8^+$ T cells play in infection may be associated with their location in the host [106]. When located in the draining lymph node, $CD8^+$ T cells produce IFNγ and are protective [107]. In contrast, when migrating to the lesion site during infection, $CD8^+$ T cells produce lower levels of IFNγ and exhibit cytolytic activity, leading to cell death and an exaggerated inflammatory response that further promotes tissue damage [108]. This is supported by findings from a mouse model showing $CD8^+$ T cells that had migrated to the skin, produced lower levels of IFNγ and instead exhibited cytolytic activity promoting disease progression [103]. There is substantial evidence for a pathogenic role of $CD8^+$ T cells in patients infected with *L. braziliensis* [109–111]. As the disease progresses from small nodules to larger skin lesions, an increase in $CD8^+$ T cells and a decrease in CD4+ T cells was observed in the histopathological analysis of human skin lesions [112]. In CL patients a link between $CD8^+$ T cell mediated cytotoxicity and IL-1β inflammasome activation was observed [111]. This activation of NLRP3 inflammasome pathway and its promotion of disease inflammation is currently targeted for host-directed therapy [88, 106].

3.3 Regulatory T cells

The role of T_{reg} cells in *Leishmania* infection is still being elucidated, although though they have been shown in rodent models to be involved in disease pathology and parasite persistence depending on the experimental model used. $CD4^+$ $CD25^+$ T_{reg} cells have been shown to suppress $CD4^+$ T cell activity in *L. major*-infected C57BL/6 mice, thereby favoring parasite persistence [98, 113, 114]. T_{reg} cells influence both primary and secondary infections with *L. major*, as they render otherwise non-susceptible mice susceptible to infection [115]. However, their activity may also be dependent on the infecting *Leishmania* species. For example, T_{reg} cells play a protective role during infection with New World *Leishmania* species, such as *L. amazonensis* [95, 116]. Transferring T_{reg} cells from an *L. amazonensis*-infected mouse

to a naïve mouse prior to infection with *L. amazonensis* reduced the development of lesions suggesting that they may also contribute to the control of immunopathogenic responses [116]. Understanding how T_{reg} cells are involved in human *Leishmania*-infections is still being explored, with evidence so far suggesting that these cells play a role at the infection site and contributing directly to parasite persistent as the main source of IL-10 production [95, 98].

3.4 B cells and antibodies

The function of B cells in CL has not conclusively been shown. During the initial *Leishmania* infection, antibody production by B cells themselves are not believed to play a role, in controlling parasites as *Leishmania* are intracellular. However, some studies indicate that B cells may regulate both protective and pathogenic immune responses during *Leishmania* infection, depending on the infecting species and model used. Production of *L. major* antibodies was shown to be important for DCs to phagocytose parasites, as the absence of antibodies by B cells resulted in larger lesions in B cell$^{-/-}$ mice, higher parasite load, low production of IFNγ and a decreased cell-mediated immune response [117]. Moreover, IgG$^{-/-}$ BALB/c mice infected with *L. major* resulted in larger lesions and higher parasite load compared to IgG^{+} BALB/c mice [118]. In contrast, a study using a BALB/c mice deficient in IgM transmembrane domain (μMT), thereby lacking mature B cells, observed that these mice were resistant to *L. major* infection [119]. Other studies using BALB/c mice lacking IL-4Rα expression specifically on B cells, mbicreIL-4Rα$^{-/lox}$ BALB/c mice, resulted in a protective host immunity [29, 119, 120].

There is still a lot of knowledge to gain on B cells' function and whether they play a part in protection or pathology during infection with *Leishmania* parasites.

4. Persistent *Leishmania* infection and emulating concomitant immunity

Naturally and experimental infection with cutaneous *Leishmania* species is controlled following the development of an adaptive T_H1 immune response. After induction of this response, parasite numbers decline in infected tissues, lesions heal and lifelong immunity against the infecting *Leishmania* species is gained [121]. Though recovery from cutaneous disease has been reached, a small number of *Leishmania* parasites normally remain indefinitely in the host at the initial site of infection; known as persistent parasites [122, 123]. These parasites play an important role in maintaining protective immunity in the event of reinfection by providing a constant source of *Leishmania* antigen for immune stimulation [121, 124]. Both mice and humans who recover from CL maintain chronic subclinical infection at the lesion site and have been shown to be highly resistant to second challenge through sandfly transmitted infections [125]. Though, the immune response is unable to clear the primary infection, the immune system can facilitate concomitant immunity by IFNγ secreting CD4^{+} T_H1 cells [126]. However, reactivation of disease causing infection has been documented for leishmaniasis when the immune system is no longer able to control this low level chronic parasite infection [127, 128]. This is frequently observed when persistently infected individuals become immunosuppressed, such as during infection with the human immunodeficiency virus (HIV) [127, 129].

Currently, vaccine programs have been unsuccessful to emulate the protective responses mediated by concomitant immunity as observed during subclinical infections with persistent parasites (reviewed in [130]). Similar, a sterile cure whereby the parasites are completely eliminated has not been achieved without consequently the loss of long-term immunity [131].

In the past the leishmanization live vaccine practice was employed by inoculating virulent *Leishmania* parasites into individuals, however this has since fallen out of practice due to safety concerns regarding development of non-healing lesions [132]. Since then, vaccine-candidates have failed to provide protection against natural exposure even though they demonstrate protective cell-mediated immunity in rodent models. It is thought that this is due to differences in experimental delivery versus the natural route of infection via the bite of a sandfly. Other challenges are observed when using whole killed parasites or subunit protein vaccine candidates only short-term protection in rodent models has been observed [123, 131].

The difference in protective immunity induced following natural infection and inoculation of whole killed parasites is not fully understood but it has been hypothesized that there is a difference in the immunologic memory responses, which is influenced by the presence of live versus killed parasites. Moreover, the adjuvant dose-quantity tested to date may not be sufficient to generate a memory T cell population [123, 128]. It is possible that vaccines utilizing live-attenuated parasites will most closely mimic natural infection, potentially providing long-term protection against infection and disease [131].

Recently, vector-associated factors have been identified to have an important impact on challenge models in vaccine-mediated immunity [130]. Following needle versus infected sandfly challenge in mice showed that various protein/adjuvant-based vaccines provided intermediate protection against needle challenge whereas sandfly challenge failed to provide protection. Despite generating antigen-specific T_H1 immune responses prior to and following challenge, vaccines failed to protect against infected sandfly challenge [125, 133]. The sandfly vector challenge model clearly emphasizes important factors induced by the sandfly, such as the impact of recruited inflammatory cells and immune-mediated host cell activation by the vector.

5. Concluding remarks

The leishmaniases are one of the most important groups of neglected tropical diseases estimated to affect 1 million people annually in nearly 100 countries [1]. The fact that an effective vaccine has yet to be developed reflects the gap in our understanding of host responses to *Leishmania* species, disease pathogenesis and what actually constitutes a protective immune response. The different *Leishmania* parasites inducing different host immune responses, which are further impacted by host genetics, have made it difficult to achieve consensus among experimental studies regarding the role of the different immune components in *Leishmania* infection. Furthermore, research to date highlights the inadequacies of small animal models in understanding human host responses to *Leishmania*. The increase in *in vitro*/*ex vivo* characterization of *Leishmania*-specific immune responses using samples derived from human clinical studies has provided more information on human CL, however more efforts towards human clinical studies (cohort and case control-studies) including human *ex vivo* infection models should be emphasized to gain a better understanding of the human immune response to *Leishmania* parasites. An interesting recent focus has been the use of humanized mice to further examine the role of specific immune cells and responses in *Leishmania* infection; this could further inform the development of novel vaccine strategies [134].

Additionally, there are other important vector and parasite-derived components affecting host immune responses, which were outside the scope of this chapter but are important to consider in terms of host-parasite interactions. Recent experimental studies are providing new insights into host immune responses by employing a sandfly challenge model using the natural route of parasite inoculation via phlebotomine sandflies [46]. Vector-derived components have been shown to

contribute to early immune responses in infection [14]. For example, tissue damage caused by the phlebotomine sandfly's proboscis and the delivery of sandfly saliva triggers the rapid recruitment of neutrophils which induce inflammation [41]. *Leishmania*-derived components have also been shown to play a role during inoculation and *Leishmania* exosomes have been shown to modulate immune cells and host responses through direct and indirect contact [135].

This chapter has highlighted the complexity associated with CL and how host immune cells can both be protective and pathogenic depending on the interaction with *Leishmania* species parasite and host genetic. Employing a human CL model that provides a better understanding and more accurately represents parasite-host interactions will be critical for the development of an effective vaccine capable of inducing long-lasting protective immunity.

Conflict of interest

The authors declare no conflict of interest.

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