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CD8+ T cells in cutaneous leishmaniasis: The good, the bad and the ugly

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Abstract

CD8$^+$ T lymphocytes are components of the adaptive immune response and play an important role in protection against many viral and bacterial infections. However, their role in parasitic infections is less well understood. In leishmaniasis, a disease caused by intracellular protozoan parasites of the genus *Leishmania*, CD8$^+$ T cells have been shown to be protective. However, increasing evidence indicates that CD8$^+$ T cells may also exacerbate disease. In this review, we will describe the situations where CD8$^+$ T cells are either good or bad for the outcome of the infection, and attempt to reconcile the dual role played by CD8$^+$ T cells in cutaneous leishmaniasis.

Keywords

CD8$^+$ T cells; CD4$^+$ T cells; cytotoxicity; dendritic cells; immunotherapy; immunopathology; Leishmania; macrophages; MHC class I; phagolysosome; Th1 cells and vaccine

Introduction

CD8$^+$ T lymphocytes play two main roles in the adaptive immune response. Most often CD8$^+$ T cells are associated with a lytic function–hence they are also known as cytolytic T cells. Upon TCR recognition of a cell expressing peptides bound to MHC Class I, granules containing perforin and granzymes are released at the immunologic synapse, leading to lysis of the target cell. These responses are essential in eliminating some viral infections, as well as contributing to control of tumors (3). However, CD8$^+$ T cells also produce cytokines and chemokines, which can enhance immunity to pathogens. The most important of these is IFN-γ, which promotes a strong type 1 immune response. While individual CD8$^+$ T cell can be both cytolytic and produce IFN-γ, at times their effector function can be restricted (4–6). How these distinct functions lead to protection against viral and bacterial infections is fairly well understood, however how they function in protozoal infections is less clear. For example, following infection with the intracellular protozoan parasite *Leishmania*, CD8$^+$ T cells are activated, but the effector functions they exhibit, and whether they are protective, pathologic or irrelevant, depends upon several factors that are just beginning to be defined.

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Understanding these factors will be critical in considering how $CD8^+$ T cells might be targeted for a leishmanial vaccine, or alternatively for immunotherapy to lessen $CD8^+$ T cell-induced pathology. In this review, we explore the role $CD8^+$ T cells play in different clinical manifestations of cutaneous leishmaniasis and discuss the circumstances where $CD8^+$ T cells are good or bad for disease.

Immunity to cutaneous leishmaniasis

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* that are delivered into the skin by the bite of an infected sand fly. Subsequently, several phagocytic innate cells, including macrophages, inflammatory monocytes, dendritic cells and neutrophils, take up the flagellated forms of the parasite, called promastigotes, which then differentiate into non-flagellated forms called amastigotes (8). What happens to the parasite following phagocytosis depends upon the host cell, since early uptake by neutrophils may protect the parasites (10), while inflammatory monocytes are leishmanicidal (11). Macrophages predominantly support parasite maintenance, since it is in these cells that parasites survive and replicate within the phagolysosome. Depending upon the species of *Leishmania*, the parasites may primarily be limited to the skin, causing a cutaneous disease that exhibits varying degrees of severity, or visceralize leading to a potentially fatal infection (see Box 1). The life cycle is completed when a sand fly takes a blood meal from an infected host.

A protective immune response requires the generation of *Leishmania*-specific $CD4^+$ T cells that make IFN-γ to control parasite burden, although low numbers of parasites appear to persist following resolution of the disease (12). *L. major* infections induce the expansion of distinct $CD4^+$ T cell subsets in resistant and susceptible mouse strains, and extensive studies by immunologists have led to a fairly clear understanding of the role $CD4^+$ T cells play in leishmaniasis. Thus, experimental infections in mice with *Leishmania major* showed that IFN-γ-secreting $CD4^+$ Th1 cells, which develop in the presence of IL-12, are critical in controlling the parasites (13, 14). While *Leishmania* survives and replicates in resting macrophages, macrophages that are activated by IFN-γ are able to kill the parasites. In the mouse, the primary macrophage effector mechanism for killing *Leishmania* is the production of nitric oxide, although reactive oxygen species also contribute to parasite control (15). However, nitric oxide is much less important in humans, and reactive oxygen species appear to be the major mediators of parasite killing (16).

In contrast to the protective role of $CD4^+$ Th1 cells, experimental murine studies found that $CD4^+$ Th2 cells, which make high levels of IL-4, as well as $CD4^+$ T cells making IL-10 (both Tr1 and Treg cells), promote parasite growth and susceptibility (17–20). BALB/c mice are normally highly susceptible to *L. major*, but they are able to resolve their lesions in the absence of IL-4 or IL-10 (21, 22), and even in resistant mice (e.g. C57BL/6 mice) IL-10 plays an important role. Thus, C57BL/6 mice lacking IL-10 resolve their infections much faster, and following low dose infections can eliminate all of the parasites (23). Interestingly, at high parasite doses IL-10 deficient mice also control the parasites much better than controls, but develop increased pathology due to the development of a $CD4^+$ Th17 cell population (24). While the role of IL-4 in promoting susceptibility is less clear in human patients, IL-10 is strongly linked to lack of parasite control in visceral leishmaniasis

(25). Elevated levels of IL-17 in *L. braziliensis* patients, particularly those with mucosal disease, suggest that IL-17 may be pathologic in cutaneous leishmaniasis (26–28), although in visceral leishmaniasis the presence of IL-17 has been correlated with resistance (29–31).

Several other cells contribute to the outcome of infection with *Leishmania*. For example, NK cells respond soon after *L. major* infection, and promote increased resistance, apparently due to their production of IFN-γ (32, 33). Neutrophils may play the most controversial role, since in some studies they are essential for initiating an infection with *L. major*, while in other studies they promote parasite killing (10, 34–36). Several studies have examined the role of B cells and antibodies in leishmaniasis, which taken together suggest that antibodies play no role in protection, although in some situations antibodies may be detrimental (22, 37, 38). Finally, while $CD4^+$ Th1 cells are essential for resistance to *Leishmania*, $CD8^+$ T cells also provide a level of control through their production of IFN-γ. Thus, for some *Leishmania* species, such as *L. major*, $CD8^+$ T cells contribute to resistance against primary and secondary infection. However, recent studies show that $CD8^+$ T cells may paradoxically increase pathology. It is this dual role that $CD8^+$ T cells play in cutaneous leishmaniasis that is covered in depth below.

Leishmania recognition by $CD8^+$ T cells

$CD8^+$ T lymphocytes recognize peptides bound to MHC class I molecules, which are classically loaded in the endoplasmic reticulum with proteasome-generated peptides that were transported from the cytoplasm by the transporter associated with antigen processing (TAP). However, since *Leishmania* is not present in the cytoplasm, other mechanisms must be involved in MHC I loading of leishmanial peptides. One possibility is that phagosomes are self-sufficient to present exogenous antigens, which has been shown in other models (39). Another possibility is that parasite antigens or peptides escape into the cytoplasm, processed by the proteasome, and presented by the classical pathway. Consistent with this idea, a study revealed that recognition of the *Leishmania* antigen GP46/M-2 by $CD8^+$ T cells interacting with *L. amazonensis* infected cells is proteasome-dependent (40). However, in another study *L. major* antigen presentation to $CD8^+$ T cells was found to be purely phagosomal, and $CD8^+$ T cell activation occurred in a TAP independent manner (41). Because peptide generation in phagosome-restricted and TAP-independent MHC I loading is less efficient than the classical cytosolic/proteasome dependent pathway, it was suggested that this is a strategy used by *L. major* to minimize $CD8^+$ T cell activation in vivo (41). However, another possibility is that this is a host strategy to control exuberant $CD8^+$ T cell responses that might lead to severe disease in certain circumstances, as we will discuss later.

Currently there are no well-defined *Leishmania* $CD8^+$ T cell epitopes, which has made it difficult to investigate how $CD8^+$ T cell activation occurs in leishmaniasis. Once such epitopes are defined, and the tools to study them are developed, we should be able to answer several questions related to $CD8^+$ T cell function in leishmaniasis, including which DC subsets cross-present *Leishmania* antigen, whether individual *Leishmania* species can promote or block MHC I loading of leishmanial peptides, and if similar mechanisms occur within human antigen presenting cells. Answering these questions will be critical for future therapeutic design approaches to promote or inhibit $CD8^+$ T cell activation in leishmaniasis.

CD8+ T cell effector mechanisms in leishmaniasis

Following infection with *Leishmania* $CD8^+$ T cells respond to the MHC I-peptide complexes by proliferating, leading to a population of *Leishmania*-specific T cells. As discussed above, it is not fully understood how $CD8^+$ T cells get activated in leishmaniasis, but it is clear that they do respond during *Leishmania* infection. Thus, the critical question is what $CD8^+$ T cells do once they are activated. However, the answer turns out to be complicated, as $CD8^+$ T cell effector function seems to depend on the *Leishmania* species and/or mouse model employed, as well as their location in the host.

The good CD8+ T cells

The first few studies to address the importance of $CD8^+$ T cells in leishmaniasis suggested that $CD8^+$ T cells had no role in protection, as CD8-deficient mice, as well as β2-microglobulin deficient mice, resolved infections with *L. major* or *L. mexicana* as well as wild-type mice (42–44). This was somewhat surprising, since at that time $CD8^+$ T cells were known to play a major role in the control of other intracellular parasites, such as *Trypanosoma cruzi* (45). However, in 1998, a new mouse model for the study of leishmaniasis was established, in which injection of lower doses of parasites (100–1,000 infective stage promastigotes) and intradermal inoculation (the ear dermis), better mimicked a natural infection (46). In contrast to previous studies, newer studies utilizing the more physiologic infection conditions revealed that $CD8^+$ T cells do indeed play a role in protection (46, 47). Later, a comparative study between high and low doses of *L. major* found that the requirement for $CD8^+$ T cells was limited to low infection doses. As has been observed in other models, low doses of parasites (or peptide) appear to favor the generation of Th2 responses, whereas high doses favor Th1 cell development (48). This bias towards a Th2 response at low doses was overcome when IFN-γ producing $CD8^+$ T cells were present in intact animals (47). Thus, when CD8-deficient mice were infected with low doses of *L. major* they developed a Th2 response and uncontrolled disease. However, adoptive transfer of $CD8^+$ T cells, but not IFN-γ deficient $CD8^+$ T cells, into these mice resulted in the development of a Th1 response and resistance. Thus, following experimental infection with physiological doses of *L. major*, IFN-γ production by $CD8^+$ T cells blocks the development of Th2 cells, thus promoting the development of protective Th1 responses (Fig. 1).

Mice that have resolved a primary infection with *L. major* are highly resistant to reinfection, and several studies have shown that $CD8^+$ T cells contribute to this immunity (49, 50). As with a primary infection, this protective role is due to the ability of $CD8^+$ T cells to produce high levels of IFN-γ. Consistent with this function, $CD8^+$ T cells from cured mice were able to adoptively transfer delayed-type hypersensitivity to recipient mice challenged with *L. major* (50). $CD8^+$ T cells have been reported to exhibit cytotoxic activity in a secondary infection (51), although it is unclear if such cytolysis is protective in a natural secondary infection. However, since perforin-deficient mice are as resistant as wild-type mice to reinfection with *L. major*, it would seem that cytolysis plays little role in protection in secondary infections (52).

Since $CD8^+$ T cells are protective in mice that have resolved a primary infection with *L. major*, they have been targeted for vaccine development. As indicated above, this is most

likely due to their ability to proliferate and produce large amounts of IFN-γ very rapidly after challenge, thereby eliminating the majority of parasites before lesions develop (53). Indeed, many studies indicate that $CD8^+$ T cells are essential for vaccine-induced immunity. For example, DNA vaccination with a *Leishmania* antigen, LACK, leads to a $CD8^+$ T cell-dependent protection against challenge with *L. major* (54). Other vaccine strategies for cutaneous leishmaniasis also demonstrated a critical role for $CD8^+$ T cells in protection against challenge with live parasites, as depletion of $CD8^+$ T cells abrogated protection induced by vaccination (55, 56). While most studies examining the role of $CD8^+$ T cells in vaccines indicate that their protective capacity is due IFN-γ production, one study showed that the production of both perforin and IFN-γ by $CD8^+$ T cells was crucial for vaccine-induced immunity to *L. amazonensis* (57).

There is similarly strong evidence that $CD8^+$ T cells are essential for the control of primary and secondary infection in visceral leishmaniasis (reviewed by (58)). In mouse models of visceral leishmaniasis, $CD8^+$ T cells are activated, produce chemokines and are a source of IFN-γ (59–61). $CD8^+$ T cells also help with the formation of granulomas and parasite control, and are involved in the control of visceral leishmaniasis after therapy (59–62). Immunization of mice with A2, a virulence factor in *Leishmania*, reduced the number of parasites in the spleen and liver after subsequent challenge with *L. donovani*. Protection in vaccinated mice was associated with the production of IFN-γ by $CD8^+$ T lymphocytes and their ability to induce lysis of A2 pulsed targets in vivo (63). Hence, it is clear from mouse studies that $CD8^+$ T cells play an essential protective role during visceral leishmaniasis by reducing parasite burden.

The bad $CD8^+$ T cells

The development of cutaneous leishmaniasis is promoted by replicating parasites in the skin, as well as by the host immune response to infection. Activation of T cells leads to a robust inflammatory response, which promotes the development of cutaneous lesions. The critical requirement for T cells in lesion development in leishmaniasis is strikingly apparent in immunodeficient mice. Specifically, RAG knockout (KO) mice, which lack both B and T cells, infected with *L. major* develop lesions at a much slower rate than do wild-type mice (46) and minimal lesions are observed following infection with either *L. braziliensis* or *L. amazonensis* (64, 65), despite the fact that they have impaired parasite control. The lack of pathology combined with high numbers of parasites in infected RAG KO mice demonstrates that adaptive immunity is not only essential for the control of *Leishmania* parasites in the skin, but also promotes lesion development. Thus, when RAG KO mice are reconstituted with a combination of both $CD4^+$ and $CD8^+$ T cells and infected with either *L. major*, *L. braziliensis* or *L. amazonensis*, both parasite control and lesion development are restored (46, 64, 65). Most interestingly, while the reconstitution of RAG KO mice with $CD4^+$ T cells alone is protective, RAG KO mice that received $CD8^+$ T cells alone developed severe pathology (46, 64). This increased pathology was unrelated to parasite numbers, as similar numbers were observed in unreconstituted RAG KO mice and RAG KO mice that received $CD8^+$ T cells. Enhanced lesion size promoted by $CD8^+$ T is not limited to adoptive transfer studies of RAG KO mice, as MHC I-deficient mice that are unable to mount a $CD8^+$ T cell response develop smaller lesions than do MHC I sufficient mice following infection with *L.*

amazonensis (65). Furthermore, in contrast to $CD8^+$ T cell depletion studies in *L. major* infections, depletion of $CD8^+$ T cells in BALB/c mice infected with *L. braziliensis* resulted in reduced lesion size (64). This difference in lesion development was independent of parasite load, as CD8-depleted and control mice had similar levels of *L. braziliensis* in the skin (64). Taken together, these results indicate that $CD8^+$ T cells alone cannot eliminate *Leishmania*, and that under certain circumstances $CD8^+$ T cells promote increased pathology during infection with *Leishmania*.

Some of the most severe lesions in cutaneous leishmaniasis are found in patients infected with *L. braziliensis*, and there is substantial evidence for a pathogenic role for $CD8^+$ T cells in these patients. For example, as disease progresses from small nodules to large skin ulcers in these patients, there is an increase in the frequency of $CD8^+$ T cells and a decrease in $CD4^+$ T cells in the skin (66). Surprisingly, while $CD4^+$ T cells obtained from lesions of *L. braziliensis* infected humans made both IFN-γ mRNA and protein, $CD8^+$ T cells did not (67–69). The lack of IFN-γ production by $CD8^+$ T cells that have migrated to the skin was also observed in mice infected with either *L. major* or *L. braziliensis* (70)(Novais et al., unpublished observations). In contrast, $CD8^+$ T cells isolated from the skin of patients exhibit markers of granule-dependent cytotoxicity, including perforin and granzymes, and the levels of $CD8^+$ T cells expressing granzyme A were higher in patients with large skin ulcers than those with nodular lesions (66). A genome wide transcriptional analysis that compared cutaneous lesions from *L. braziliensis* patients with normal skin also found that cytotoxicity is a main signature of the disease (64), and transcriptional profiling that compared lesions from *L. braziliensis* patients with cutaneous leishmaniasis and the more severe form of the disease, mucosal leishmaniasis, showed greater mRNA levels for cytolytic markers in mucosal patients (71). Furthermore, $CD8^+$ T cells from the lesions of *L. braziliensis* patients were degranulating within lesions, as assessed by expression of CD107a on the cell surface (64, 68). This correlation between cytotoxicity and disease severity was also seen in mice, where susceptible mice expressed more cytotoxicity markers in the skin in comparison to resistant mice (72)(Novais et al., unpublished observations). Taken together, these observations suggest that cytolytic activity of $CD8^+$ T cells promotes disease progression, a pathologic mechanism first suggested by Brodskyn and collaborators in 1997 (73).

While studies in *L. braziliensis* patients strongly suggested that cytolytic activity of $CD8^+$ T cells promotes disease, it remained possible that the observed cytolysis was a consequence of severe disease, rather than its cause. To address this question, the course of *L. braziliensis* infection in RAG KO mice reconstituted with either wild-type or perforin KO $CD8^+$ T cells was compared (64). While wild-type $CD8^+$ T cells induced severe pathology in infected RAG KO mice, there was minimal pathology in RAG KO mice that received perforin KO $CD8^+$ T cells (64). In contrast, when $CD8^+$ T cells deficient in either IFN-γ or IL-17 were transferred to *L. braziliensis* infected RAG KO mice, the animals still developed severe pathology. While these experiments demonstrated that cytolytic $CD8^+$ T cells are pathologic, they surprisingly showed that $CD8^+$ T cells also promote metastatic lesion development. Thus, in addition to promoting severe pathology at the primary infection site, RAG KO mice reconstituted with wild-type $CD8^+$ T cells developed lesions in distant

cutaneous sites, including the nose, tail, footpad and contra-lateral ear, which were absent in RAG KO mice reconstituted with perforin KO $CD8^+$ T cells. These results suggest that $CD8^+$ T cell-mediated cytotoxicity is involved in the development of more severe forms of leishmaniasis, such as mucosal and disseminated leishmaniasis. While the mechanism by which metastasis occurs is still unclear, one possibility is that parasites metastasize independent of $CD8^+$ T cells, but lesions only become visible when $CD8^+$ T cells are recruited to these sites, thereby initiating cell death and tissue damage.

A question that remains is how cytolytic $CD8^+$ T cells mediate such severe pathology. Some insights into this question came from a transcriptional analysis that identified key pathways induced in *L. braziliensis* lesions compared to normal skin (74). In addition to a cytotoxic gene signature, the lesions from *L. braziliensis* patients were abundant in genes associated with the immunoproteasome, mRNA for T cell-recruiting chemokines, such as CXCL9 and CXCL10, as well as inflammasome-related genes. Together, this information suggested a hypothetical model of immunopathology in cutaneous leishmaniasis (74). In this model, CCXCL9 and CXCL10 help to recruit $CD8^+$ T cells that, once in the skin, adopt a cytolytic profile after activation by antigens processed through the immunoproteasome. $CD8^+$ T cell-mediated cytolysis of infected cells would then release damage-associated molecular patterns (DAMPs), which amplify the inflammatory response by activating the inflammasome and promoting release of IL-1β (75), an inflammatory cytokine present in high levels in lesions from *L. braziliensis* patients. Besides activating the inflammasome, DAMPs can also provide a positive feedback loop for $CD8^+$ T cell cytolytic activity (76) (Fig. 2).

Collectively, these observations show that $CD8^+$ T cell induced cytotoxicity is extremely detrimental in *L. braziliensis* infected lesions, and that targeting these bad $CD8^+$ T cells should be considered for immunotherapy. It remains to be determined if this is unique to the disease caused by *L. braziliensis* in humans, or if other species of *Leishmania* promote severe disease by a similar mechanism. For *L. major* infection, cytotoxicity has only been evaluated in the peripheral blood mononuclear cells from patients, and has been associated with protection (77). However, systemic responses do not necessarily reflect what is occurring at effector sites such as the skin, as differences in the frequency and phenotype of $CD8^+$ T cells in the blood and lesion can be observed (64, 78, 79). In order to determine if cytotoxicity is a conserved mechanism promoting pathology in cutaneous leishmaniasis, additional studies with other *Leishmania* species need to be performed.

The ugly $CD8^+$ T cells

Both effector and memory T cells are recruited to sites of inflammation independent of their specificity. Thus, it may not be surprising that $CD8^+$ T cells isolated from leishmanial lesions of *L. braziliensis* patients who were seropositive for *Toxoplasma* contained both leishmanial- and *Toxoplasma*-specific T cells (80). However, the role that potentially large numbers of bystander T cells play in modulating disease, if any, is unclear. Our group has recently addressed this question experimentally, and found that recruitment of bystander $CD8^+$ T cells to the skin exacerbated lesion development after *L. major* infection (70). For these studies, mice were infected with an acute strain of lymphocytic choriomeningitis virus

(LCMV), which leads to the development of a large memory $CD8^+$ T cell pool that remains once the virus has cleared. When the mice were challenged with *L. major* several weeks following viral clearance, a large number of LCMV-specific $CD8^+$ T cells were recruited to the *L. major* infected skin. Furthermore, the LCMV immune mice developed substantially larger lesions compared to mice that were infected with *L. major* alone, although the number of parasites was the same between groups. Importantly, depletion of $CD8^+$ T cells was sufficient to protect LCMV immune mice from developing larger leishmanial lesions than controls, indicating that bystander $CD8^+$ T cells are detrimental in leishmaniasis.

Because LCMV-specific $CD8^+$ T cells are unable to recognize *Leishmania*, it remains unclear how bystander $CD8^+$ T cells modulate disease. When the LCMV-specific $CD8^+$ T cells in the lesions were characterized, they were clearly activated, exhibiting high levels of granzyme B, but low levels of IFN-γ, similar to what has been observed in patients (67–69). Furthermore, some of them expressed CD107a, showing that they are degranulating within the lesions, and suggesting that they may be promoting disease by their cytolytic function. Thus, these bystander $CD8^+$ T cells exhibited the same effector functions as leishmanial-specific $CD8^+$ T cells in RAG KO mice (46, 64). However, how LCMV-specific bystander $CD8^+$ T cells were activated in mice that had cleared the virus remained puzzling until it was discovered that they expressed NKG2D (70). NKG2D is an activation receptor found on NK cells and $CD8^+$ T cells that can act as a costimulatory molecule and also mediate target cell lysis. Importantly, one of the ligands for NKG2D, Rae1γ, was expressed in a large number of the cells within the *L. major* infected lesions. These observations suggested that NKG2D-dependent cytolysis of Rae1γ expressing cells promotes pathology. In support, in vivo blockade of NKG2D in *L. major* infected LCMV-immune mice prevents cytolysis and lesion development (70) (Fig. 2). Thus, like *Leishmania*-specific $CD8^+$ T cells, memory $CD8^+$ T cells that recognize an irrelevant epitope can participate in the development of severe disease in leishmaniasis.

Concluding remarks

The relative ability of $CD8^+$ T cells to contribute to protective or pathologic mechanisms in cutaneous leishmaniasis is directly related to their effector functions. $CD8^+$ T cells are protective when they produce IFN-γ, but promote pathology when they are cytolytic. Thus, in a primary infection $CD8^+$ T cells in the draining lymph nodes make IFN-γ critical for $CD4^+$ Th1 cell development and inducing macrophage activation for parasite killing. In contrast, $CD8^+$ T cells that migrate to the lesions in a primary infection make little IFN-γ, and instead exhibit a cytolytic phenotype that increases cell death, leading to an exaggerated inflammatory response that further promotes tissue damage. Therefore, vaccines that target IFN-γ -producing $CD8^+$ T cell should be protective, whereas immunotherapies that dampen $CD8^+$ T cell cytotoxicity or its downstream mediators are a promising strategy to lessen disease. Why the $CD8^+$ T cells in the skin develop a cytolytic profile (which is not evident in the draining lymph node), and why they make little IFN-γ, is unknown. In order to successfully target $CD8^+$ T cells it will be important to address these issues and define the factors that determine effector $CD8^+$ T cell function during the development of cutaneous leishmaniasis.

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Abbreviations

DCs	dendritic cells
MHC	major histocompatibility complex
TCR	T cell receptor
Th	T helper

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Disease manifestation in leishmaniasis

Leishmaniasis is a disease with three major clinical manifestations: cutaneous, mucosal and visceral leishmaniasis. Generally, cutaneous leishmaniasis patients develop skin ulcers with elevated borders and a necrotic center. In the Americas, cutaneous leishmaniasis is caused by a variety of species and among the most common etiological agents are *L. braziliensis*, *L. mexicana*, *L. panamensis*, *L. amazonensis* and *L. guyanensis*, while in the Old World, the species implicated in cutaneous leishmaniasis disease are *L. major*, *L. aethiopica* and *L. tropica*.

Cutaneous leishmaniasis can manifest as a single ulcer in the skin, named localized cutaneous leishmaniasis, or in rare cases by large numbers of lesions, called disseminated leishmaniasis (1). Disseminated leishmaniasis has only been described in *L. braziliensis* infected patients (2). In the lesions of patients with disseminated leishmaniasis, parasites are rarely found and a strong immune response is present. Another form of severe cutaneous leishmaniasis is diffuse cutaneous leishmaniasis. In contrast to disseminated leishmaniasis, the diffuse lesions are nodular and consist of macrophages containing many parasites without other immune cells (7). Diffuse cutaneous leishmaniasis has been described in patients infected with *L. amazonensis*, *L. mexicana* and *L. aethiopica*. In some cutaneous leishmaniasis cases, parasites metastasize to the nasal-pharyngeal mucosa, causing a severe form of leishmaniasis called mucosal leishmaniasis, which can occur in a small percentage of patients infected with *L. braziliensis*, *L. amazonensis*, *L. guyanensis* and *L. panamensis* (1).

Visceral leishmaniasis or kala azar is caused by *L. donovani* or *L. infantum* (*L. chagasi* in the New World). The parasite replicates in the spleen, liver and bone marrow, and in the absence of drug treatment the disease is fatal (9).

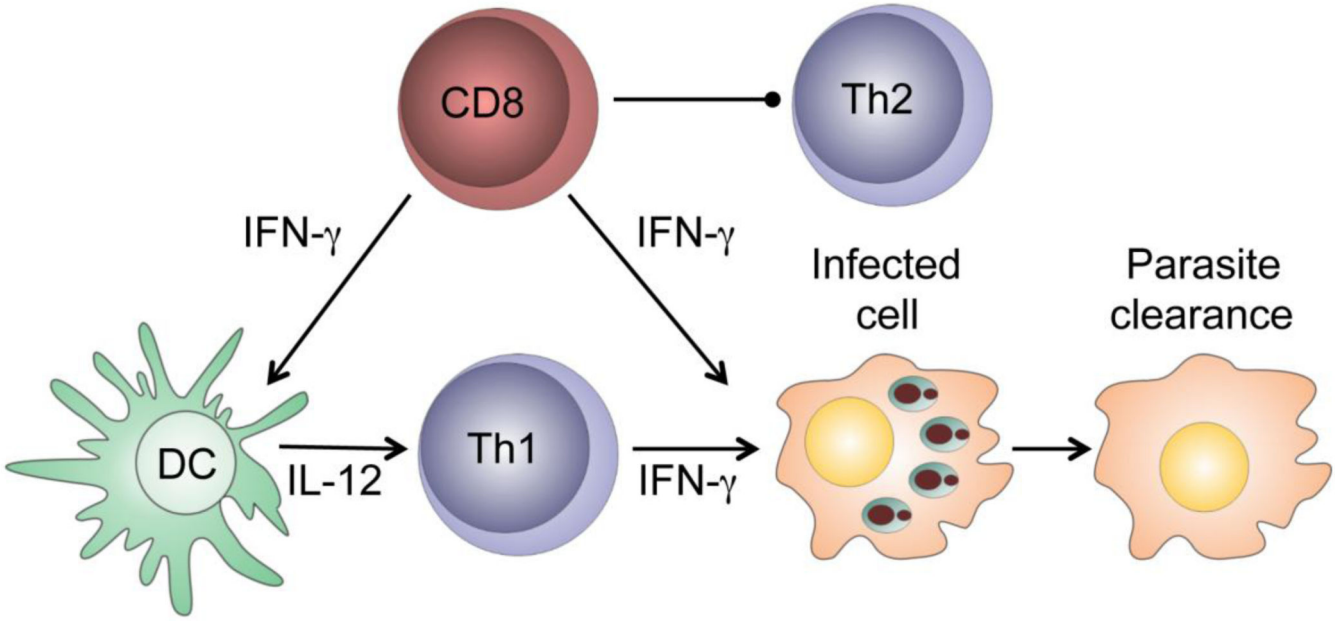

Figure 1. Protective CD8$^+$ T cells in leishmaniasis

CD8$^+$ T cells producing IFN-γ activate macrophages, leading to parasite clearance. In addition, the IFN-γ produced by CD8$^+$ T cells promotes increased production of IL-12, which amplifies the development of protective CD4$^+$ Th1 cells. With low doses of parasites, CD8$^+$ T cells are essential for blocking CD4 Th2 cells development.

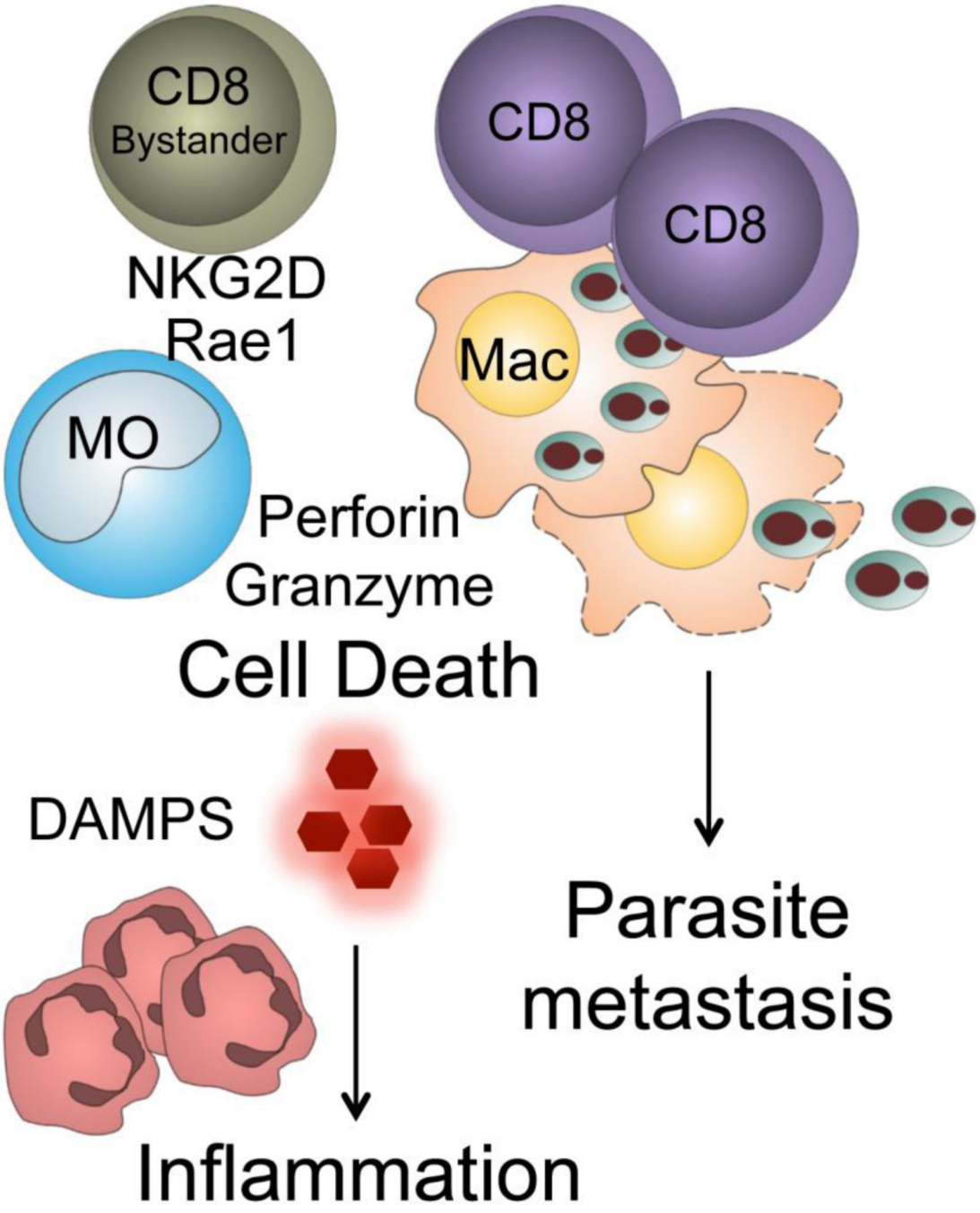

Figure 2. Pathologic CD8$^+$ T cells in leishmaniasis

Leishmania-specific CD8$^+$ T cells migrate to leishmanial lesions and lyse infected cells, leading to the release of proinflammatory molecules, including molecules with damage-associated molecular patterns (DAMPS). Lysis of infected cells leads to release of parasites, which may promote increased metastasis of the parasites. Memory CD8$^+$ T cells generated from prior non-leishmanial infections are also recruited to leishmanial lesions. *Leishmania* infection leads to upregulation of ligands for NKG2D, such as Rae1γ, and thus if the

recruited $CD8^+$ T cells express NKG2D they lyse target cells, also leading to cell death and increased inflammation.