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Eosinophils and mast cells in leishmaniasis

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Abstract

Leishmania spp. are parasitic protozoa endemic in tropical and subtropical regions and the causative agent of leishmaniasis, a collection of syndromes whose clinical manifestations vary according to host and pathogen factors. *Leishmania* spp. are inoculated into the mammalian host by the bite of an infected sand fly, whereupon they are taken up by phagocytosis, convert into the replicative amastigote stage within macrophages, reproduce, spread to new macrophages and cause disease manifestations. A curative response against leishmaniasis depends in the classical activation of macrophages and the IL-12-dependent onset of an adaptive type 1 response characterized by the production of IFN- γ . Emerging evidence suggests that neutrophils, dendritic cells and other immune cells can serve as either temporary or stable hosts for *Leishmania* spp. Furthermore, it is becoming apparent that the initial interactions of the parasite with resident or early recruited immune cells can shape both the macrophage response and the type of adaptive immune response being induced. In this review, we compile a growing number of studies demonstrating how the earliest interactions of *Leishmania* spp. with eosinophils and mast cells influence the macrophage response to infection and the development of the adaptive immune response, hence, determining the ultimate outcome of infection.

Keywords

Eosinophils; Mast cells; Leishmania; Innate immunity

Introduction to leishmaniasis

Protozoa belonging to the genus/subgenuses *Leishmania* *Leishmania* spp. and *Leishmania* *Viannia* spp. are classified into at least 24 species (reviewed in: [1, 2]). Each *Leishmania* species characteristically causes a particular clinical syndrome, the most common of which are visceral, cutaneous and mucocutaneous leishmaniasis. There is considerable overlap, and many examples of *Leishmania* species cause atypical clinical manifestations. Novel forms continue to emerge, underscoring the fact that we do not yet know the full spectrum of

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disorders due to the *Leishmania* spp. protozoa [3–6]. Leishmaniasis, therefore, refers to a collection of disorders with varying degrees of severity [3].

Many *Leishmania* spp. are found only in certain geographic locations, leading to regional variations in the types of leishmaniasis found in each of the 88 countries in which *Leishmania* spp. are endemic (reviewed in Desjeux: [7]). Contributing to the distribution, the insect vectors and mammalian reservoirs vary among different regions (reviewed in [1, 2, 7]). Sand flies of the genus *Phlebotomus* are the insect vectors for leishmaniasis in the Old World, whereas members of the genus *Lutzomyia* are the vectors in the New World. Foxes and domestic dogs are thought to be the major animal reservoirs for *L. infantum*, one of the two causative agents of visceral leishmaniasis, which is prevalent in Latin America, China and the Mediterranean littoral including some countries in North Africa and Southern Europe. Rodents serve as frequent reservoirs for many *Leishmania* species causing cutaneous leishmaniasis in Africa, Latin America and the Middle East, whereas humans are a major reservoir for *L. donovani* infection in Afghanistan, India, Bangladesh and Nepal (reviewed in: [1, 8]). As such, the epidemiologic patterns of *Leishmania* infections vary from sylvatic to peri-domestic to anthroponotic depending on the host-reservoir proximity.

The life cycle of *Leishmania* spp. protozoa

Leishmania parasites exhibit two main life forms, the promastigote and the amastigote. The flagellated metacyclic promastigote (10–20 µm) is introduced into the mammalian skin during the bite of an insect vector and transforms into the obligate intracellular, nonmotile amastigote (3–7 µm), a form that is found throughout mammalian infection (reviewed in: [1]). The female sand fly ingests amastigote-laden macrophages upon taking a blood meal from an infected host. In the insect gut (midgut or hindgut depending on the subgenus), the amastigotes are released from the macrophages, transform to procyclic promastigotes and through other intermediate forms (nectomonad, haptomonad, leptomonad) to ultimately become infectious metacyclic promastigotes [9–11]. These parasite stages replicate and acquire virulence while avoiding expulsion from the midgut by attaching to the gut wall, through expression of stage-specific forms of the surface glycolipid lipophosphoglycan (LPG) [12]. After about 7–10 days, the promastigote achieve [11] the nondividing, virulent metacyclic stage, which coincides with the time that the sand fly takes a new blood meal [10].

Murine models of leishmaniasis

Murine models of leishmaniasis have been developed to reflect the biological differences between the species and the different types of human clinical syndrome that develops. Frequently studied models are mentioned below. Many parasites that typically lead to localized cutaneous leishmaniasis without dissemination to mucosal sites are members of the *Leishmania* subgenus. Old World causes of localized cutaneous leishmaniasis include *L. L. major* and *L. L. tropica* among others. New World species include *L. L. mexicana* and *L. L. amazonensis*. Visceral leishmaniasis is caused by two species belonging to the *Leishmania* subgenus, *L. L. donovani* and *L. L. infantum*. Prior publications also refer to *L. L. chagasi*, but genome sequencing has made it clear that this is the same parasite as *L. L. infantum* [13]. Models of cutaneous leishmaniasis usually include introduction of the

parasite in the footpad or ear, followed by measuring of localized lesions throughout infection. Remarkably, the BALB/c mouse infected with *L. major* has a unique combination of immunodominant parasite antigen (LACK) and murine H-2 haplotype, leading to a predominant TH2 expansion that mediates vigorous local expansion and wide dissemination of the parasite. This contrasts with expansion of TH1 type clones and self-resolution of *L. major* infection in other mouse strains, causing *L. major* to become a prototype model of the biological consequences of TH1 versus TH2 type responses [14]. TH2 clonal expansion does not occur during *L. major* infection of other mouse strains, or with other *Leishmania* species infection models. Although the dichotomous TH1–TH2 response is unique to the *L. major*–BALB/c model, there is a clear genetic contribution to the outcome of leishmaniasis in mouse strains [15].

Models of the visceralizing species often include introduction of parasites as either amastigotes [16] or promastigotes [17] intravenously. Mice infected with these parasites develop a characteristic pattern of parasite expansion first in the liver, followed by local resolution in the liver and steady growth of the parasite in the spleen [18]. Parasites of the *Leishmania Viannia* subspecies (e.g., *L. V. braziliensis*, *L. V. guyanensis* and *L. V. panamensis*) are endemic in Latin America and lead to localized cutaneous lesions followed by later dissemination to mucosal sites as well as other more unusual or emerging syndromes [19]. It has been difficult to develop murine models of these infections without artificial manipulation such as delivery of TGF- β during *L. V. braziliensis* infection [20], although recently, groups have successfully developed models of all three of the abovementioned species [21]. Below we will refer to these parasites by genus and species names, omitting subgenus name, since this omission leads to no confusion.

Developmental changes in *Leishmania* spp. parasites affecting disease progression

The developmental migration of *L. major* parasites from midgut forward in the sand fly infected with *L. major* has been attributed to lectin-mediated attachment parasite LPG to the sand fly gut epithelium. Developmental changes in surface LPG cause virulent metacyclic *L. major* promastigotes to be unable to attach to the gut wall and migrate to the insect proboscis (reviewed in: [22, 23]). When the *Leishmania*-infected sand fly takes a blood meal from a noninfected mammalian host, it regurgitates promastigotes from its midgut and/or foregut into the mammalian dermis. Estimated numbers of promastigotes inoculated vary from 1–1000 to 10^3 to 10^5 ([11] and reviewed in: [22, 24]).

Both parasite extracellular components and sand fly salivary contents are transmitted with the parasite, influencing the local immune response to and likely the ultimate course of infection. Sand fly saliva includes the protein maxadilan, which causes vasodilation and consequently enhances the flow of mononuclear phagocytes to the region [25, 26]. There are also direct immunomodulatory effects of maxadilan, including inhibition of delayed type hypersensitivity (DTH) responses, decreased production of TNF- α and IL-12p70, with increased IL-6, IL-10 and TGF- β from monocytes [26–28]. Other components in sand fly saliva may also be immunomodulatory. Several groups have hypothesized that antibody responses to sand fly salivary components not only serve as an epidemiologic marker of sand fly exposure, but also provide protective immunity to naturally transmitted *Leishmania* spp.

infections [29, 30]. In the host dermis, promastigotes are phagocytosed by host cells and begin their promastigote-to-amastigote conversion, a process that takes 2–5 days to complete in mononuclear phagocytes (reviewed in: [22]). This transformation is characterized by morphologic and biochemical changes including loss of the externalized flagellum, expression of stage-specific proteophosphoglycans (PPGs), loss of the major parasite surface glycolipid lipophosphoglycan (LPG) and down-modulation with relocalization of the major surface protease [MSP, also known as GP63 or promastigote surface protease (PSP)]. The lowered or absent MSP or LPG, respectively, uncovers the dense layer of glycoinositolphospholipids (GIPLs) on the parasite surface [31]. Conversion into amastigotes allows the parasite to replicate, disseminate to other macrophages and cause disease [32–35].

Despite early beliefs that mononuclear cells were the sole cell of the mammalian host harboring intracellular amastigotes, a growing number of studies suggest that other cells of the immune system can also serve as either temporary or long-term host cells for *Leishmania* spp., and also modify the initial inflammatory response to the parasite. Several reviews and publications outline our emerging understanding of the role of neutrophils (PMNs) in the early stages of leishmaniasis [36]. The aim of this review is to examine the contribution of eosinophils and mast cells to the earliest host immune interactions with *Leishmania* spp. parasites, and put this in the context of other local immune responses.

Resident and recruited immune cells at the site of *Leishmania* spp. infections

A low intradermal inoculum of *L. major* promastigotes (100 parasites) was found to lead to a protective delayed-type hypersensitive (DTH) immune response to the parasite, which protected against subsequent challenge infection. This contrasts with higher doses, which induced an exacerbating immune response [37–39]. Cutaneous lesions that develop in an immune protected host contain a variety of inflammatory cells including macrophages and neutrophils [38]. Murine models of visceral leishmaniasis show a biphasic pattern, with parasite expansion over 4 weeks in liver tissue, followed by spontaneous healing of parasite infection in the liver, but expansion in the spleen [18, 40]. The kinetics of parasite growth in the spleen is parallel but prolonged relative to the liver [17]. A parallel “silent expansion” period has also been documented in the low-dose experimental model of *L. major* infection [37]. In the early stages of this model, the inflammatory infiltrate at the site of a *L. major* lesion consists of neutrophils, macrophages and eosinophils. Remarkably, the silent expansion phase and the composition of the inflammatory infiltrate of several immunodeficient mice, [IL-12p40 knock-out (KO), CD40L KO and SCID], were similar to that of wild-type C57BL/6 mice. The development of skin lesions overlaps with the period of type 1 cell expansion in the lymph nodes, around 6 weeks after infection. Mice deficient in either IFN- γ or iNOS experienced an influx of mostly neutrophils correlating with the development of necrotic lesions, whereas SCID mice experienced a scant cellular infiltrate with little lesion development [37]. These observations are reminiscent of studies of IL-12-deficient mice infected with *L. infantum* in which the effects of IL-12 were manifest only after 4 weeks of infection [41]. In both models, after the initial “silent” phase, the

requirement for IL-12 as well as CD40/CD40L and CD4 + mediated T cells for healing was evident [37, 39].

Studies of early lesions illuminate the fact that cells other than macrophages serve as hosts for parasites at different times during infection. Indeed, a model of natural infection transmitted by sand fly bites demonstrated that during the initial 2 h of infection, neutrophils accumulating at the site of *L. major* inoculation readily take up parasites [42]. Indeed, during the initial 18 h of infection, the majority of infected cells are neutrophils. However, between 48 h and 7 days after infection, the proportion of infected neutrophil decrease and macrophages become the predominant infected cells [42]. Similar results have been documented in a model of *L. infantum* infection [43], in which intradermal parasite inoculation resulted in a local cellular infiltrate composed primarily of neutrophils, a majority of which were infected. As the infection progressed over 48 h, the composition of the cellular influx, and the majority of infected cells, switched from mostly neutrophils to mostly macrophages. Macrophages were the predominant infected host cell 2–7 days after infection [43]. Taken together, these studies suggest that (1) “tissue injury” caused by either natural infection or experimental inoculation enhances the local early inflammatory infiltrate and (2) that the formation of skin lesions correlates with the development of adaptive immunity [37, 42].

Eosinophils

For a long time, eosinophils were considered nonspecific cells whose anti-microbial activity was mediated through the secretion of granules. It is now evident that eosinophils can influence the developing adaptive immune response in at least three ways. First, eosinophils can serve as antigen-presenting cells [44, 45]. In a model of *Strongyloides stercoralis* infection, eosinophils increase their MCH II expression, prime naïve T cells and bias T cell differentiation toward a type 2 phenotype [44]. Accumulating evidence also supports the antigen-presenting ability of eosinophils during allergic inflammation [46]. Second, eosinophils can facilitate innate immune responses through the activation of neutrophils, basophils and mast cells [45, 46]. Third, eosinophils can shape the adaptive immune responses by inducing the maturation of dendritic cells and modulating type 1 versus type 2 T cell responses. For instance, eosinophil synthesis of IL-4 facilitates the differentiation of CD4 + T cells into a Th2 phenotype. IL-4 production by Th2 cells in turn facilitates the recruitment of more eosinophils suggesting a positive feedback (reviewed in [45–47]). Eosinophils also produce the enzyme indoleamine 2,3 dioxygenase (IDO), which promotes apoptosis of Th1 cells further favoring a type 2 environment [48]. One contrasting report suggests that eosinophils produce the Th1-promoting cytokines IFN- γ and IL-12, but this has been only documented during experimental infection with the fungus *Cryptococcus neoformans* [49]. Hence, more studies are needed to elucidate the spectrum of eosinophil activity in T cell development.

Eosinophils in leishmaniasis

Eosinophils have been associated with immune responses to helminthic parasites [45]. Usually, eosinophils are not implicated in the immune response against protozoa because

their levels are not elevated during these infections. However, several lines of evidence support the involvement of eosinophils during infection with the *Leishmania* spp. protozoa. In vitro, eosinophils are chemoattracted toward *L. amazonensis* promastigotes, and in vivo studies have demonstrated eosinophil recruitment to the infection site of *L. amazonensis* and *L. major* [50, 51]. During the initial hours of *L. mexicana* infection, eosinophils have been observed close to degranulating mast cells at the site of parasite inoculation, suggesting a role in parasite clearance [52]. Finally, it has been demonstrated that eosinophils have anti-microbial activity against *L. mexicana* and *L. donovani* promastigotes [53, 54].

A contribution of eosinophils during leishmaniasis has recently been examined in more detail by de Oliveira et al. [50]. This group showed that infection with *L. amazonensis* causes an eosinophil-rich infiltrate in primary lesions and draining lymph nodes. An abundance of local eosinophils is observed from 3 h to at least 120 days, suggesting a role throughout infection. Interestingly, lesions from resistant mice show more local eosinophils at the lesion site than mice with a susceptible background. In resistant mice, the increased presence of eosinophils correlates with a lower parasite load and less ulceration in *L. mexicana* infection [50]. The inverse correlation between cure and eosinophil levels was also observed in studies of canine *L. infantum* infection, in which dogs with a more robust eosinophil infiltration and higher expression of the eosinophil recruiting chemokine, CCL24, had a lower parasite density [55]. Similarly, *L. braziliensis* patients in the early phase of lymphadenopathy exhibit a higher proportion of neutrophils and eosinophils in their cellular infiltrate than those from patients showing skin ulcers and in the late phases of lymphadenopathy [56]. However, it cannot be ruled out that additional factors such as co-infections with parasitic helminthes could contribute to the increased eosinophil levels seen in these patients.

Related experiments demonstrated that mice deficient in toll-like receptor 2 (TLR-2) recruit more eosinophils to the site of *L. amazonensis* infection than their wild-type counterparts. The increased eosinophil infiltration correlates with the presence of eosinophil-rich granulomas and lower parasite load [57]. It is hypothesized that eosinophils could directly phagocytose and kill *Leishmania* spp. Furthermore, it has also been proposed that eosinophils might enhance the anti-microbial activity of macrophages [51, 52, 58, 59].

In the mouse, macrophage stimulation with IFN- γ leads to the production of nitric oxide (NO $^{\circ}$), the most potent anti-leishmanial molecule [60–63]. Similarly, stimulation of peritoneal eosinophils with IFN- γ results in the killing of *L. major* in a NO $^{\circ}$ dependent manner [58]. In contrast, studies in an *L. amazonensis* infection model showed that the microbicidal activity of eosinophils is independent of NO $^{\circ}$ production [59]. It is unclear whether these discrepancies are due to differences between *Leishmania* species (*L. major* vs. *L. amazonensis*) or between hosts (rat vs. mouse).

Finally, some studies suggest that eosinophils are influenced by the host background. During *L. amazonensis* infection, the eosinophil infiltrate is greater in resistant C3H mice than in susceptible strains [50]. In contrast, during *L. major* infection, the influx of eosinophils is higher in susceptible BALB/c than in resistant C57BL/6 mice [64]. The discrepancies seen between different studies might be a consequence of the different species of parasites used.

Interplay of eosinophils and cytokines during *Leishmania* spp. infection

In a study of cytokine production by peripheral blood cells from subjects with various phenotypes of infection, Peruhype-Magalhaes et al. [65] attempted to discern responses of neutrophils, eosinophils, NK cells and monocytes to soluble *Leishmania* antigen (SLA). The study included individuals with (1) active or (2) cured visceral leishmaniasis, (3) individuals with skin test evidence of asymptomatic *L. chagasi* infection and (4) noninfected controls. The identity of blood cells and their intracellular cytokine expression was analyzed by flow cytometry. The authors stated that eosinophils in all groups produced higher IL-10 and lower TNF- α in response to SLA. Furthermore, eosinophils from subjects with symptomatic visceral leishmaniasis down-modulated the type 1 cytokines IL-12 and IFN- γ in response to SLA. Overall, this study suggested that the eosinophil cytokine pattern mirrored the clinical status. However, since the method used to identify cells as eosinophils was based on CD16 stain and scatter characteristics, more studies are needed before drawing further conclusions.

IL-5 stimulates eosinophil maturation and migration [66], and its role in eosinophil responses has been studied using the IL-5 transgenic C3H/HeN mice in which eosinophils constitute 50 % of peripheral leukocytes [67]. Infection of wild-type C3H/HeN mice with *L. amazonensis* results in gradually enlarging, non-healing lesions with large parasite loads. In contrast, IL-5 transgenic mice have footpads that are closer in size to noninfected controls, and their lesions showed very few parasites [59]. The IL-5-mediated resistance to infection could not be over-ridden by administration of IL-4, a strong suppressor of Th1 development, suggesting a role for eosinophils in suppressing the developing lesion and diminishing the parasite load. The addition of either IL-4 or IFN- γ to *L. amazonensis*-infected eosinophils results in increased parasite killing, suggesting eosinophils might directly kill the parasite. Inclusion of inhibitors indicated that the anti-leishmanial activity of eosinophils is independent of superoxide and nitric oxide; instead, it is exerted through H₂O₂ [59].

Some studies have shown an initial influx of eosinophils at the site of *Leishmania* spp. infection [50, 57, 64]. Because eosinophils can express a variety of cytokines, including TNF- α , IL-4, IL-5, IL-10, IL-12 and IL-13, they could play important roles in the onset of local immune responses [68] (and reviewed in [47, 68]). However, the role of eosinophils, later in the immune response, has not been established. In fact, patients with active visceral leishmaniasis suffer from leukopenia reflected in all major subpopulations including eosinophils [65, 69].

Overall, these studies suggest that the initial eosinophil influx at the infection site may participate in the immune response against *Leishmania* spp. possibly by controlling the parasite load, impacting the functions of other innate immune cells, limiting the damage associated with the corresponding local inflammatory response as well as shaping the onset of adaptive immunity. Yet further studies are needed to delineate the mechanisms by which the initial influx of eosinophils influences the downstream disease status.

Mast cells

The initial lines of defense against invading pathogens are physical and chemical barriers. Examples of chemical barriers include the characteristic pH of the skin and stomach,

whereas physical barriers include the skin and mucous membranes, both of which are lined with mast cells. In particular, it is estimated that the density of mast cells in the skin is about 3,000–7,000 cells/mm³ ([70] and reviewed in [71]). Hence, mast cells are located at the interface of the host and its environment, which is the foremost setting to initiate immune responses. For a long time, mast cells were only implicated in mediating allergic responses, but emerging evidence indicates that they play key early roles in innate and adaptive immune responses. First, mast cells can produce nitric oxide and a large array of pro-inflammatory molecules, many of which are stored within cytoplasmic granules and released within minutes of activation [72–74]. Second, mast cells can recognize and phagocytose microbes through CD48, complement receptors and toll-like receptors [22, 75–77]. In fact, increasing evidence demonstrates that exposure of mast cells to bacterial, viral and parasite products induces secretion of preformed mediators and/or the synthesis of cytokines and chemokines. The nature of the particular mast cell receptors and pathogen products implicated in these responses is an area of active investigation [72, 75, 77–79] and reviewed in [71, 73, 74, 76].

In vitro studies of mast cells in *Leishmania* infection

Bone marrow-derived mast cells (BMMC) can be generated by incubating bone marrow cells for 21 days in the presence of IL-3. Confirmation of mast cell differentiation is attained by identification of metachromatic granules upon staining with toluidine blue solution [75]. Bidri et al. [75] have shown that BMMC are able to bind and internalize *L. major* and *L. infantum* promastigotes. Although the receptor(s) mediating these interactions have not been identified, receptor-mediated interactions are suggested by data showing that attachment of non-opsonized stationary phase promastigotes is saturable and plateaus at a multiplicity of infection (MOI) of ten. BMMC can sustain *Leishmania* infection as demonstrated by their ability to support amastigote conversion and abundant parasite replication. In fact, after 72 h of infection with either *L. major* or *L. infantum*, about 80 % of BMMC have ruptured due to parasite replication. Furthermore, infection with live, virulent promastigotes leads to the release of preformed β -hexosaminidase and TNF- α , and to *de novo* synthesis of TNF- α . In contrast, incubation with attenuated or heat-killed *L. major* or *L. infantum* promastigotes fails to induce the release of preformed mediators or induce cytokine production. These results led Bidri et al. [75] to suggest that mast cells are important cells in the first line of defense against dermally inoculated *Leishmania* spp.

In addition, cytokines released from mast cells can have long-term effects on *Leishmania* infection. Degranulation or priming of mast cells leads to the release of an array of cytokines whose patterns vary according to the activation stimuli. The products released by mast cells include TNF- α , IFN- γ and IL-12p70 which are associated with type 1 responses as well as IL-4, IL-10 and TGF- β which are associated with type 2 responses [73, 80, 81]. Products from type 1 responses correlate with a curative outcome, whereas those associated with type 2 are associated with disease [73, 80–82]. Upon infection, mast cells are among the first cells to interact with *Leishmania* spp.; hence, it can be speculated that their ability to secrete cytokines known to bias the adaptive immune response will have major implications in the outcome of disease.

In vivo studies suggesting that mast cells exacerbate leishmaniasis

In vivo infection with 10^5 *L. major* promastigotes subcutaneously in the footpad as well as intradermally in the ear pinna induces extensive degranulation of dermal mast cells in both genetically resistant and susceptible mouse strains [83]. The kinetics of cutaneous lesion development is similar between mast cell-deficient WBB6F, – *WTW*^v and wild-type WWBB6F mice. However, the lesions are significantly smaller in mast cell-deficient mice. However, intradermal inoculation of syngeneic mast cells prior to infection, significantly increased lesion size in mast cell-deficient mice. Since cutaneous leishmaniasis symptoms are a result of a local inflammatory response, these results suggest that mast cells are in part responsible for mediating the vigorous local inflammatory lesion induced by *L. major* infection [83].

There is evidence that mast cells favor type 2 responses through histamine, IL-4 and IL-13 release [78] (and reviewed in [73]). Based upon this scenario, Romao et al. [84] hypothesized that mast cells could exacerbate leishmaniasis in the murine model. To test this prediction, mice were treated with the G-protein activating agent 48/80 to degranulate mast cells, releasing histamine and depleting mast cell activity before *L. major* infection [85]. Prior degranulation of mast cells with 48/80 significantly reduced *L. major* lesion size in both resistant C57BL/6 and susceptible BALB/c mice. 48/80 treatment also increased transcripts implicated in type 1 responses (IFN- γ , CCL2, CCL5 and iNOS) and decreased the abundance of transcripts encoding the type 2 cytokine IL-4 [84]. The decrease in *L. major* lesion size induced by mast cell degranulation was also observed in IL-4 KO BALB/c mice, suggesting that the effect is independent of IL-4. These results led the authors to hypothesize that mast cell degranulation before *L. major* infection reduces not only their ability to exacerbate inflammation through histamine release, but also their ability to promote type 2 adaptive responses, which would antagonize the development of a curative type 1 immune response to the parasite [84].

In vivo studies implicating mast cells in protection against leishmaniasis

An effective immune response in *Leishmania* spp. infection involves the formation of granulomas around the parasite [18, 86]. TNF- α plays an essential role in skin inflammation by recruiting leukocytes to the sites of cutaneous granulomas [87–89]. Using mast cell-deficient Kit^W/Kit^{W-v} mice, von Stebut et al. [79] showed that deficiency in either mast cells or the mast cell product TNF- α , causes a decrease in both macrophage and neutrophil recruitment to polyacrylamide gel (PAG)-induced cutaneous granulomas. Intradermal inoculation of mast cells derived from wild-type, but not TNF- α ^{-/-} mice, restored the influx of neutrophils to cutaneous granulomas. The release of MIP-1 α and MIP-1 β (CCL3) by neutrophils, in turn, facilitates the recruitment of macrophages to the developing cutaneous granulomas. These results have led the authors to conclude that mast cells support granuloma formation through neutrophil recruitment [79].

Consistent with the hypothesis that mast cells contribute to the development of granulomas and subsequent control of *L. major* infection, mast cell-deficient Kit^W/Kit^{W-v} mice develop larger skin lesions and higher parasite loads than control Kit^{+/+} mice [90]. Mast cell deficiency is associated with decreased migration of neutrophils, macrophages and dendritic

cells into infected lesions. In addition, mast cell deficiency leads to a decreased production of the Th1-promoting cytokine IL-12. Consistently, the systemic immune response in Kit^W/Kit^{W-ν} mast cell-deficient mice is biased toward a type 2 cytokine profile with reduced IFN-γ and increased IL-4 [90] (and reviewed in [91]). Reconstitution of Kit^W/Kit^{W-ν} mice with competent bone marrow-derived mast cells either at the lesion site or in the opposite ear is sufficient to restore the wild-type phenotype [90]. Based on these results, Maurer et al. [90] hypothesize that mast cells guide the systemic immune response to *Leishmania* spp. through the sequential recruitment of neutrophils and macrophages to the lesion site.

Host genetic background as a factor affecting mast cell functions

Several studies suggest that variations in host background influence the recruitment and functional role of mast cells during infection. For example, after *L. donovani* infection, the percentage of mast cells in the spleen infiltrate varies between resistant C57BL/6 and susceptible BALB/c mice. In particular, the infiltrate of mast cells is low in C57BL/6 but high in BALB/c mice. In BALB/c mice, the increase in mast cell numbers is IL-3 dependent [92]. These results agree with studies showing that IL-3 exacerbates *L. major* infection in susceptible BALB/c mice [22, 93, 94].

To examine the interplay between cytokines and mast cell functions, *L. donovani*-infected macrophages of BALB/c origin were incubated with supernatants from either BALB/c or C57BL/6 mast cells. Supernatants from C57BL/6 mast cells exhibited high levels of TNF-α and decreased parasite load in BALB/c macrophages. In contrast, supernatants from BALB/c mast cells increased the macrophage parasite load in an IL-3- and IL-4-dependent manner [22]. Similar host-specific variations were observed in the recruitment and function of mast cells after *L. major* infection. In particular, the upper dermis of susceptible BALB/c has a higher and more prolonged mast cell infiltrate than that of resistant CBA/T6T6 mice. However, despite a lower mast cell infiltrate, CBA/T6T6 mice have higher numbers of degranulating mast cells [22]. Taken together, these results suggest that intrinsic host-specific differences drive the immune responses of mast cells and their effects on macrophages.

Similarly, studies of *L. mexicana* infections further support the hypothesis that the host background frames the immune response of mast cells [95]. First, mast cells from C57BL/6 mice express higher levels of TLR2, TNF-α and IL-10 than those from BALB/c in basal conditions as well as upon stimulation with *L. mexicana* LPG. Second, the numbers of mast cells infiltrating and degranulating at the lesion site differ between susceptible and resistant mice. Mast cell infiltration peaks at around the third day in susceptible BALB/c mice, but in genetically resistant C57BL/6 mice, mast cell infiltration peaks after 1 week and remains high for at least 8 weeks. These results have led the authors to hypothesize that a high influx of mast cells in the initial stages of infection facilitates disease progression [95]. These observed differences in the kinetics of mast cell infiltration and mast cell gene expression lead us to hypothesize that there is a direct relationship between mast cell phenotype and host susceptibility.

Additional pathogen and host factors affecting mast cell functions

The complexity in the functions of mast cells during *Leishmania* infection goes beyond differences in host background. In a clinical study of human cutaneous leishmaniasis, higher numbers of intralesion mast cells correlated with faster healing [96]. However, the relative numbers of mast cells varied depending on the causative species of *Leishmania*. In particular, lesions from *L. V. braziliensis* show the highest numbers of mast cells, whereas lesions caused by *L. amazonensis* show the lowest. The authors speculate that variations in the chemokines and/or cytokines induced by the parasite might result in the differences observed in mast cell infiltrates. However, it cannot be excluded that the genetic background of the patients was also a factor. To complicate matters, another factor influencing the number of intralesion mast cells is the patient's age [96]. Indeed, this and several other reports indicate that the number of mast cells decreases in older subjects [97, 98]. Both murine and human clinical studies indicate that mast cells play a role in the inflammatory response during cutaneous leishmaniasis, but the extent of infiltration and the functional phenotype of mast cells varies depending on both the *Leishmania* species and the host origin.

Histamine

The role of mast cells and their hallmark product, histamine, has expanded beyond allergic reactions to include varied aspects of the immune response. Binding of IgE antibodies to surface IgE receptors triggers a signaling cascade that results in the release of histamine from mast cells granules [99]. However, it is becoming apparent that after cytokine stimulation, other immune cells such as dendritic cells (DC), T cells, neutrophils and macrophages can synthesize histamine “*de novo*.” This newly formed histamine is synthesized by histidine decarboxylase (HDC) (for a detailed review see [100, 101]). HDC expression, and hence the potential for histamine production, varies according to host-specific differences. For instance, susceptible and resistant mice differentially regulate HDC RNA during *L. major* infection. The differences in HDC expression become apparent in the chronic phase, i.e., 2–6 months after infection. Resistant C57BL/6 mice express low levels of HDC in the lymph nodes, spleen and liver. In contrast, susceptible BALB/c express higher levels of HDC which correlates with high parasite loads. However, it still needs to be determined whether the increased expression of HDC is a cause or an indicator of disease in susceptible animals [102].

Histamine can also influence *Leishmania* infection through its effects on other immune cells. Neutrophils, basophils, B cells, DC and T cells express histamine receptors, and as such, their immune functions could be responsive to histamine [103, 104]. In particular, signaling through the histamine receptor H₁ favors the differentiation of T cells into Th1, whereas signaling through the histamine receptors H₂–H₄ is mostly associated with the development of Th2 cells [101, 104, 105]. Given the importance of type 1 curative and type 2 exacerbatory responses in cutaneous leishmaniasis due to *L. major* [19], the receptors involved in histamine signaling could bias the adaptive immune response during leishmaniasis.

Histamine release can facilitate Th2-type responses by modulating cytokine production, i.e., promoting IL-10 while inhibiting IL-12 [105, 106]. In addition, histamine is a chemoattract for neutrophils, a phagocytic cell that is hypothesized to serve as a “safe haven” early in *Leishmania* infection [103, 107]. Considering this capacity, histamine release during the initial stages of *Leishmania* infection could facilitate a robust neutrophilic cell infiltrate that could promote parasite survival [90, 107] (and reviewed in [73]). In resistant mice, in which the infiltration and degranulation of mast cells occur at later times than susceptible mice, this role would differ. Indeed, data from *L. mexicana*, *L. donovani* and *L. major* infection show that there are host-specific differences in mast cell recruitment, degranulation and cytokine secretion. It is hypothesized that these differences facilitate parasite survival in susceptible mice but promote parasite clearance from their resistant counterparts [22, 95]. In contrast, a study of *L. amazonensis* infection showed that both resistant and susceptible mice had similar levels of mast cell infiltration during the initial stages of infection [50]. Whether these discrepancies merely reflect different immune responses to different *Leishmania* species or to other factors remains to be defined.

Histamine modulates the cytokine environment

During *L. major* infection, the decision whether the predominant CD4 + T cell response will develop into either a protective type 1 or an exacerbatory type 2 responses is established during the initial 48 h infection, highlighting the importance of early events in the outcome of infection [109] and (reviewed in: [108]). The local cytokine environment in which cells are exposed to an antigenic stimulus can lead to divergent results. Interestingly, during *L. major* infection, IL-4 can have differential effects during activation of CD4 + T cells and dendritic cells [110]. Similarly, histamine can have divergent effects in the adaptive immune response when present at different times. It has been documented that histamine can support type 2 immune responses by inhibiting IL-12 and increasing IL-10 production [101, 105]. However, the net effect of histamine on T cell differentiation depends on (1) the timing of histamine exposure with respect to T cell differentiation and (2) the particular histamine receptor expressed on the target cell [103]. Signaling through the histamine receptor H1 favors the differentiation of CD4 + T cells into antigen-responsive Th1 cells. In contrast, an early histamine signal through the histamine receptors H2–H4 leads to the induction of type 2 responses [103, 111]. However, later in the immune response, histamine signaling through the H₂ receptor inhibits the differentiation of both Th1 and Th2 cells [101, 105] and reviewed in [100, 104].

Chronic *L. major* infection in susceptible BALB/c mice leads to production of the type 2 cytokines IL-13 and IL-4. Both IL-13 and IL-4 stimulate the proliferation of mast cells and prevent their apoptosis [82, 112]. IL-4 also induces high levels of B cell-derived IgE, which has the dual effects of increasing mast cell proliferation and mast cell activation through ligation of FcεR receptors. Mast cell activation through their FcεRI receptors increases HDC gene expression, the enzyme involved in histamine synthesis [101, 113]. If *Leishmania* lesions indeed have high numbers of IgE secreting plasma cells as a small clinical study suggested [114], one could hypothesize that during the chronic phase of cutaneous leishmaniasis, there is a positive feedback between mast cell effector functions and adaptive type 2 responses.

At least four lines of evidence support the role of histamine in deterring pro-type 1, curative responses during *Leishmania* infection. First, histamine destabilizes IL-18-induced interactions between monocytes and T cells and inhibits the production of IL-12, TNF- α and IFN- γ [113, 115]. Second, histamine decreases IFN- γ transcription in cultures of human peripheral blood mononuclear cells as well as in T cell-enriched fractions [111]. Third, studies in human monocytes have shown that histamine decreases bioactive IL-12 p70 while increasing IL-10 secretion [115]. IL-12 is necessary for a curative response, whereas IL-10 is an immunosuppressive cytokine that exacerbates leishmaniasis [116]. Finally, ligation of mast cell Fc ϵ RI receptors leads to the release of IL-4, IL-5, IL-6 and IL-13, which promote type 2 responses (reviewed in [73]). Based on these results, de Oliveira et al. [78] have speculated that the release of histamine and IL-4 resulting from the interactions between mast cells and promastigotes facilitates parasite survival and prolongs the course of *L. major* infection.

To complicate matters, interactions between mast cell and *L. major* or *L. infantum* promastigotes induce the release of preformed TNF- α and lead to TNF- α *de novo* synthesis [75]. TNF- α is a priming signal for macrophage classical activation characterized by pro-inflammatory responses and the induction of microbicidal products (reviewed in [117, 118]), but it is elevated during visceral leishmaniasis, and its overproduction drives some of the clinical wasting symptoms of visceral leishmaniasis [119]. Furthermore, histamine has been reported to facilitate Th1 differentiation through H1 receptor signaling [101, 105, 106] (and reviewed in [100]). It has been well documented that in *L. major* infection, differentiation of CD4⁺ T cells into Th1 promotes a curative response, whereas differentiation into Th2 exacerbates disease [82, 120]. Overall, one could surmise that through its effects on other immune cells, particularly T cells and their cytokine expression, mast cells can have major implications in driving immune responses throughout *Leishmania* infection.

Concluding remarks

It is becoming apparent that the initial interactions between a pathogen and its host set the stage for the development of long-term immunity or lack thereof. Among the earliest interactions of a pathogen with the immune system will be those involving mast cells as they are part of the resident cells at the interface of the host and its environment. In addition to support *Leishmania* spp. infection, the ability of mast cells to release preformed molecules and synthesize inflammatory mediators upon parasite contact suggests that they can profoundly impact the recruitment and activation of other immune cells. Cells recruited by the fast action of mast cells preformed mediators include neutrophils, a phagocytic cell with the ability to host *Leishmania* spp. at least temporarily, and macrophages, the ultimate host cells of the parasite. In addition, the early inflammatory infiltrate at the site of *Leishmania* spp. infection includes eosinophils. Besides their anti-leishmanial activity, the secretion of immunomodulatory cytokines by eosinophils makes them an important force in shaping the local and systemic response to leishmaniasis. In summary, eosinophils and mast cells influence the immune response to *Leishmania* spp. infection through their direct anti-microbial actions, recruitment and activation of other immune cells, controlling the tissue damage caused by the local inflammatory response, and biasing the development of the systemic, long-term, adaptive immune response.

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Abbreviations

BMMC	Bone marrow-derived mast cells
CR3	Complement receptor 3
DC	Dendritic cells
DTH	Delayed-type hypersensitivity
GIPLs	Glycoinositolphospholipids
HDC	Histidine decarboxylase
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
KO	Knock out
LCF	Leishmania chemotactic factor
LPG	Lipophosphoglycan
MIP	Macrophage inhibitory protein
MOI	Multiplicity of infection
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSP	<i>Leishmania major</i> surface protease, also called GP63
NO	Nitric oxide
PAG	Polyacrylamide gel
PPGs	Proteophosphoglycans
SLA	Soluble Leishmania antigen
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor- α
TGF-β	Transforming growth factor- β

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