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Minireview

Type 1 and type 2 responses to *Leishmania major*

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Abstract

Leishmania major is a protozoan parasite that is transmitted to the mammalian host by its sand fly vector when the fly probes in the host's skin for a blood meal and injects the parasite within its saliva. In mice experimentally infected with *L. major*, outgrowth of CD4 type 1 (Th1) cells leads to resolution of the infection, but outgrowth of type 2 (Th2) cells exacerbates disease. To design an effective vaccine against the parasite (and other pathogens that induce polarized Th1 and Th2 responses), we must determine the mechanism underlying this phenomenon so that we can design the vaccine to elicit the appropriate (i.e., protective) Th cell. Recent work indicates that Th bias is influenced by a number of signals delivered by antigen-presenting cells, including cytokines and co-stimulatory molecules. Moreover, recent work also suggests that sand fly saliva influences the immune response to *L. major* and Th polarization. Determining the mechanisms that lead to polarized Th responses should expand our knowledge regarding immunity to *L. major*, and should add to our understanding of immunoregulation in general.

Keywords: Leishmaniasis; Th cell; Antigen-presenting cell; Cytokine; Co-stimulation; Sand fly

1. *Leishmania* and leishmaniasis

Members of the genus *Leishmania* are sand fly-transmitted protozoan parasites that cause leishmaniasis in their vertebrate hosts. The parasite is transmitted to the host when the sand fly vector probes in the skin for a blood meal and injects the promastigote form of the parasite within its saliva. Importantly, the saliva dramatically enhances the infectivity of the parasite for the host. When a large number of parasites (10^4–10^6) are injected into experimental mice, saliva markedly enhances infection compared to the infection in saliva-free control animals. When the number of parasites injected by the sand fly ($\sim$100) is injected, the parasite does not survive unless it is co-injected with sand fly saliva [1]. Thus, saliva may be critical for natural transmission of *Leishmania* by sand flies. Sand flies that transmit the parasite in the Old World are of the genus *Phlebotomus* and those that transmit *Leishmania* in the New World are of the genus *Lutzomyia*.

Leishmaniasis currently afflicts some 12 million individuals, with 350 million at risk [2]. In addition, HIV has compounded the acquisition/re-activation of leishmaniasis, and recent epidemics of leishmaniasis in such places as Sudan have been particularly devastating [2]. Moreover, there are still no effective control measures for the disease.

Within the mammalian host *Leishmania* resides as an amastigote in phagocytic cells such as macrophages, dendritic cells and neutrophils (reviewed in [3–5], space limitations do not permit a thorough survey of the literature). The clinical manifestations of leishmaniasis depend not only upon the species of parasite infecting the host, but the general health and genetic constitution of the infected individual. In general, parasites that cause cutaneous leishmaniasis (*Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica* in the Old World and *Leishmania braziliensis* and *Leishmania mexicana* in the New World) induce

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a lesion at the site of the insect bite, which takes months to heal.

2. The Th1/Th2 paradigm in experimental mice infected with *L. major*

Infection of experimental mice with *L. major* promastigotes is perhaps the best-studied model of a chronic infectious disease that involves activation of CD4 type 1 (Th1) and type 2 (Th2) cells. Th1 and Th2 cells can be distinguished by the cytokines they secrete: Th1 cells secrete activators of cell-mediated immunity such as interferon (IFN)-γ, while Th2 cells secrete cytokines such as interleukin (IL)-4 which promote antibody responses. Most strains of mice (C57BL/6, C3H, CBA) develop a self-limiting cutaneous disease when infected with *L. major*. In these mice, resolution of infection is mediated by Th1 cells that produce IFN-γ. IFN-γ induces production of nitric oxide (NO) in phagocytic cells that harbor *L. major* (principally macrophages) which leads to destruction of the parasite. Therefore, infection with *L. major* in these strains of mice resembles self-limiting cutaneous leishmaniasis in humans.

In contrast, other strains of mice (notably, BALB/c) develop a Th2 response following infection with *L. major*. Within minutes to hours after infection with *L. major*, Vβ4Vα8 CD4 T cells in BALB/c mice produce IL-4 mRNA in response to a single parasite antigen, LACK (*Leishmania* homolog of receptors for activated C kinase). IL-4 down-regulates expression of the β2 subunit of IL-12 receptors on potentially protective Th1 T cells. As a result the cells become unresponsive to IL-12 and production of IFN-γ and NO is inhibited. Thus, *L. major* parasites within macrophages are not killed. Rather, IL-4 promotes the outgrowth of Th2 T cells, which stimulate the production of parasite-specific antibodies [3–5].

It should be noted however that the explanation for the susceptibility of BALB/c mice to infection with *L. major* may be more complicated than that outlined above. For example, in certain cases IL-4-deficient BALB/c mice (e.g., see [6]) are still susceptible to infection with the parasite. This observation may in part be explained by the fact that IL-4-deficient BALB/c mice are more or less susceptible to infection with *L. major* depending upon the substrain of parasite used to infect the mice, since some strains of *L. major* induce a full-blown infection in IL-4-deficient BALB/c mice while others do not [7]. Thus, the susceptibility of BALB/c mice to infection with *L. major* may be dependent upon the ability of the parasite to induce not only the production of IL-4, but also other cytokines such as IL-13 [8] and IL-10 [9,10], which also promote susceptibility to infection with the parasite. Finally, it has been reported that BALB/c mice that bear a transgenic β2 subunit of the IL-12 receptor are still susceptible to infection with *L. major*, which calls into question the notion that down-regulation of the β2 subunit of the IL-12 receptor is a key prerequisite for the susceptibility of BALB/c mice to infection with the parasite [11].

Nevertheless, in general, Th1 cell-mediated immune responses control intracellular infections such as leishmaniasis and tuberculosis, while Th2 antibody-mediated immune responses are best suited for extracellular pathogens such as intestinal worms.

2.1. The influence of co-stimulatory molecules on Th bias

Activation of T cells requires two signals: cognate recognition of the antigen for which the T cell is specific (which can be accomplished by interactions between the major histocompatibility complex and the T cell receptor) and a co-stimulatory signal(s), normally provided by antigen-presenting cells. Since *L. major* resides in the phagolysosome of antigen-presenting cells and the phagolysosome also contains major histocompatibility complex class II molecules, the predominant T cell response to the parasite is a CD4; however, other T lineage cells are involved in this response such as CD8, NK and γδ T cells.

Dendritic cells and macrophages are antigen-presenting cells that play a central role in leishmaniasis. Dendritic cells harbor few parasites but are highly efficient at stimulating naive T cells. In contrast, macrophages are avid scavengers of *Leishmania* but are permissive to infection unless they are stimulated by cytokines (e.g., IFN-γ; [12]). It has been shown that skin dendritic cells (known as Langerhans cells) can migrate from a site of infection with *L. major* to the draining lymph node where they can stimulate *L. major*-specific T cells [12]. During this migration and their subsequent interaction with T cells, dendritic cells mature into potent antigen-presenting cells. This is in part mediated through interactions between CD40 (expressed on dendritic cells) and CD40 ligand (CD40L, expressed on T cells) and this leads to changes in expression of major histocompatibility complex class II and B7s (CD80 and 86 [13]). B7s are expressed on antigen-presenting cells and their ligands (CD28 and CTLA-4) are expressed on T cells. Ligation of CD28 by B7s activates T cells, however ligation of CTLA-4 can deliver a negative signal to the T cell, thus limiting T cell expansion. A summary of the role of molecules such as CD40 and B7 as well as cytokines in inducing *L. major*-specific Th1 and Th2 cells is presented in Fig. 1.

It has been shown that CD40 and CD40L are required for resistance to *L. major* infection since CD40 and CD40L-deficient mice are highly susceptible to infection with *L. major* [14,15]. In contrast, the role of B7s (CD80 and 86) is less clear. Although B7–CD28 interactions appear to have little effect on the outcome of *L. major* infection in mice, treating with CTLA-4Ig reversed the disease phenotype in susceptible but not in resistant mice, suggesting that interactions between B7s and CTLA-4 are important in the immune response to *L. major*. Sub-

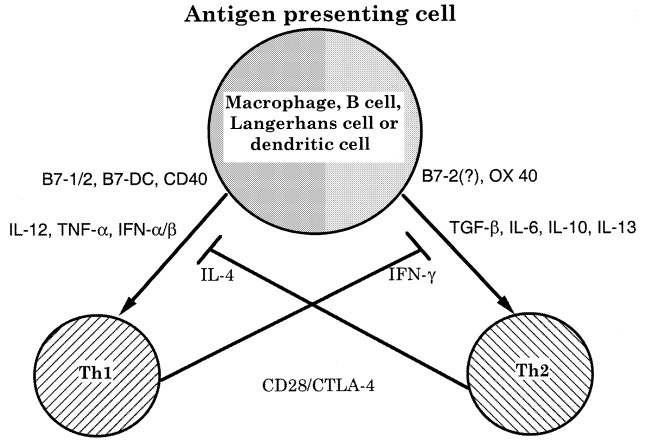

Fig. 1. Co-stimulatory molecules and cytokines that influence *L. major*-specific Th priming. B7-1, B7-2 (and their receptors on T cells: CD28 and CTLA-4) and CD40 are important in Th1 priming. In most but not all cases B7-2 is important in Th2 priming (see text); OX40 is also important for Th2 priming. IL-12, TNF-α and IFN-α/β promote the development of Th1 responses, while transforming growth factor (TGF)-β, IL-6, IL-10 and IL-13 promote the development of Th2 responses. IFN-γ (an important component of a Th1 response) can block the outgrowth of Th2 cells, while IL-4 (an important component of a Th2 response) can block the outgrowth of Th1 cells.

sequent work (with one exception, [16]) confirmed that both CD80 and 86 are involved in activation of T cells in mice infected with *L. major* (discussed in [17]).

Indeed our own recent work supports an important role for dendritic cells/Langerhans cells and B7 expression in immunity to *L. major* [18]. When we infected mouse epidermal cells enriched for Langerhans cells with *L. major* (which we feel is the best model to study since *L. major* would encounter all of these cell types when it is injected into the skin of mice), we found that CD40 expression was unchanged, but B7-1 expression was reduced on BALB/c (susceptible) Langerhans cells while B7-2 expression was reduced on C3H (resistant) epidermal cells.

Interestingly, while resistant C3H T cells did not produce IL-4 unless stimulated by BALB Langerhans cells/epidermal cells, BALB T cells made IL-4 whether stimulated by syngeneic or congenic Langerhans cells/epidermal cells (Fig. 2). However, in all cases blockade of B7-2 inhibited IL-4 production. Taken as a whole, these data suggest that while both B7-1 and B7-2 on Langerhans cells are involved in co-stimulating IFN-γ production, B7-2 alone co-stimulates IL-4 production. This result agrees with our previous results, which showed that treating *L. major*-infected mice with anti-B7-2 was protective for the mice and decreased IL-4 production [19]. The results discussed above also demonstrate that dynamic interactions can occur between skin antigen-presenting cells and responding T cells. Indeed, when resistant C3H T cells are stimulated with C3H skin antigen-presenting cells, they, as expected, do not produce IL-4, but the T cells do make IL-4 when they are stimulated with susceptible BALB.K skin antigen-presenting cells. Therefore, the antigen-presenting cell environment can reverse the phenotype of responding T cells [18].

Finally, recent experiments have shown that the co-stimulatory molecule OX40 (Fig. 1) is critical for Th2 development in susceptible BALB/c mice infected with *L. major* [20], and B7-DC (which is expressed exclusively on dendritic cells) is a potent signal for IFN-γ production [21].

2.2. The influence of cytokines on Th bias

Perhaps the most important cytokine involved in resistance to *L. major* in mice is IL-12. IL-12 induces IFN-γ production by T cells and NK cells and IFN-γ can induce the production of NO and parasite clearance by macrophages. Dendritic cells are perhaps the most important source of IL-12 (discussed in [22]). *L. major* has been shown to induce IL-12 production by dendritic cells and

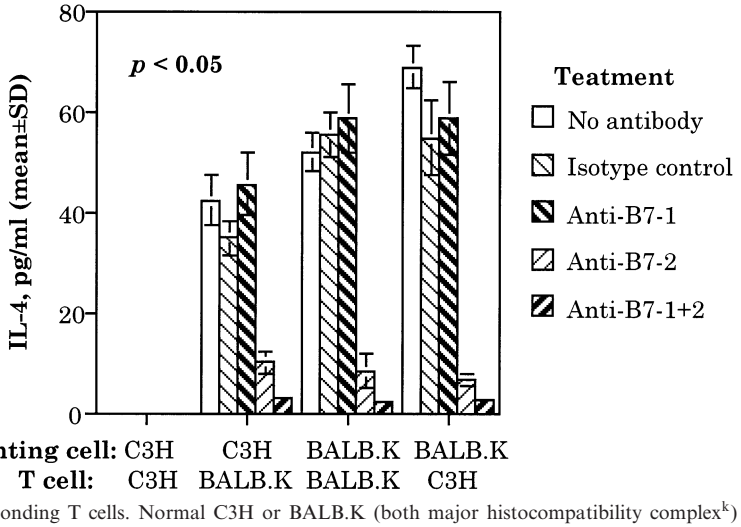

Fig. 2. B7-2 induces IL-4 production by responding T cells. Normal C3H or BALB.K (both major histocompatibility complex^k) T cells were stimulated with *L. major*-infected syngeneic or congenic Langerhans cells/epidermal cells in the presence or absence of B7-1- or B7-2-blocking antibodies. The resulting IL-4 was measured by ELISA. Anti-B7-2-blocking antibody significantly ($P < 0.05$) inhibits the production of IL-4. Drawn from reference [18].

Table 1
Known immunomodulatory effects of sand fly salivary-gland lysate (Old World *P. papatasi* and New World *Lutzomyia longipalpis*) and *L. longipalpis* MAX

Effect	*P. papatasi*	*L. longipalpis*	MAX
Exacerbation of *L. major* infection	Yes	Yes	Yes
Inhibition of T cell activation	n.d.	Yes	Yes
Up-regulation of IL-4	Yes	n.d.	n.d.
Up-regulation of prostaglandin E_2, IL-10 and IL-6	n.d.	n.d.	Yes
Inhibition of macrophage activation (down-regulation of TNF-α, NO, H_2O_2)	Yes	Yes	Yes
Vaccine candidate	Yes	Yes	Yes

n.d. = not done. Summarized from [1,30–35].

in turn these cells can stimulate many T cells due to the large surface area of the dendritic cells. In contrast to the beneficial effects of IL-12, IL-4 is perhaps most responsible for disease progression in mice infected with *L. major*. As discussed above (Section 2), an early anti-LACK response in susceptible BALB/c mice leads to production of IL-4, down-regulation of IL-12 receptors and ultimately death of the mice [3–5]. In addition, treating with IL-12 or with anti-IL-4 allows BALB/c mice to heal an infection with *L. major* [3–5], which demonstrates how these two cytokines can literally have life or death effects in infected mice.

However, in addition to IL-12 and IL-4, several other cytokines have marked effects on infection with *L. major* in mice (Fig. 1). For instance, tumor necrosis factor (TNF)-α is critical for resolution of a *L. major* infection since infection with the parasite in TNF-α knockout mice is fatal [23]. Among the many ways in which TNF-α may play a role, the most obvious is its ability to enhance macrophage activation, NO production and thus parasite clearance. Similar to IL-12 and TNF-α, IFN-α/β is also produced by antigen-presenting cells. IFN-α/β (also known as type 1 IFN) can induce cell activation, including activation of macrophages to produce NO to kill *L. major* (discussed in [24]). As a result, treating mice infected with *L. major* with a neutralizing anti-IFN-α/β was detrimental for the course of infection. Taken as a whole, these observations suggest that several cytokines produced by antigen-presenting cells (IL-12, TNF-α and IFN-α/β) can promote the development of a protective Th1/IFN-γ response to *L. major* infection

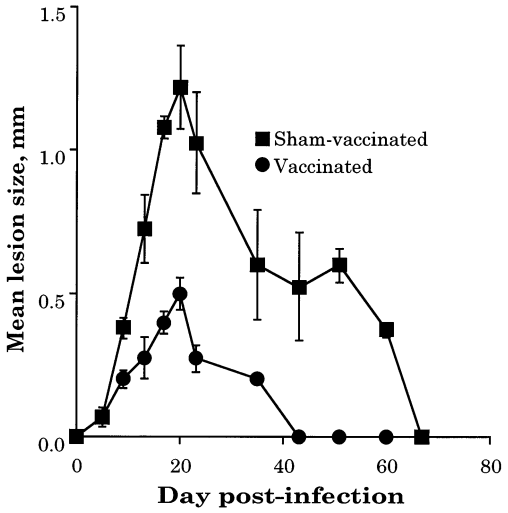

Fig. 3. Vaccination against *L. longipalpis* MAX protects mice from a subsequent challenge with *L. major* admixed with whole *L. longipalpis* salivary-gland lysates. Groups of C3H mice were vaccinated with synthetic MAX emulsified in Freund's adjuvant or were sham-vaccinated (received adjuvant alone). Both groups were then challenged in the footpad with *L. major* parasites mixed with salivary-gland lysate of *L. longipalpis*. The lesions were monitored by comparing the thickness of the infected footpad with the contralateral control footpad. See reference [35] for further experimental details.

There are also cytokines (which again can be produced by antigen-presenting cells) that promote the development of a Th2 response to infection with *L. major* in mice. Transforming growth factor-β can inhibit the production of IFN-γ and can 'deactivate' macrophages, making them more permissive to infection with *Leishmania* [25]. Likewise, IL-10 can also inhibit the production of IFN-γ and thus has been referred to as a cytokine that favors Th2 development. However, injecting mice with IL-12 triggers both IFN-γ and IL-10 gene expression [26]. In addition, when infected with *L. major*, resistant mice produce more IL-10 than susceptible mice [27]. Taken together these observations suggest that IL-10 may also participate in a feedback loop to prevent overproduction of IFN-γ and possible tissue damage. Finally, IL-6 has been proposed to favor the development of Th2 responses [28]. However, when IL-6-deficient mice on a susceptible BALB/c background were infected with *L. major*, the course of infection was not different from control animals. The absence of IL-6 led to down-regulation of both Th1- (IL-12) and Th2-associated (IL-4, IL-10 and IL-13) cytokines. Thus, in mice infected with *L. major*, IL-6 may promote the development of both Th1 and Th2 responses [29].

3. The influence of vector sand fly saliva on Th bias

Saliva is co-injected with *Leishmania* into the skin of the vertebrate host when the sand fly takes a blood meal. It is now well established that the lysates of the salivary gland of the sand fly vector for *Leishmania* (both Old and New World species) exacerbate infection with the parasite in the mammalian host [1,30,31]. For a recent review of the im-

munomodulatory properties of sand fly saliva that are involved in this phenomenon, the reader is referred to Gillespie et al. [32] or Kamhawi [33].

The immunomodulatory properties of whole saliva (from either Old or New World sand flies) or of maxadilan (or MAX, a potent vasodilator/immunomodulator present in the saliva of New World sand flies) would be expected to exacerbate leishmaniasis (summarized in Table 1), and thus could be the explanation for the effect of saliva/MAX on infection with *L. major*. For example, saliva increases IL-4 production, and IL-4 is one of the most important factors that leads to disease progression in *L. major*-infected mice (discussed in Section 2.2 above). In addition, saliva/MAX up-regulates the production of prostaglandin E_2, IL-10 and IL-6, which can inhibit macrophage activation. Indeed the activation of saliva/MAX-treated macrophages is inhibited since the cells are less capable of producing TNF-α, NO and H_2O_2 (Table 1), all of which are markers for macrophage activation.

One of the most exciting aspects in Table 1 is the recent discovery that salivary-gland proteins can be used to vaccinate against infection with *L. major* [34,35]. Using Old World *Phlebotomus papatasi* sand flies, Valenzuela et al. [34] showed that a protein in the saliva of the fly (SP-15) was highly immunogenic for mice. They therefore vaccinated mice against the protein and then challenged the mice with *L. major* admixed with whole *P. papatasi* salivary-gland lysate. The result was marked protection against infection with the parasite. Using New World *Lutzomyia longipalpis* MAX, Morris et al. [35] also showed that MAX-vaccinated mice were markedly protected against challenge with *L. major*. An example of the results achieved with MAX vaccination is shown in Fig. 3. Vaccinated animals had lesions considerably smaller than control (sham-vaccinated) mice and these lesions healed approximately 4 weeks in advance of the controls. Interestingly, in both the work of Valenzuela and Morris, an analysis of the mechanism of protection showed that cell-mediated responses in the vaccinated mice (delayed type hypersensitivity and IFN-γ) were at least partly responsible for the protection observed.

The advantage to this approach towards vaccination is that, theoretically, vaccinating against the salivary proteins of a given vector would protect the host from infection with any pathogen the vector transmits. Since there are numerous vector-borne diseases (malaria, filariasis, trypanosomiasis, leishmaniasis and Lyme disease, to name but a few), this type of vaccine would be both novel and efficient. The efficacy of this type of vaccine in humans, however, has yet to be explored.

4. Human leishmaniasis

The immune response of humans to infection with *Leishmania* is not as well characterized as the response of mice. However, we will attempt to summarize the commonalities of the human response to *Leishmania* with respect to the role of cytokines, co-stimulatory molecules and sand fly saliva. It should be noted that this summary will encompass the human response to several species of *Leishmania*, with an emphasis on those that cause cutaneous leishmaniasis.

The clinical outcome of infection with *Leishmania* in humans ranges from a relatively mild to a severe life-threatening disease depending on several host and parasite factors. Among these are the species/isolate of *Leishmania* that is involved, however, it is clear that a single strain of *Leishmania* can give rise to more than one clinical form of the disease, which include cutaneous, mucocutaneous, and visceral. These differences are also likely influenced by the patient's own immune response [36].

Information on the nature of the cell infiltrate in humans with cutaneous leishmaniasis has been obtained principally through the analysis of lesion biopsies. This approach has limitations because it only allows for a single 'snapshot' of the infectious process. Nevertheless, the predominant cells found in the lesion are CD4 and CD8 T cells, with lower numbers of macrophages, granulocytes and a few B cells [37].

Although there are exceptions, lymphocytes from patients with active leishmaniasis produce mainly IFN-γ and some IL-4 in response to stimulation with the parasite; cure of the infection is associated with the production of IFN-γ only, while IL-10 may prolong the course of disease [38–40]. A small portion of individuals infected with *L. braziliensis* develop mucosal lesions, which are associated with a strong cellular response and the expression of IFN-γ, TNF-α and IL-10 [41].

In an effort to gain more control over experimental conditions and the timing and dose of infective parasites, some investigators have studied the response of peripheral blood mononuclear cells (PBMC) from *Leishmania*-unexposed normal donors to infection with the parasite in vitro. A similar approach (using spleen or lymph node cells) in a murine system was shown to mimic closely the immune response that occurs to infection with *L. major* in an intact mouse [27]. Hviid et al. [42] showed that phenotypic changes (e.g., CD3 and CD25 expression) occurred in PBMC when the cells were exposed to *Leishmania donovani* in vitro, and Kurtzhals et al. [43] reported that PBMC produced IFN-γ in response to stimulation with *L. donovani*. More recently, we [44,45] and Rogers and Titus, unpublished, showed that most donors developed a Th1 or Th0 (Th0 cells display a mixed Th1–Th2 phenotype) response to stimulation with *L. major* in vitro. Moreover, we found that expression of CD80 and CD40 was enhanced on macrophages exposed to *L. major* and responding lymphocytes, and that blocking CD80 and/or CD86 interfered with the production of IL-5 and IFN-γ by T cells and IL-12 by *L. major*-infected macrophages. Finally, we also showed (Rogers and Titus unpublished) that sand fly sa-

liva and/or salivary MAX inhibits IFN-γ production by human PBMC infected with *L. major* while it stimulates the production of IL-6 by human macrophage/monocytes. This suggests that MAX affects the human and mouse immune systems similarly and that therefore MAX may be useful in the development of anti-*Leishmania* vaccines for humans.

Taken as a whole, these results using normal human PBMC stimulated with *Leishmania* in vitro appear also to yield results that mimic infection in humans just as the murine in vitro system mimics infection in the mouse. Therefore, the human in vitro system may prove useful for dissecting the immune response of humans to *Leishmania*, especially during the first few hours and days of infection, which may prove to be critical to understanding the immune response of humans to the parasite.

5. Conclusions and future directions

It is clear that polarization of Th responses to either Th1 or Th2 can lead to life or death outcomes to infection with *L. major* in mice. However, this is the downstream effect of the infection. What needs to be explored is the upstream events that lead to this polarization. Recent work has revealed that the antigen-presenting cell/co-stimulatory molecule/cytokine environment in which T cells are primed influences Th priming. Many of the cells that deliver these signals to T cells (e.g., antigen-presenting cells such as dendritic cells and macrophages) are members of the innate immune system. Therefore, further work is needed to analyze the interactions that occur between the innate and adaptive immune systems in animals infected with *L. major*. In addition, the effects that sand fly salivary proteins have on these interactions should also be explored since these salivary proteins have such dramatic effects on the immune response of the host and therefore might be useful as components of a subunit vaccine directed against *Leishmania*.

Acknowledgements

Due to the publisher's space limitations, it was not possible to cite all the relevant literature; rather, we tried to cite either reviews that would lead the reader to all the relevant literature or we cited recent publications that would discuss previous relevant work. This work was supported by NIH Grants AI27511 and 29955.

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