Dendritic cells and immunity to leishmaniasis and toxoplasmosis
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There is increased recognition that dendritic cells (DCs) are an important source of the IL-12 required to initiate protective immunity to protozoa, such as Leishmania and Toxoplasma. This article reviews the advances made in the last two years in understanding the pathways that lead to DC activation after infection with these organisms. Interestingly, there appear to be differences in the DC activation pathways utilized by these two intracellular protozoa which also may differ from the pathways utilized by bacteria.

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Abbreviations
DC dendritic cell
LeIF Leishmania elongation and initiation factor
STAg soluble Toxoplasma antigen
TLR Toll-like receptor

Introduction
Over the last decade, our view of dendritic cells (DCs) has been transformed: whereas previously we thought of them as simple antigen-presenting cells (albeit extremely efficient ones), now we consider them to be the architects of immunity (reviewed in [1–4]). DCs not only present antigen efficiently — and therefore determine the magnitude of the immune response — but also influence the quality of the response, contribute to deletional tolerance, promote cross-priming and may be important in the downregulation of effector responses and the maintenance of memory.

The recognition that there are different types of DCs (e.g. follicular, myeloid, lymphoid and plasmacytoid) may in part explain these pleiotropic effects and attempts have been made to ascribe specific functions to different DC subsets. However, the instructions that DCs receive at the start of an immune response may be the critical component in shaping subsequent events. Thus, DCs are able to distinguish different pathogens, which allows them to provide signals to T cells to ensure that an appropriate immune response is initiated. In the case of bacteria, pathogen-specific molecules — such as LPS, peptidoglycans and lipoproteins — trigger DC activation and maturation through Toll-like receptors (TLRs), with concomitant production of IL-12 and other cytokines, increased expression of costimulatory molecules, and responsiveness to chemokines that promote migration of DCs from peripheral tissues to lymphoid organs. The pathways of DC activation following infection with parasites are less well understood. In this review, we discuss recent advances in understanding the role of DCs in the initiation of the immune response to the intracellular protozoa, Leishmania and Toxoplasma.

Although Leishmania and Toxoplasma are both intracellular pathogens that require a host Th1 response for effective resistance to infection, the biology associated with these parasites is distinct. In the case of Leishmania, the parasites are primarily found in macrophages or DCs, although infection of other cell types has been reported. Once infection is initiated, Leishmania invade macrophages in the skin, multiply within a phagolysosome and eventually rupture the host cell and reinfect other cells. Whereas expansion of antigen-specific T cells can be evident within the first week of infection, the development of resistance can take several weeks to occur. In contrast, Toxoplasma infects all cells, replicates rapidly and in the absence of IFN-γ causes a fatal infection within days. Thus, a clear distinction is apparent between these two protozoa with regard to their requirements for the rapid development of immunity: Toxoplasma needs to induce a rapid immune response to prevent the infection from overwhelming the host, whereas the kinetics of the response can be delayed following Leishmania infection, possibly due to slower replication of these parasites and/or the limitation imposed by the inability of Leishmania to infect all cell types.

Production of IL-12
The production of IL-12 is critical for the development of a protective Th1-type of immune response following infection with Toxoplasma or Leishmania. Early studies focused on identifying the source of IL-12 during these infections and, since Leishmania and Toxoplasma infect macrophages, this cell type was a logical candidate. However, infection of macrophages with Leishmania failed to stimulate IL-12 production and indeed the parasite was able to selectively inhibit the capacity of macrophages to produce IL-12 in response to other stimuli [5–7]. How IL-12 suppression is mediated is unknown, although infection results in activation of macrophage phosphotyrosine phosphatases that may downregulate a variety of cell functions [8]. Recently, it was found that infection of macrophages with Toxoplasma also inhibits their ability to produce IL-12 in response to other stimuli, such as LPS. The reduced capacity of infected cells to produce IL-12 has been linked to the ability of this parasite to inhibit the activation of the NF-κB family of transcription factors [9,10].

Subsequent in vitro studies have shown that DCs infected with Leishmania produce IL-12. Murine fetal-skin-derived DCs [11,12], murine splenic DCs [13,14] and blood-derived human DCs [15] produce IL-12 following
infection with *Leishmania*. Interestingly, it was also found that some IL-12 may be preformed, available for release immediately after exposure to *L. donovani* [16•]. Although most of these studies have been done *in vitro*, *L. donovani* was found to stimulate DCs in the spleen to produce IL-12 [13]. In addition, a recent study has shown that treatment of *L. major*-infected BALB/c mice with the hematopoietic cytokine Flt3 ligand, known to expand the DC population, resulted in a significant increase in IL-12p40 production, and control of the infection in 40% of the mice, whereas all of the untreated animals succumbed to disease [17].

DCs (as well as neutrophils [18,19•]) have also been shown to produce IL-12 in response to *Toxoplasma*. Several studies were done with an extract of *Toxoplasma* — termed soluble *Toxoplasma* antigen (STAg) — which stimulates IL-12 production by splenic DCs [20]. Notably, in addition to IL-12 production, STAg also induced the migration of DCs from the red pulp and marginal zones of the spleen into the periarteriolar lymphoid sheath — the T cell region [20]. Although recent studies have shown that myeloid DCs are the main source of IL-12 in the brains of mice with toxoplasmonic encephalitis [21••], there is a surprising lack of data on DC responses during infection with *Toxoplasma* in *vivo*. However, *in vitro* studies with human DCs have shown that live parasites stimulate IL-12 production in a process that is partially dependent on the CD40–CD40L interaction (see below), whereas a *Toxoplasma* parasite lysate was a poor inducer of IL-12 [22••].

In studies designed to define the chemokines involved in the DC migration seen following STAg injection, it was observed that ligation of CCR5 on DCs provided a stimulus for IL-12 production [23••]. Treatment of DCs with pertussis toxin, which blocks the G-protein signaling pathways used by chemokine receptors, blocked almost all of the IL-12 induced by STAg, but had no effect on LPS-induced IL-12 production. Paradoxically, other studies found that pertussis toxin enhanced IL-12 production by DCs [24••]. The *in vivo* relevance of this effect was tested in *L. major*-infected BALB/c mice, where pertussis toxin treatment promoted enhanced IL-12 production and resistance [24••] (Figure 1). These data highlight the complexity of DC activation associated with different protozoa.

Evidence of the complexity of DC activation is increased when different parasite strains or species are studied. Thus, a direct comparison of the ability of human DCs to produce IL-12 following infection with different *Leishmania* parasites found that *L. major*, in conjunction with CD40L, induced IL-12, but no IL-12 was evident when DCs were exposed to *L. tropica* or *L. donovani* with CD40L (M McDowell, M Marovich, R Lira, M Braun, D Sacks, personal communication). An important observation in this study was that these differences were only evident when IL-12p70 was measured. Similarly, *L. mexicana* parasites failed to stimulate IL-12 production by murine DCs [25], contrasting with the results reported with *L. major*. On the other hand, infection of DCs with *L. amazonensis* followed by ligation of CD40 induced IL-12 production [26•]. The increase in IL-12 was only observed in DCs from C3H mice; DCs from BALB/c mice were found to make IL-4 and to promote a Th2 response following transfer to naïve mice.

**Regulation of DC activation**

Studies with human DCs indicate that production of IL-12 following exposure to either *Leishmania* or *Toxoplasma* may...
be dependent on ligation of CD40 [15••,22••,27]. Similarly, vaccine studies combining CD40L with leishmanial antigen demonstrated the in vivo importance of CD40 ligation for IL-12 production [28]. This would suggest that production of IL-12 requires two signals, one of microbial origin and the other from the host. In some situations this may require cytokines that work in concert with CD40 ligation, such as IFN-γ. Alternatively, microbial signals may provide the needed priming signal [29•]. Indeed, the requirement for such a priming signal would ensure that DCs do not make IL-12 every time CD40 is ligated, but only when appropriate. However, CD40–CD40L interactions may provide signals other than IL-12 that may influence DC function. This was recently illustrated in a helminth model in which DCs exposed to Schistosoma mansoni egg extract and adaptively transferred to naïve recipients promoted a Th2 response that was dependent on expression of CD40 [30••].

Although CD40–CD40L interactions may be important, they may not always be required. For example, studies with STAg indicate that IL-12 production by DCs can proceed independently of CD40L [20], and CD40 deficient mice infected with Toxoplasma control the acute phase of this infection [31]. However, the CD40–CD40L interaction does appear to be important in the ability of human DCs to make optimal levels of IL-12 and this may account for the susceptibility of hyper-IgM patients to Toxoplasma [22••]. Similarly, although early experiments demonstrated a requirement for CD40–CD40L interactions in healing Leishmania infection [32–34], more recent studies show that CD40 deficient 129/B6 mice can control L. major with lower challenge doses or in the absence of CD28 [35••] and that resistance to reinfection in C57BL/6 mice is independent of CD40 (P. Scott, unpublished data). Whether other host molecules compensate to promote IL-12 production in the absence of CD40–CD40L in either of these situations is unknown. A molecule cloned from Leishmania (termed Leishmania elongation and initiation factor [LeIF]) is reported to be a potent inducer of IL-12 production [45•,46]. This molecule was shown to stimulate IFN-γ production by scid spleen cells in an IL-12 dependent manner [45•]. No receptors have yet been identified that recognize LeIF, but TLR4 has been excluded because cells from C3H/HeJ mice, which lack TLR4, respond to LeIF stimulation [45•].

In the case of Toxoplasma, many of the studies done to elucidate the pathways of DC activation have used STAg and so far the molecules involved in its function have not been defined. Nevertheless, evidence that the pathways involved in TLR signaling are important for the recognition of Toxoplasma is provided by studies from two groups which found that mice lacking MyD88 — an adapter protein required for TLR-mediated activation of NF-κB and production of IL-12 [47] — are defective in their ability to make IL-12 responses to Toxoplasma (C Scanga, J Alberti, A Sher, D Sibley, personal communications). However, recent studies have shown that although the NF-κB family member c-Rel is required for TLR-mediated production of IL-12, it is not required for the production of IL-12 by DCs, macrophages or neutrophils during infection with Toxoplasma or in response to STAg [48••]. A possible explanation for this disparity is provided by a role for MyD88 in the activation of mitogen-activated protein kinases and AP-1, as well as NF-κB.

From an analysis of the studies to date with Leishmania and Toxoplasma, one might conclude that they are in fact quite poor inducers of IL-12 on their own, as compared with microbial stimuli. Thus, human DCs require an additional stimulus (such as CD40) to produce IL-12 when exposed to these protozoa [15••,22••]. Similarly, although in vitro exposure of murine DCs to Leishmania or Toxoplasma...
induces IL-12, the levels are often less than observed with bacteria ([11–14]; CA Hunter, unpublished data). On the other hand, the Th1 responses observed in vivo are substantial, particularly in the case of Toxoplasma. This suggests that other pathways of activation may be important. In addition to recognizing specific molecules of Leishmania or Toxoplasma, other mechanisms may alert DCs to the presence of invading parasites, thus amplifying the response. For example, both Toxoplasma and Leishmania are cytolytic and the cellular damage associated with these infections may provide a non-specific ‘danger’ signal that enhances the development of a Th1 type response [49].

Conclusions/summary

The pathways leading to DC activation following infection are just beginning to be elucidated and many questions remain. Some of the more important include: defining the pathogen-derived molecules, and the DC receptors, that are responsible for activating DCs; defining the role DCs play in the maintenance of immunity; and assessing whether definable subsets of DCs are responsible for priming cells to become Th1 or Th2 cells.

Previous studies indicated that particular DC subsets promoted either a Th1 or Th2 response [50,51], although cytokines can modulate that function [52]. An alternative view is that the microbe determines whether the DC promotes a Th1 or Th2 response. That this can be the case was clearly demonstrated in two studies this last year. Thus, following injection of Brucella, IL-12 production was exclusively seen in CD8+ splenic DCs, whereas, as previously reported, Toxoplasma antigen stimulated IL-12 production in CD8- DCs [53••]. On the other hand, exposure of bone-marrow-derived DCs to helminth antigens resulted in a DC population that primed for a Th2 response, whereas the same population of DCs exposed to bacteria promoted a Th1 response [30••]. Taken together, these data indicate that although DCs may be the architects of immunity, it is the nature of the microbial stimuli that directs DC responses.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
**of outstanding interest

16. Quinones M, Ahuja SK, Melby PC, Pate L, Reddick RL, Ahuja SS: Preformed membrane-associated stores of interleukin (IL)-12 are a previously unrecognized source of bioactive IL-12 that is mobilized within minutes of contact with an intracellular parasite. J Exp Med 2000, 192:507-516.

Important study demonstrating that human DCs make IL-12 when primed with Leishmania and triggered with CD40L.

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Interesting study demonstrating that live parasites are critical for the induction of IL-12 by human DCs and that DC–T cell interactions are required for optimal IL-12 responses.


Important paper demonstrating that the chemokine receptor CCR5 is involved in the production of IL-12 in mice following exposure to a Toxoplasma extract.


Important paper demonstrating that Gi protein signaling regulates IL-12 production. A comparison of the results in this paper with [23++] demonstrates the differences in IL-12 pathways that may exist between Leishmania and Toxoplasma.


This study suggests that DCs from BALB/c and C3H mice may differ in their response to infection with Leishmania.


This study indicates the importance of two signals for the ability of DCs to produce IL-12—a microbial signal as well as stimulation through CD40.


This is the first study to demonstrate that DCs can be primed by a helminth extract to promote the Th2 responses and that this activity requires CD40.


This study demonstrates that CD40 may not always be required for a Th1 response following infection with Leishmania and therefore the study sets the stage to look for alternative pathways of DC activation.


This study demonstrates that the in vitro observation that IL-4 can promote IL-12 production can be recapitated in vivo and have a dramatic effect on the course of infection with Leishmania.


This is an important study that demonstrates that protozoa can signal the innate immune system through TollRs.


This study continues a characterization of the ability of LeIF to stimulate the innate immune response.


The NF-κB transcription factors are important for IL-12 production in response to many microbial stimuli, but this manuscript indicates the presence of NF-κ-B-independent pathways for the recognition of Toxoplasma.


This study demonstrates that the microbe, rather than a particular DC subset, may determine the role of DCs with regard to their ability to promote Th1 or Th2 responses.