

Dendritic cells and immunity to leishmaniasis and toxoplasmosis

Phillip Scott* and Christopher A Hunter

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.

Addresses

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA
*e-mail: pscott@vet.upenn.edu

Current Opinion in Immunology 2002, 14:466–470

0952-7915/02/\$ – see front matter
© 2002 Elsevier Science Ltd. All rights reserved.

Published online 24 April 2002

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 reinvade 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.

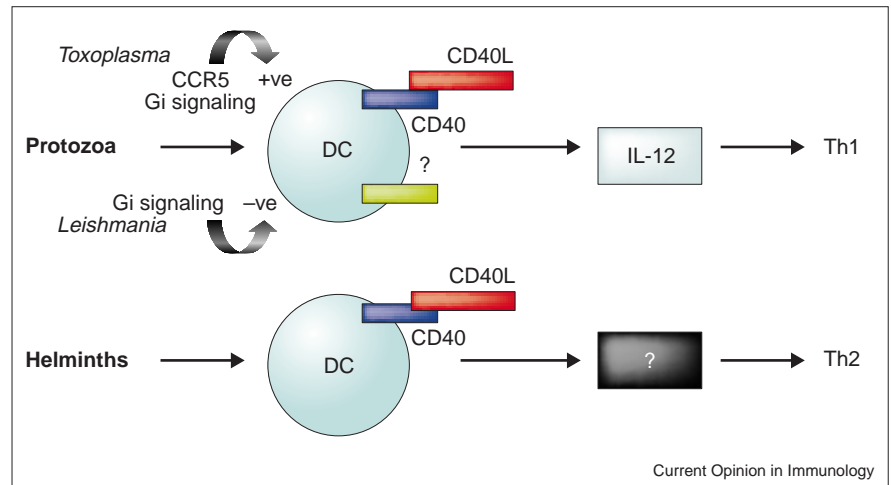
Production of IL-12

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- κ B 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

Figure 1

Stimulation of Th1/Th2 responses by protozoa and helminths. *Toxoplasma* and *Leishmania* prime DCs, allowing responsiveness to CD40 ligation, which promotes IL-12 production [15••,22••,27]. In the case of *Toxoplasma*, this priming may involve signaling through the chemokine receptor, CCR5 [23••]. Treatment of DCs with pertussis toxin (which blocks G-protein [Gi] signaling) blocks nearly all IL-12 production stimulated by *Toxoplasma* STAg; on the other hand, in other situations (such as *L. major* infection in BALB/c mice), pertussis toxin enhances IL-12 production [24••]. Exposure to helminth molecules apparently provides a different priming signal that does not promote Th1 responses [30••]. Nevertheless, the development of the Th2 response by antigen-pulsed DCs is dependent on CD40, although little is yet known about this pathway or the critical cytokine(s) involved. In contrast, with protozoal (and bacterial) priming, other molecules may compensate in the absence of CD40, and/or direct IL-12 production may occur after exposure to the microbial stimulus.



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 toxoplasmic 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 adoptively 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.

Another regulator of IL-12 production is IL-4, a Th2 cytokine that paradoxically increases the production of IL-12 [36]. An interesting paper recently demonstrated how IL-4 might promote IL-12 production *in vivo* [37[•]]. Treatment of BALB/c mice with IL-4 during the first eight hours of infection with *L. major* promoted a healing infection, which was correlated with an increase in IL-12 production by DCs within draining lymph nodes. Interestingly, increased expression of the IL-4 receptor was found on Langerhans cells from BALB/c mice infected with *L. major* [38]. These results are reminiscent of earlier studies demonstrating that under certain circumstances IL-4 could augment lesion resolution in leishmaniasis [39].

Once activated with LPS, DCs undergo a period of desensitization. Similarly, after exposure to STAg, there is a period of 5–7 days when DCs are unresponsive to further stimulation [40]. The mechanism involved in this paralysis was recently elucidated, when it was discovered that

lipoxin A4, an arachidonate-derived inhibitor of acute inflammation, was produced following injection of STAg [41]. Lipoxin is produced by macrophages and appears to act on DCs to promote paralysis of DC function following exposure to STAg. In mice lacking the ability to produce lipoxin, no paralysis was evident. Interestingly, this pathway of DC paralysis might be unique to *Toxoplasma*, since lipoxin had no inhibitory effect on IL-12 production in response to LPS.

Parasite molecules that activate DCs

Implicit in the findings that *Leishmania* and *Toxoplasma* can induce IL-12 production is that DCs can recognize parasite-derived molecules. The best-characterized molecules involved in the recognition of microbial stimuli are the TLRs, which are triggered by bacterial products [42]. It is possible that members of the TLR family are involved in the recognition of protozoa and evidence in support of this idea is provided by studies that implicated TLR2 in recognition of *Trypanosoma cruzi* glycosylphosphatidylinositol anchors [43[•], 44].

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- κ B 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- κ B 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- κ B.

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.

Acknowledgements

The authors thank D Sacks, A Sher and D Sibley for providing unpublished data for this review, and Edward Pearce for reviewing the manuscript.

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

1. Reid SD, Penna G, Adorini L: **The control of T cell responses by dendritic cell subsets.** *Curr Opin Immunol* 2000, **12**:114-121.
2. Banchereau J, Steinman RM: **Dendritic cells and the control of immunity.** *Nature* 1998, **392**:245-252.
3. Bell D, Young JW, Banchereau J: **Dendritic cells.** *Adv Immunol* 1999, **72**:255-324.
4. Reis e Sousa C: **Dendritic cells as sensors of infection.** *Immunity* 2001, **14**:495-498.
5. Carrera L, Gazzinelli RT, Badolato R, Hieny S, Muller W, Kuhn R, Sacks DL: ***Leishmania* promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice.** *J Exp Med* 1996, **183**:515-526.
6. Sartori A, Oliveira MA, Scott P, Trinchieri G: **Metacyclogenesis modulates the ability of *Leishmania* promastigotes to induce IL-12 production in human mononuclear cells.** *J Immunol* 1997, **159**:2849-2857.
7. Belkaid Y, Butcher B, Sacks DL: **Analysis of cytokine production by inflammatory mouse macrophages at the single-cell level: selective impairment of IL-12 induction in *Leishmania*-infected cells.** *Eur J Immunol* 1998, **28**:1389-1400.
8. Forget G, Siminovich KA, Brochu S, Rivest S, Radzioch D, Olivier M: **Role of host phosphotyrosine phosphatase SHP-1 in the development of murine leishmaniasis.** *Eur J Immunol* 2001, **31**:3185-3196.
9. Butcher BA, Kim L, Johnson PF, Denkers EY: ***Toxoplasma gondii* tachyzoites inhibit proinflammatory cytokine induction in infected macrophages by preventing nuclear translocation of the transcription factor NF- κ B.** *J Immunol* 2001, **167**:2193-2201.
10. Shapira S, Speirs K, Gerstein A, Caamano J, Hunter CA: **Suppression of NF- κ B activation by infection with *Toxoplasma gondii*.** *J Infect Dis* 2002, **185**:S66-S72.
11. von Stebut E, Belkaid Y, Nguyen BV, Cushing M, Sacks DL, Udey MC: ***Leishmania major*-infected murine Langerhans cell-like dendritic cells from susceptible mice release IL-12 after infection and vaccinate against experimental cutaneous leishmaniasis.** *Eur J Immunol* 2000, **30**:3498-3506.
12. von Stebut E, Belkaid Y, Jakob T, Sacks DL, Udey MC: **Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity.** *J Exp Med* 1998, **188**:1547-1552.
13. Gorak PM, Engwerda CR, Kaye PM: **Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection.** *Eur J Immunol* 1998, **28**:687-695.
14. Konecny P, Stagg AJ, Jebbari H, English N, Davidson RN, Knight SC: **Murine dendritic cells internalize *Leishmania major* promastigotes, produce IL-12 p40 and stimulate primary T cell proliferation *in vitro*.** *Eur J Immunol* 1999, **29**:1803-1811.
15. Marovich MA, McDowell MA, Thomas EK, Nutman TB: **IL-12p70 production by *Leishmania major*-harboring human dendritic cells is a CD40/CD40 ligand-dependent process.** *J Immunol* 2000, **164**:5858-5865.
- Important study demonstrating that human DCs make IL-12 when primed with *Leishmania* and triggered with CD40L.
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.
- This study demonstrates that some of the IL-12 required to develop a Th1 response may come from preformed stores in DCs.
17. Kremer IB, Gould MP, Cooper KD, Heinzel FP: **Pretreatment with recombinant Flt3 ligand partially protects against progressive cutaneous leishmaniasis in susceptible BALB/c mice.** *Infect Immunol* 2001, **69**:673-680.
18. Bliss SK, Marshall AJ, Zhang Y, Denkers EY: **Human polymorphonuclear leukocytes produce IL-12, TNF- α , and the chemokines macrophage-inflammatory protein-1 α and -1 β in response to *Toxoplasma gondii* antigens.** *J Immunol* 1999, **162**:7369-7375.
19. Bliss SK, Butcher BA, Denkers EY: **Rapid recruitment of neutrophils containing prestored IL-12 during microbial infection.** *J Immunol* 2000, **165**:4515-4521.
- Important paper because of the demonstration that neutrophils represent an alternative source of IL-12 during *Toxoplasma* infection and that IL-12 may be immediately released after infection.
20. Sousa CR, Hieny S, Schariton-Kersten T, Jankovic D, Charest H, Germain RN, Sher A: ***In vivo* microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas.** *J Exp Med* 1997, **186**:1819-1829.
21. Fischer HG, Bonifaz U, Reichmann G: **Phenotype and functions of brain dendritic cells emerging during chronic infection of mice with *Toxoplasma gondii*.** *J Immunol* 2000, **164**:4826-4834.
- Important study since it demonstrates that DCs are a major source of IL-12 in the brain during *Toxoplasma* infection.

22. Subauste CS, Wessendarp M: Human dendritic cells discriminate between viable and killed *Toxoplasma gondii* tachyzoites: dendritic cell activation after infection with viable parasites results in CD28 and CD40 ligand signaling that controls IL-12-dependent and -independent T cell production of IFN- γ . *J Immunol* 2000, 165:1498-1505.
- 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.
23. Aliberti J, Reis e Sousa C, Schito M, Hieny S, Wells T, Huffnagle GB, Sher A: CCR5 provides a signal for microbial induced production of IL-12 by CD8 α^+ dendritic cells. *Nat Immunol* 2000, 1:83-87.
- Important paper demonstrating that the chemokine receptor CCR5 is involved in the production of IL-12 in mice following exposure to a *Toxoplasma* extract.
24. He J, Gurunathan S, Iwasaki A, Ash-Shaheed B, Kelsall BL: Primary role for Gi protein signaling in the regulation of interleukin 12 production and the induction of T helper cell type 1 responses. *J Exp Med* 2000, 191:1605-1610.
- 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*.
25. Bennett CL, Misslitz A, Colledge L, Aebischer T, Blackburn CC: Silent infection of bone marrow-derived dendritic cells by *Leishmania mexicana* amastigotes. *Eur J Immunol* 2001, 31:876-883.
26. Qi H, Popov V, Soong L: *Leishmania amazonensis*-dendritic cell interactions *in vitro* and the priming of parasite-specific CD4 $^+$ T cells *in vivo*. *J Immunol* 2001, 167:4534-4542.
- This study suggests that DCs from BALB/c and C3H mice may differ in their response to infection with *Leishmania*.
27. Seguin R, Kasper LH: Sensitized lymphocytes and CD40 ligation augment interleukin-12 production by human dendritic cells in response to *Toxoplasma gondii*. *J Infect Dis* 1999, 179:467-474.
28. Chen G, Darrah PA, Mosser DM: Vaccination against the intracellular pathogens *Leishmania major* and *L. amazonensis* by directing CD40 ligand to macrophages. *Infect Immun* 2001, 69:3255-3263.
29. Schulz O, Edwards AD, Schito M, Aliberti J, Manickasingham S, Sher A, Reis e Sousa C: CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells *in vivo* requires a microbial priming signal. *Immunity* 2000, 13:453-462.
- 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.
30. MacDonald AS, Straw AD, Dalton NM, Pearce EJ: Th2 response induction by dendritic cells: a role for CD40. *J Immunol* 2002, 168:537-540.
- This is the first study to demonstrate that DCs can be primed by a helminth extract to promote Th2 responses and that this activity requires CD40.
31. Reichmann G, Walker W, Villegas EN, Craig L, Cai G, Alexander J, Hunter CA: The CD40/CD40 ligand interaction is required for resistance to toxoplasmic encephalitis. *Infect Immun* 2000, 68:1312-1318.
32. Soong L, Su J-C, Grewal IS, Kima P, Sun J, Longley BJ, Ruddle NH, McMahon-Pratt D, Flavell RA: Disruption of CD40-CD40 ligand interactions results in enhanced susceptibility to *Leishmania amazonensis* infection. *Immunity* 1996, 4:263-274.
33. Kamanaka M, Yu P, Yasui T, Yoshida K, Kawabe T, Horii T, Kishimoto T, Kikutani H: Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. *Immunity* 1996, 4:275-282.
34. Campbell KA, Ovendale PJ, Kennedy MK, Fanslow WC, Reed SG, Maliszewski CR: CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. *Immunity* 1996, 4:283-290.
35. Padigel UM, Perrin PJ, Farrell JP: The development of a Th1-type response and resistance to *Leishmania major* infection in the absence of CD40-CD40L costimulation. *J Immunol* 2001, 167:5874-5879.
- 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.
36. Takenaka H, Maruo S, Yamamoto N, Wysocka M, Ono S, Kobayashi M, Yagita H, Okumura K, Hamaoka T, Trinchieri G *et al.*: Regulation of T cell-dependent and -independent IL-12 production by the three Th2-type cytokines IL-10, IL-6, and IL-4. *J Leukoc Biol* 1997, 61:80-87.
37. Biedermann T, Zimmermann S, Himmelrich H, Gumy A, Egeter O, Sakrauskis AK, Seegmuller I, Voigt H, Launois P, Levine AD *et al.*: IL-4 instructs TH1 responses and resistance to *Leishmania major* in susceptible BALB/c mice. *Nat Immunol* 2001, 2:1054-1060.
- This study demonstrates that the *in vitro* observation that IL-4 can promote IL-12 production can be recapitulated *in vivo* and have a dramatic effect on the course of infection with *Leishmania*.
38. Moll H, Scharner A, Kampgen E: Increased interleukin 4 (IL-4) receptor expression and IL-4-induced decrease in IL-12 production by Langerhans cells infected with *Leishmania major*. *Infect Immun* 2002, 70:1627-1630.
39. Carter KC, Gallagher G, Baillie AJ, Alexander J: The induction of protective immunity to *Leishmania major* in the BALB/c mouse by interleukin 4 treatment. *Eur J Immunol* 1989, 19:779-782.
40. Reis e Sousa C, Yap G, Schulz O, Rogers N, Schito M, Aliberti J, Hieny S, Sher A: Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology. *Immunity* 1999, 11:637-647.
41. Aliberti J, Hieny S, Reis e Sousa C, Serhan CN, Sher A: Lipoxin-mediated inhibition of IL-12 production by DCs: a mechanism for regulation of microbial immunity. *Nat Immunol* 2002, 3:76-82.
42. Medzhitov R, Janeway CA Jr: Innate immunity: the virtues of a nonclonal system of recognition. *Cell* 1997, 91:295-298.
43. Campos MA, Almeida IC, Takeuchi O, Akira S, Valente EP, Procopio DO, Travassos LR, Smith JA, Golenbock DT, Gazzinelli RT: Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *J Immunol* 2001, 167:416-423.
- This is an important study that demonstrates that protozoa can signal the innate immune system through TLRs.
44. Ropert C, Almeida IC, Cselos M, Travassos LR, Ferguson MA, Cohen P, Gazzinelli RT: Requirement of mitogen-activated protein kinases and I kappa B phosphorylation for induction of proinflammatory cytokines synthesis by macrophages indicates functional similarity of receptors triggered by glycosylphosphatidylinositol anchors from parasitic protozoa and bacterial lipopolysaccharide. *J Immunol* 2001, 166:3423-3431.
45. Borges MM, Campos-Neto A, Sleath P, Grabstein KH, Morrissey PJ, Skeiky YA, Reed SG: Potent stimulation of the innate immune system by a *Leishmania brasiliensis* recombinant protein. *Infect Immun* 2001, 69:5270-5277.
- This study continues a characterization of the ability to LelF to stimulate the innate immune response.
46. Probst P, Skeiky YA, Steeves M, Gervasi A, Grabstein KH, Reed SG: A *Leishmania* protein that modulates interleukin (IL)-12, IL-10 and tumor necrosis factor-alpha production and expression of B7-1 in human monocyte-derived antigen-presenting cells. *Eur J Immunol* 1997, 27:2634-2642.
47. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R: Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2001, 2:947-950.
48. Mason N, Aliberti J, Caamano JC, Liou H-C, Hunter CA: Identification of c-Rel-dependent and -independent pathways of IL-12 production during infectious and inflammatory stimuli. *J Immunol* 2002, 168:2590-2594.
- The NF- κ B 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*.
49. Matzinger P: An innate sense of danger. *Semin Immunol* 1998, 10:399-415.
50. Maldonado-Lopez R, Moser M: Dendritic cell subsets and the regulation of Th1/Th2 responses. *Semin Immunol* 2001, 13:275-282.
51. Maldonado-Lopez R, De Smedt T, Michel P, Godfroid J, Pajak B, Heirman C, Thielemans K, Leo O, Urbain J, Moser M: CD8 α^+ and CD8 α^- subclasses of dendritic cells direct the development of distinct T helper cells *in vivo*. *J Exp Med* 1999, 189:587-592.
52. Maldonado-Lopez R, Maliszewski C, Urbain J, Moser M: Cytokines regulate the capacity of CD8 α^+ and CD8 α^- dendritic cells to prime Th1/Th2 cells *in vivo*. *J Immunol* 2001, 167:4345-4350.
53. Huang LY, Reis e Sousa C, Itoh Y, Inman J, Scott DE: IL-12 induction by a TH1-inducing adjuvant *in vivo*: dendritic cell subsets and regulation by IL-10. *J Immunol* 2001, 167:1423-1430.
- 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.