Commentary

Interleukin-12, Dendritic Cells, and the Initiation of Host-protective Mechanisms against *Toxoplasma gondii*

By Lawrence L. Johnson and Peter C. Sayles

From the Trudeau Institute, Inc., Saranac Lake, New York 12983

The principal job of the immune system is to provide protection against infectious pathogens. One of the immunologist's goals is to determine how the cellular and cytokine components of the immune system are functionally organized to provide this protection. In vitro studies can suggest which components of the immune system may be involved in resistance to a given pathogen, but ultimately the immunologist must look in vivo to see whether these components truly contribute to protection. In recent years, this task has been made easier by the advent of gene knockout mice and antibodies that can be used to selectively deplete or neutralize components of interest from infected experimental animals so that the consequences of a missing entity can be observed. A more demanding task has been to identify where and under what circumstances an entity that may be important in host protection performs its functions. Reis e Sousa et al. take on this task in a study published in this issue of this journal (1). They show that dendritic cells in spleen are the key initial producers of IL-12 in response to *Toxoplasma gondii* antigens or LPS. Their demonstration that macrophages are not significant sources of IL-12 in the in vivo setting they describe is quite surprising, because other studies have shown that activated macrophages are excellent producers of IL-12 in vitro in response to *T. gondii* antigens. In this commentary, we discuss how these findings may relate to innate and acquired pathways of resistance to *Toxoplasma*.

Pathogenesis of T. gondii Infection and Components of Innate and Acquired Resistance. *T. gondii* is a widespread protozoan parasite found in nature encysted in infected animal tissue or as oocysts in feces of infected cats, the definitive host. Infections are initiated when tissue cysts or oocysts are eaten. Rapidly dividing single-cell forms of the parasites (tachyzoites) develop after emergence from cysts in intestine and infect gut cells by active penetration. In fact, tachyzoites can probably infect any nucleated mammalian or avian cell. Within a few days to a week in experimentally infected mice, tachyzoites can be found in Peyer's patches, spleen, and mesenteric lymph nodes, and have also spread to nonlymphoid tissues, such as liver, lung, and brain (2, 3). The difficulty in detecting parasites in the blood, except by sensitive PCR-based techniques (4), suggests that the early spread of tachyzoites may be predominantly via lymphatics (3). This early tachyzoite proliferation and dissemination defines the acute phase of infection. With time, fewer tachyzoites are found in most infected tissues, and cysts appear that contain the quiescent bradyzoite form of the parasite (2). Cysts are particularly evident in the brain. The appearance of cysts marks the onset of the chronic phase of infection, which probably persists for the life of the host.

Toxoplasma infections in immunocompetent humans and in many strains of mice are largely asymptomatic (5). However, it is clear that this depends on intact host-resistance mechanisms. Antibody depletion studies and studies with gene knockout mice have identified a number of essential components of resistance during the acute phase of infection. Unlike control mice, mice depleted of IFN-γ (6), TNF-α (7), or IL-12 (8–10) die quite soon after inoculation of parasites. So, too, do mice depleted of Thy-1$^+$ non-CD4$^+$ or -CD8$^+$ cells (most of which are NK cells; reference 11), neutrophils (12), and mice treated with antibody against CR3 (13). In vitro experiments have revealed interconnections between some of the essential components of resistance. IL-12, produced by activated macrophages, along with TNF-α, induces NK cells to produce IFN-γ (14, 15), and IFN-γ plus TNF-α can activate anti-*Toxoplasma* activity in macrophages (15–17). However, it is clear that neither CD4$^+$ nor CD8$^+$ T cells are necessary for mice to survive acute toxoplasmosis (11, 18, 19).

In contrast to acute infection, survival of chronic *Toxoplasma* infection and resistance to a rechallenge infection clearly depends on acquired cell-mediated immunity, in which CD8$^+$ T cells and IFN-γ play major roles (20–24). Thus, acquired immunity to *T. gondii* exhibits the characteristics associated with a Th1-type response. Despite the advances that have been made in identifying important components of innate and acquired resistance to *T. gondii*, we still have only a sketchy understanding of how these components are functionally orchestrated to provide resistance during acute and chronic infection with this parasite. However, there can be little doubt that production of IL-12 is a crucial early event in the generation of both innate and acquired resistance mechanisms after oral administration of cysts in experimental animal models.

Dendritic Cells May Be the Key Source of IL-12 in the Initiation of Response to T. gondii Antigen. The striking dependence on both innate resistance mechanisms during the acute phase of infection and on T cell–dependent acquired immunity during the chronic phase makes *Toxoplasma* an especially attractive and useful pathogen with which to reveal the workings of host resistance. Thus it is understandable that Reis e Sousa et al. have used *Toxoplasma* antigens to study events that may be important to initiation of both innate and acquired resistance mechanisms (1). They have

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/12/1799/04 $2.00
Volume 186, Number 11, December 1, 1997 1799–1802
http://www.jem.org

found that dendritic cells, not macrophages, are the first cells to make IL-12 in the spleens of mice given soluble *Toxoplasma* antigen or LPS intravenously. This is surprising in that macrophages have been assumed, on the basis of the in vitro studies cited above, to be a primary source of IL-12. Reis e Sousa et al. also show that after administration of *Toxoplasma* antigens, dendritic cells increase in number, redistribute to T cell areas of the spleen, and express markers characteristic of interdigitating dendritic cells. Moreover, they show, using IFN-γ knockout mice, that the production of IL-12p40 by spleen cells and IL-12p40 expression by dendritic cells (based on in vivo staining) does not require IFN-γ. Furthermore, by the use of CD40L knockout mice and SCID mice, it was found that IL12p40 production does not depend on activation by T cells that express CD40L.

On the basis of these findings, Reis e Sousa et al. propose that dendritic cells, not macrophages, are the key initiators of innate responses as well as dictators of the response class (Th1 versus Th2) of cells expressing acquired immunity. Underlying this proposal are the indications that macrophages may not be able to produce IL-12 in response to microbial antigens unless previously activated (25), an observation confirmed for splenic macrophages in Reis e Sousa et al. Also, dendritic cells, but not macrophages, are efficient transporters of antigen from infected tissue sites to the T cell areas of draining lymph nodes (26), where potentially responsive naive T cells are located. How might this proposal translate into specifics in regard to initiation of innate and acquired immunity following ingestion of infective *T. gondii* cysts by the natural peroral route?

Interface between Host Cells and Toxoplasma in the Gut. As mentioned above, *Toxoplasma* infections are ordinarily acquired by ingestion of cysts. Therefore, it is most relevant to consider events that may be happening locally in the gut and associated lymphoid tissue. Unfortunately, in the case of primary *Toxoplasma* infection, we have little detailed information regarding which sites are the first to be infected and where pathogen-responsive host cells first encounter parasite antigens. We know that *T. gondii* tachyzoites can be found in Peyer's patches within a day or two after cysts are fed to experimental mice (2, 3), but we don't know whether the predominant entrance pathway of tachyzoites from the gut is into Peyer's patches via M cells (27), or via direct infection of nonlymphoid mucosal cells. Furthermore, we don't know which of several *T. gondii* antigens that have been identified may be most important in stimulating innate protective mechanisms or in initiating lymphocyte-dependent acquired immunity.

If *T. gondii* enters the host predominantly via Peyer's patches through M cells, it is likely that dendritic cells are involved in the initial presentation of antigens to T cells, since Peyer's patches are rich in dendritic cells but relatively poor in macrophages (28–30). If, on the other hand, tachyzoites invade other gut sites, dendritic cells in gut epithelia and in lamina propria may acquire *T. gondii* antigens and thence traffic to mesenteric lymph nodes to stimulate T cells (31, 32). If *Toxoplasma* reach the lamina propria, then macrophages may be among the first antigen-presenting cells they encounter, since the lamina propria is rich in macrophages but relatively poor in dendritic cells (30). However, lamina propria T cells exhibit characteristics indicative of prior activation (28), and macrophages may not be efficient at priming naive T cells. Furthermore, macrophages, unlike dendritic cells, are not likely to traffic from lamina propria to lymph nodes where naive T cells are located. Thus, the initial activation of naive T cells probably occurs either in Peyer's patches after stimulation by dendritic cells, or in mesenteric nodes, rather than in the lamina propria after stimulation by macrophages. Of course, dendritic cells, having acquired antigens from *T. gondii* in Peyer's patches, may then traffic to mesenteric lymph nodes for stimulation of naive T cells. Therefore, it seems most likely that dendritic cells are the initiators of responses from naive T cells in Peyer's patches or mesenteric nodes, in mice orally infected with *T. gondii*. This is the scenario that would seem to be favored by the findings of Reis e Sousa et al. (1). The principal role for macrophages in this scenario would then be to restimulate already activated T cells in the lamina propria, and other sites for that matter. Restimulated T cells would thus provide activation signals necessary for macrophages to carry out effector functions against parasites that may have invaded at sites other than Peyer's patches or have spread to distant sites.

What about NK cell–dependent innate resistance? Although NK cells can be found in uninfected rodent Peyer's patches and lamina propria, their numbers may be limited in those sites. In contrast, NK cells are more abundant in spleen and blood. Since spleen, along with Peyer's patches, is one of the earliest sites at which *Toxoplasma* tachyzoites can be detected after oral infection of mice, it is probable that much of the initial *Toxoplasma*-induced, IL-12–dependent activation of NK cells takes place in the spleen. Activated NK cells would then traffic, via blood, to local sites of infection, where they could become further stimulated by IL-12–producing macrophages to produce protective IFN-γ, as suggested (1). This model is consistent with what we have found regarding temporal kinetics of accumulation of NK cells and NK cell–dependent IFN-γ production at a primary site of infection in the peritoneal cavity (11). Significant accumulation of NK cells and IFN-γ production does not occur until about day 3 after intraperitoneal inoculation of *T. gondii* cysts, which is later than the first appearance of tachyzoites in the spleen (3). These kinetics are compatible with the notion that NK cells may be immigrants into the infection site after initial activation elsewhere. Certainly, the diminished numbers of NK cells in peritoneal cavitites of *Toxoplasma*-infected mice treated with anti-CR3 antibody (13), which inhibits the recruitment of CR3-expressing cells such as NK cells into inflamed sites (33), is consistent with this possibility.

Important Questions to be Answered. There is no doubt that much remains to be learned before we can confidently conclude that dendritic cells are the first producers of IL-12 in *T. gondii* infections in vivo, and that dendritic cells, rather than tissue macrophages, are the cells most instru-

mental in initiating innate NK cell–dependent and T cell responses to *Toxoplasma*. For example, IL-12–dependent mechanisms may be bypassed altogether, as suggested by a report that the ts-4 vaccine strain of *T. gondii*, which is an excellent inducer of protective T cell–dependent immunity, may nonetheless elicit IFN-γ in vivo by an IL-12–independent, as well as an IL-12–dependent, pathway (34). It seems clear that detailed studies of early events occuring locally in intestines and associated lymphoid tissue, as well as in spleen, after oral administration of *Toxoplasma* cysts are needed to answer key questions regarding initiation of host-protective responses to this parasite. At which sites do parasites first enter host tissue? Where are dendritic cells, macrophages, and natural killer cells localized in relation to initial infection sites? Where and when do these host cells first express cytokines indicating that they are responding to the parasite? Are they responding to the intact parasite itself or to one or more antigens secreted by the parasites? What is the relative importance of responses that occur in the Peyer's patches versus mesenteric lymph nodes versus spleen for the generation of innate responses or acquired immunity? Are dendritic cells and the IL-12 they make involved in protection of chronically infected mice which ingest more cysts? These are just a few of the questions that could profitably be addressed using the highly informative *Toxoplasma* oral infection model.

Address correspondence to Lawrence L. Johnson, Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983. Phone: 518-891-3080; FAX: 518-891-5126; E-mail: ljohnson@northnet.org

Received for publication 8 October 1997 and in revised form 16 October 1997.

References

1. Reis e Sousa, C., S. Hieny, T. Scharton-Kersten, D. Jankovic, H. Charest, R.N. Germain, and A. Scher. 1997. 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.* 186:1819–1829.
2. McLeod, R., R.G. Estes, D.G. Mack, and H. Cohen. 1984. Immune response of mice to ingested *Toxoplasma gondii*: a model of toxoplasma infection acquired by ingestion. *J. Infect. Dis.* 149:234–244.
3. Sumyuen, M.H., Y.J. Garin, and F. Derouin. 1995. Early kinetics of *Toxoplasma gondii* infection in mice infected orally with cysts of an avirulent strain. *J. Parasitol.* 81:327–329.
4. Paugam, A., J. Dupouy-Camet, M.-H. Sumuyen, S. Romand, J. Lamoril, and F. Derouin. 1995. Detection of *Toxoplasma gondii* parasitemia by polymerase chain reaction in perorally infected mice. *Parasite.* 2:181–184.
5. McCabe, R.E., and J.S. Remington. 1990. *Toxoplasma gondii*. *In* Principles and Practices of Infectious Diseases, 3rd edition. G.L. Mandell, R.G. Douglas, Jr. and J.E. Bennett, editors. Churchill Livingstone, Inc., New York. 2090–2103.
6. Suzuki, Y., M.A. Orellana, R.D. Schreiber, and J.S. Remington. 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. *Science (Wash. DC).* 240:516–518.
7. Johnson, L.L. 1992. A protective role for endogenous tumor necrosis factor in *Toxoplasma gondii* infection. *Infect. Immun.* 60:1979–1983.
8. Gazzinelli, R.T., M. Wysocka, S. Hayashi, E.Y. Denkers, S. Hieny, P. Caspar, G. Trinchieri, and A. Sher. 1994. Parasite-induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection with *Toxoplasma gondii*. *J. Immunol.* 153:2533–2543.
9. Khan, I.A., T. Matsuura, and L.H. Kasper. 1994. Interleukin-12 enhances murine survival against acute toxoplasmosis. *Infect. Immun.* 62:1639–1642.
10. Hunter, C.A., E. Candolfi, C. Subauste, V. Van Cleave, and J.S. Remington. 1995. Studies on the role of interleukin-12 in acute murine toxoplasmosis. *Immunology.* 84:16–20.
11. Johnson, L.L., F.P. VanderVegt, and E.A. Havell. 1993. Gamma interferon–dependent temporary resistance to acute *Toxoplasma gondii* infection independent of $CD4^+$ or $CD8^+$ lymphocytes. *Infect. Immun.* 61:5174–5180.
12. Sayles, P.C., and L.L. Johnson. 1997. Exacerbation of toxoplasmosis in neutrophil-depleted mice. *Natural Immunity.* In press.
13. Johnson, L.L., G.W. Gibson, and P.C. Sayles. 1996. CR3-dependent resistance to acute *Toxoplasma gondii* infection in mice. *Infect. Immun.* 64:1998–2003.
14. Sher, A., I.P. Oswald, S. Hieny, and R.T. Gazzinelli. 1993. *Toxoplasma gondii* induces a T-independent IFN-γ response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor–α. *J. Immunol.* 150:3982–3989.
15. Gazzinelli, R.T., S. Hieny, T.A. Wynn, S. Wolf, and A. Sher. 1993. Interleukin 12 is required for the T-lymphocyte–independent induction of interferon γ by an intracellular parasite and induces resistance in T-cell–deficient hosts [see comments]. *Proc. Natl. Acad. Sci. USA.* 90:6115–6119.
16. Langermans, J.A., M.E. van der Hulst, P.H. Nibbering, and R. van Furth. 1992. Endogenous tumor necrosis factor alpha is required for enhanced antimicrobial activity against *Toxoplasma gondii* and *Listeria monocytogenes* in recombinant gamma interferon–treated mice. *Infect. Immun.* 60:5107–5112.
17. Sibley, L.D., L.B. Adams, Y. Fukutomi, and J.L. Krahenbuhl. 1991. Tumor necrosis factor–α triggers antitoxoplasmal activity of IFN-γ primed macrophages. *J. Immunol.* 147:2340–2345.
18. Denkers, E.Y., R.T. Gazzinelli, D. Martin, and A. Sher. 1993. Emergence of $NK1.1^+$ cells as effectors of IFN-γ–dependent immunity to *Toxoplasma gondii* in MHC class I–deficient mice. *J. Exp. Med.* 178:1465–1472.
19. Sayles, P.C., and L.L. Johnson. 1996. Intact immune defenses are required for mice to resist the ts-4 vaccine strain of *Toxoplasma gondii*. *Infect. Immun.* 64:3088–3092.
20. Suzuki, Y., and J.S. Remington. 1988. Dual regulation of resistance against Toxoplasma gondii infection by $Lyt\text{-}2^+$ and $Lyt\text{-}1^+$, $L3T4^+$ T cells in mice. *J. Immunol.* 140:3943–3946.
21. Gazzinelli, R.T., F.T. Hakim, S. Hieny, G.M. Shearer, and A. Sher. 1991. Synergistic role of $CD4^+$ and $CD8^+$ T lymphocytes in IFN-γ production and protective immunity in-

duced by an attenuated *Toxoplasma gondii* vaccine. *J. Immunol.* 146:286–292.
22. Gazzinelli, R., Y. Xu, S. Hieny, A. Cheever, and A. Sher. 1992. Simultaneous depletion of $CD4^+$ and $CD8^+$ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. *J. Immunol.* 149:175–180.
23. Nagasawa, H., T. Manabe, Y. Maekawa, M. Oka, and K. Himeno. 1991. Role of $L3T4^+$ and $Lyt\text{-}2^+$ T cell subsets in protective immune responses of mice against infection with a low or high virulent strain of *Toxoplasma gondii*. *Microbiol. Immunol.* 35:215–222.
24. Parker, S.J., C.W. Roberts, and J. Alexander. 1991. $CD8^+$ T cells are the major lymphocyte subpopulation involved in the protective immune response to *Toxoplasma gondii* in mice. *Clin. Exp. Immunol.* 84:207–212.
25. Skeen, M.J., M.A. Miller, T.M. Shinnick, and H.K. Ziegler. 1996. Regulation of murine macrophage IL-12 production. Activation of macrophages in vivo, restimulation in vitro, and modulation by other cytokines. *J. Immunol.* 156:1196–1206.
26. Austyn, J.M. 1996. New insights into the mobilization and phagocytic activity of dendritic cells. *J. Exp. Med.* 183:1287–1292.
27. Neutra, M.R., A. Frey, and J.-P. Kraehenbul. 1996. Epithelial M cells: gateways for mucosal infection and immunization. *Cell.* 86:345–348.
28. Hurst, S.D., and T.A. Barrett. 1996. T-cell responses to enteric antigens. *Semin. Gastrointest. Dis.* 7:118–123.
29. Kelsall, B.L., and W. Strober. 1996. Distinct populations of dendritic cells are present in the subepithelial dome and T cell regions of the murine Peyer's patch. *J. Exp. Med.* 183:237–247.
30. Pavli, P., C.E. Woodhams, W.F. Doe, and D.A. Hume. 1990. Isolation and characterization of antigen-presenting cells from mouse intestinal lamina propria. *Immunology*. 70: 40–47.
31. Liu, L.M., and G.G. MacPherson. 1993. Antigen acquisition by dendritic cells: intestinal dendritic cells acquire antigen administered orally and can prime naive T cells in vivo. *J. Exp. Med.* 177:1299–1307.
32. MacPherson, G.G., C.D. Jenkins, M.J. Stein, and C. Edwards. 1995. Endotoxin-mediated dendritic cell release from the intestine. Characterization of released dendritic cells and TNF dependence. *J. Immunol.* 154:1317–1322.
33. Rosen, H., and S. Gordon. 1987. Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro and inflammatory cell recruitment in vivo. *J. Exp. Med.* 166:1685–1701.
34. Scharton-Kersten, T., P. Caspar, A. Sher, and E.Y. Denkers. 1996. *Toxoplasma gondii*: evidence for interleukin 12–dependent and –independent pathways of interferon-γ production induced by an attenuated parasite strain. *Exp. Parasitol.* 84: 102–114.