# Critical coordination of innate immune defense against *Toxoplasma gondii* by dendritic cells responding via their Toll-like receptors

Baidong Hou[a,1,2], Alicia Benson[b], Lili Kuzmich[a], Anthony L. DeFranco[a], and Felix Yarovinsky[b,2]

[a]Department of Microbiology and Immunology, University of California, San Francisco, CA, 94143; and [b]Department of Immunology, University of Texas Southwestern Medical Center, Dallas, TX 75390

Edited* by Bruce Beutler, The Scripps Research Institute, La Jolla, CA, and approved November 19, 2010 (received for review August 4, 2010)

**Toll-like receptors (TLRs) play an important role in host defense against a variety of microbial pathogens. We addressed the mechanism by which TLRs contribute to host defense against the lethal parasite *Toxoplasma gondii* by using mice with targeted inactivation of the TLR adaptor protein myeloid differentiation primary response gene 88 (MyD88) in different innate cell types. Lack of MyD88 in dendritic cells (DCs), but not in macrophages or neutrophils, resulted in high susceptibility to the *T. gondii* infection. In the mice deficient in MyD88 in DCs, the early IL-12 response by DCs was ablated, the IFN-γ response by natural killer cells was delayed, and the recruited inflammatory monocytes were incapable of killing the *T. gondii* parasites. The T-cell response, although attenuated in these mice, was sufficient to eradicate the parasite during the chronic stage, provided that defects in DC activation were compensated by IL-12 treatment early after infection. These results demonstrate a central role of DCs in orchestrating the innate immune response to an intracellular pathogen and establish that defects in pathogen recognition by DCs can predetermine sensitivity to infection.**

innate immunity | host-pathogen interactions | natural killer cells

The protozoan parasite *Toxoplasma gondii* is a highly successful intracellular pathogen that has evolved sophisticated mechanisms for its evasion of immune killing (1). Worldwide, it is estimated that 30–70% of the human population is infected with this parasite, and essentially the entire human population is at risk for infection (2). Type I cell-mediated immunity plays a key role in the host resistance to *T. gondii* infection. Most attention has been focused on the essential role of IFN-γ–producing T helper type 1 (Th1) effector T cells for the resolution of acute infections and for control of latent, chronic infections (3). Recently, evidence has begun to accumulate also implicating innate immune cell types in early defense against *T. gondii* infection. For example, depletion of natural killer (NK) cells or dendritic cells (DCs) rendered mice susceptible to *T. gondii* infection (4, 5). In both cases, the cause still may be related to defective activation of $CD4^+$ T cells. Interestingly, another study has found that failure to recruit $Gr\text{-}1^+$ inflammatory monocytes to *T. gondii*-infected sites also resulted in increased susceptibility despite apparently normal development of the Th1 response (6), suggesting that the innate arm of host immunity also plays a unique role in addition to activating the T-cell response. It is unclear how these different immune cell types interact to promote protection against *T. gondii*.

Toll-like receptors (TLRs) are critical innate receptors for recognition of ligands expressed by *T. gondii*, including profilin, which in the mouse stimulates TLR11 (7) (however, the human Tlr11 gene appears to contain a stop codon that would prevent expression of the protein *T. gondii*. Glycans and diacylglycerols also can stimulate TLR2 and TLR4 (8, 9). Although mice deficient in individual TLRs show only a partial defect in controlling *T. gondii* infection (7, 9–11), mice deficient for MyD88, a key adaptor for signaling by almost all TLRs, are highly susceptible to acute *T. gondii* infection. Mice deficient in MyD88 exhibit a defective Th1 response (12–14), but, because TLRs are expressed by many immune cell types and perform many functions (15), it is not clear how TLR signaling regulates different immune cells and contributes to host defense against this pathogen.

To investigate these questions, we took advantage of a recent mouse model in which specific ablation of the mouse MyD88 gene in certain cell types can be engineered using Cre-lox technology (16). We report here that MyD88 signaling in DCs, but not in macrophages or neutrophils, is required for innate immune control of parasite replication during acute *T. gondii* infection of the peritoneum before the involvement of Th1 effectors in the defense. Mice deficient in MyD88 in DCs and infected with *T. gondii* exhibited a nearly complete defect in IL-12 production, a delay in IFN-γ production by NK cells, and a failure to activate killing mechanisms of inflammatory monocytes, resulting in uncontrolled parasite growth. Although the Th1 response to *T. gondii* was slightly attenuated in the mice lacking MyD88 selectively in DCs, it was sufficient to maintain protection if the innate defect was corrected by injection of IL-12. Therefore, our results establish a critical function of MyD88 in DCs in activating type 1 innate immunity against this intracellular parasite and also provide evidence that a defect in early innate immunity may predetermine the outcome of the infection even in the presence of a largely normal adaptive immune response.

## Results

**DC-*Myd88*$^{-/-}$ Mice Are Highly Susceptible to *T. gondii* Infection.** To investigate cell-specific functions of MyD88 during experimental toxoplasmosis, we used the well-established model of i.p. infection with *T. gondii* (17, 18). Because tissue macrophages are a major innate immune cell type in the peritoneum, and DCs and neutrophils previously were found to play a crucial role in host resistance to *T. gondii*, we focused on the role of MyD88 signaling in these cell types in the defense against *T. gondii* infection. Mice lacking MyD88 selectively in DCs (DC-*Myd88*$^{-/-}$ mice) were generated by crossing the CD11c-Cre transgene into mice with two loxP sites flanking the essential exon 3 of the *Myd88* gene (*Myd88*$^{fl}$ allele), as has been reported previously (16). Furthermore, using a quantitative PCR assay as previously reported (16), we did not detect substantial deletion of *Myd88*$^{fl}$ allele in the resident peritoneal macrophage of these mice (Table S1). To generate mice lacking MyD88 in macrophages, mice with the *Myd88*$^{fl}$ allele were crossed to animals carrying the LysM-Cre transgene, which specifically deletes the targeted gene in tissue, macrophages, and neutrophils (19). In these mice, deletion of the *Myd88*$^{fl}$ allele occurred in more than 90% of resident peritoneal macrophages (henceforth referred to as "MN-*Myd88*$^{-/-}$") (Table S1).

Author contributions: B.H., A.L.D., and F.Y. designed research; B.H., A.B., L.K., and F.Y. performed research; B.H., A.L.D., and F.Y. analyzed data; and B.H., A.L.D., and F.Y. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]Present address: Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences. Beijing 100101, China.

[2]To whom correspondence may be addressed. E-mail: felix.yarovinsky@utsouthwestern.edu or baidong_hou@ibp.ac.cn.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1011549108/-/DCSupplemental.

The survival of these conditional MyD88-deficient mice and their littermate control mice was followed after i.p. injection of different numbers of Me49 *T. gondii* brain cysts. Surprisingly, there was no apparent survival deficit in *T. gondii* infection of the mice lacking MyD88 in peritoneal macrophages and neutrophils (Fig. 1*A*). In contrast, the DC-*Myd88*$^{-/-}$ mice were highly susceptible to *T. gondii* infection. About 40% of these mice died within 2–3 wk when infected with 40 brain cysts, and increasing the infecting dose of *T. gondii* organisms to 200 cysts resulted in almost 100% mortality of the infected mice during the acute phase of the infection (Fig. 1 *A* and *B*). These results revealed that MyD88 signaling in DCs plays a major role in protective immunity during acute *T. gondii* infection. Although all *Myd88*$^{-/-}$ mice died during the acute stage, some DC-*Myd88*$^{-/-}$ mice survived the lower dose of acute infection but died between 4 and 6 wk after infection (Fig. 1*A*). The difference observed between *Myd88*$^{-/-}$ and DC-*Myd88*$^{-/-}$ mice indicates a role for MyD88 signaling in DCs as well as in other cell types (e.g., T cells) (20).

**Early Defect in Controlling *T. gondii* Expansion in DC-*Myd88*$^{-/-}$ Mice.** To investigate the cause of death of DC-*Myd88*$^{-/-}$ mice during the acute phase of infection, we quantified *T. gondii* organisms after infection by measuring the amount of *T. gondii* genomic DNA in the lavaged peritoneal cells using quantitative PCR. We found that in control mice the number of tachyzoites increased slightly from day 1 to day 5 and then declined. In contrast, *T. gondii* tachyzoites expanded rapidly in the DC-*Myd88*$^{-/-}$ mice and *Myd88*$^{-/-}$ mice, with significantly more tachyzoites present in the peritoneum of the DC-*Myd88*$^{-/-}$ mice as early as day 3 after infection, and with >100-fold more tachyzoites present in these mice on day 8 (Fig. 1*C*). The DC-*Myd88*$^{-/-}$ mice contained only slightly fewer tachyzoites than the *Myd88*$^{-/-}$ mice, demonstrating a major role for DCs in the MyD88-mediated innate immune response to *T. gondii* infection (Fig. 1*C*). Substantially more tachyzoites also were detected in other organs of the DC-*Myd88*$^{-/-}$ mice (Fig. S1*A*), indicating an accelerated dissemination from the original locus of infection that may have caused death during the acute phase of the infection. Strikingly, this early defect in controlling *T. gondii* expansion was not seen in *Tcrα*$^{-/-}$ mice lacking αβ T cells or in *Rag1*$^{-/-}$ mice lacking both T and B cells (Fig. 1*D*), indicating that insufficient activation of the adaptive immune system was not the cause of the parasite expansion and dissemination seen in DC-*Myd88*$^{-/-}$ mice. Consistent with the survival data (Fig. 1), MN-*Myd88*$^{-/-}$ mice were able to control parasite expansion at a level comparable to WT mice (Fig. S1*B*). These results revealed that a deficiency of MyD88 in macrophages and neutrophils had little effect on innate defense against *T. gondii*.

**Recruitment of Inflammatory Cells in Acute *T. gondii* Infection.** To understand defects in the immune response to *T. gondii* infection caused by loss of MyD88 in DCs, we first characterized the inflammatory cells that were recruited rapidly into the peritoneal cavity after infection with the parasite. We observed that among the recruited cells, the Ly6C$^{+}$ inflammatory monocytes were the major cell type (Fig. 2 *A*–*D*). Compared with WT mice, infected DC-*Myd88*$^{-/-}$ mice often exhibited a small deficit in recruitment of inflammatory monocytes early after infection, but this deficit became less obvious as early as day 3 after infection (Fig. 2*A* and Fig. S2). Conversely, DC-*Myd88*$^{-/-}$ mice generally recruited more eosinophils, as did the *Myd88*$^{-/-}$ mice (Fig. S2*C*). At later time points, the infected DC-*Myd88*$^{-/-}$ mice recruited considerably more cells than WT mice, probably in response to increased numbers of *T. gondii*. In contrast, substantially fewer monocytes and neutrophils were recruited into the peritoneum of *Myd88*$^{-/-}$ mice than DC-*Myd88*$^{-/-}$ mice (Fig. 2*C* and Fig. S2*B*), although there were more tachyzoites in *Myd88*$^{-/-}$ mice (Fig. 1*C*). These results suggest that MyD88 signaling in DCs is not essential for the recruitment of these inflammatory cell types.

**Defective Activation of Inflammatory Monocytes in DC-*Myd88*$^{-/-}$ Mice.** Enhanced *T. gondii* replication in the presence of a nearly normal attraction of inflammatory cells in the DC-*Myd88*$^{-/-}$ mice implied a defect in the killing of intracellular parasites in the absence of MyD88 signaling in DCs. To identify the cell types that were infected by *T. gondii*, we infected mice with another type II *T. gondii* strain that expresses GFP. Defects in controlling parasite expansion and in surviving the infection observed with this strain were similar to those seen in the Me49 strain (Fig. S3). The peritoneal cells were lavaged and analyzed by flow cytometry 5 d after infection. It was evident that all immune cell types can be infected by *T. gondii*; however, inflammatory monocytes constituted the majority of the infected cells (Fig. 2*E* and Fig. S4).

Flow cytometric analysis revealed reduced surface expression of major MHC II molecules by inflammatory monocytes (Fig. 2*F*), suggesting a defect of monocyte activation in DC-*Myd88*$^{-/-}$ mice. To examine the killing machinery by these cells directly, we measured the induction of inducible nitric-oxide synthase (iNOS), a crucial factor for monocyte killing of intracellular pathogens, by staining intracellular iNOS protein in inflammatory monocytes isolated from the peritoneum on day 3 and day 5 after infection. Consistent with the results of MHC II expression, a severely delayed induction of iNOS was seen in the DC-*Myd88*$^{-/-}$ mice (Fig. 2*G*). By quantitative PCR, we did not detect deletion of *Myd88*$^{fl}$ in purified inflammatory monocyte (Table S1), ruling out the possibility that the functional defect was caused by deletion of

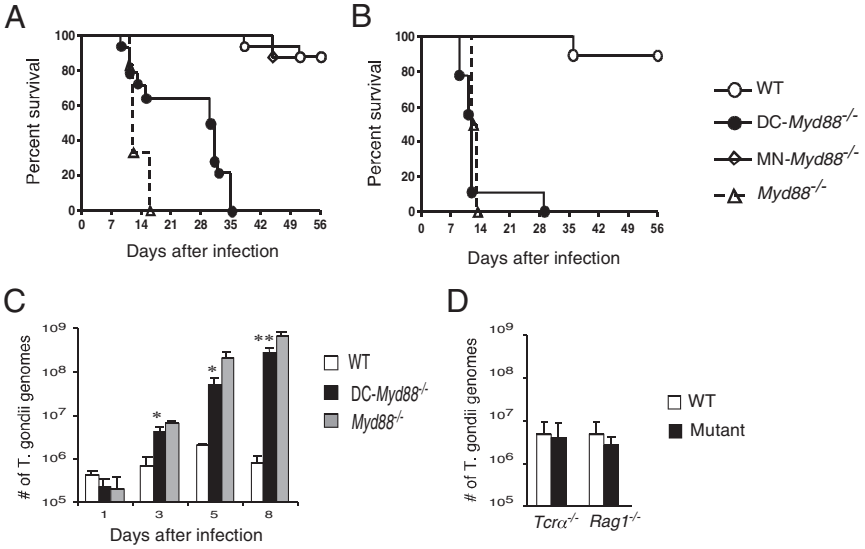


**Fig. 1.** Role of MyD88 signaling in DCs for survival of mice after *T. gondii* infection. (*A* and *B*) Female mice 7–9 wk old were infected i.p. with 40 (*A*) or 200 (*B*) cysts of the avirulent Me49 strain, and their survival was monitored daily up to 8 wk after infection. (*A*) Results of WT *Myd88*$^{fl/-}$ mice ($n$ = 17), DC-*Myd88*$^{-/-}$ mice ($n$ = 14), MN-*Myd88*$^{-/-}$ mice ($n$ = 8), and *Myd88*$^{-/-}$ mice ($n$ = 6) from two separate experiments were pooled. (*B*) Results of WT mice ($n$ = 9), DC-*Myd88*$^{-/-}$ mice ($n$ = 9), and *Myd88*$^{-/-}$ mice ($n$ = 4) from two separate experiments were pooled. Significant differences were found between WT mice and the DC-*Myd88*$^{-/-}$ mice in both low and high infecting doses (log-rank test; $P < 0.001$), and between DC-*Myd88*$^{-/-}$ mice and *Myd88*$^{-/-}$ mice at low infection dose ($P < 0.05$). (*C*) The amount of *T. gondii* genomic DNA was measured by quantitative PCR after infection with 40 cysts of the Me49 strain. *$P < 0.05$; **$P < 0.01$. (*D*) The amount of *T. gondii* in the lavaged peritoneal cells of *Tcrα*$^{-/-}$ mice, *Rag1*$^{-/-}$ mice, and WT mice at day 5 after infection. Data are expressed as mean plus SD of three or four mice and are representative of two to four separate experiments. Statistical comparison is between the DC-*Myd88*$^{-/-}$ mice and the WT control mice.

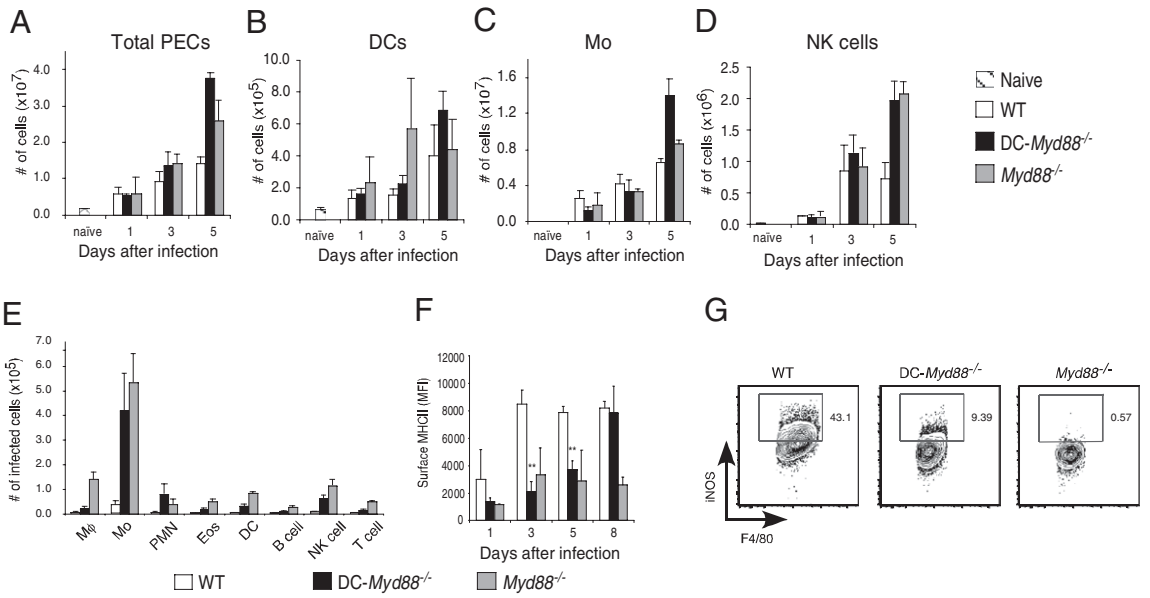


**Fig. 2.** Role of MyD88 in DCs for the recruitment of inflammatory cells in acute *T. gondii* infection. (*A–D*) Peritoneal cells were enumerated and analyzed by flow cytometry after infection with 40 cysts of the Me49 strain. The numbers of (*A*) total infiltrating cells (peritoneal exudate cells, PECs), (*B*) DCs ($CD11c^+$, MHC $II^+$), (*C*) inflammatory monocytes (Mo) ($F4/80^{int}$, $Ly6C^+$, $Ly6G^-$), and (*D*) NK cells ($NK1.1^+$, $TCR\beta^-$, $MHCII^-$, $F4/80^-$) are shown. Data are expressed as mean plus SD of three or four mice and are representative of two or three separate experiments. (*E*) Numbers of infected peritoneal cells (mean plus SD of four mice) on day 5 after infection with 100 tachyzoites of the GFP-expressing Pru$\Delta$*htp* strain. (*F*) Median fluorescence intensity (MFI) of surface MHC II on inflammatory monocytes. Statistical comparison is between the DC-*Myd88*$^{-/-}$ mice and the WT control mice. $^{**}P < 0.001$. (*G*) Intracellular iNOS expression in gated inflammatory monocytes ($Ly6G^-$, $Ly6C^+$, $F4/80^+$) on day 3 after infection as analyzed by flow cytometry.

MyD88 by CD11c-Cre in these cells. Together, these results revealed an important interaction between DCs and inflammatory monocytes; that is, MyD88 in DCs is essential for the activation of but not for the recruitment of inflammatory monocytes in *T. gondii*-infected mice.

**Delayed Induction of IFN-γ in *T. gondii*-Infected DC-*Myd88*$^{-/-}$ Mice.** IFN-γ is a key factor inducing MHC II and iNOS expression in macrophages and monocytes. Moreover, it is known that IFN-γ plays a critical role in stimulating *T. gondii* killing in both immune and nonimmune cells (21, 22). Indeed, we observed that monocyte activation, as measured by surface expression of MHC II and intracellular iNOS, also was severely defective in IFN-γ$^{-/-}$ or in IFNGR1$^{-/-}$ mice infected with *T. gondii* (Fig. S5). To examine whether lack of MyD88 in DCs affected the production of IFN-γ in response to *T. gondii* infection, the induction of IFN-γ mRNA in peritoneal cells was measured by quantitative PCR. There was a severe reduction in the production of IFN-γ in the DC-*Myd88*$^{-/-}$ mice on day 2 postinfection (Fig. 3*A*). Substantial induction of IFN-γ was detected in the DC-*Myd88*$^{-/-}$ mice beginning on day 3 postinfection (Fig. 3*A*), but *Myd88*$^{-/-}$ mice exhibited an even greater delay in IFN-γ production. A similar deficit in the serum levels of IFN-γ also was found in the DC-*Myd88*$^{-/-}$ and *Myd88*$^{-/-}$ mice (Fig. S6). These results suggested that a timely induction of IFN-γ is crucial for host defense against *T. gondii* infection and that TLR/MyD88 signaling in DCs is required for inducing the early IFN-γ expression after *T. gondii* infection.

To identify the cellular source of IFN-γ in the *T. gondii*-infected mice, the peritoneal cells were stained for intracellular IFN-γ. We found that NK cells dominated the early (days 2–5) IFN-γ response (Figs. 3 *B* and *C*), and αβ T cells also contributed to some extent. Remarkably, although a large number of NK cells in WT mice expressed intracellular IFN-γ as early as day 2 after infection, this response was ablated almost completely in the DC-*Myd88*$^{-/-}$ mice and became obvious only on day 3 (Fig. 3*D*), demonstrating a critical crosstalk between DCs and NK cells that accounted for the rapid IFN-γ response in the early defense against *T. gondii* infection. The dialogue between DC and NK cells also was observed in cell-culture experiments in which the addition of live *T. gondii* parasites to cultures of NK cells, DCs, and macrophages led to NO production by the macrophages that required DCs, NK cells, and IFN-γ (Fig. S7).

The considerable difference in the levels of IFN-γ produced by NK cells in the DC-*Myd88*$^{-/-}$ and *Myd88*$^{-/-}$ mice probably is explained by the inability of NK cells in the *Myd88*$^{-/-}$ mice to respond to IL-18, which enhances IFN-γ production (23, 24). Indeed, anti–IL-18 treatment of *T. gondii*-infected DC-*Myd88*$^{-/-}$ mice reduced IFN-γ production in these mice to the levels observed in the *Myd88*$^{-/-}$ animals (Fig. S8).

**Defective IL-12 Induction in *T. gondii*-Infected DC-*Myd88*$^{-/-}$ Mice.** Because mice lacking IL-12, a potent inducer of IFN-γ, are highly susceptible to *T. gondii* infection (25, 26), and MyD88 signaling in DCs is believed to be required for IL-12 induction by TLR ligands, we explored the effect of deletion of MyD88 in DCs on the expression of IL-12 and its relationship with the induction of IFN-γ during *T. gondii* infection. Initial analysis revealed substantially reduced the levels of the p40 subunit of IL-12 (IL-12p40) in the peritoneal lavage supernatant taken from the infected DC-*Myd88*$^{-/-}$ mice compared with that from infected WT mice (Fig. 4*A*). Moreover, intracellular staining of IL-12p40 and quantifying the mRNA level of IL12p40 in purified peritoneal cells revealed that most IL-12–producing cells were conventional DCs and not inflammatory monocytes or resident peritoneal macrophages, because the cytokine-positive cells were $CD11c^+$ and MHC $II^+$ and were $CD11b^{low-neg}$, $F4/80^-$, and $Ly6C^-$ (Fig. 4*B* and Fig. S9).

When the relationship between the amount of IL-12p40 in the peritoneum and the induction of IFN-γ mRNA in each individual mouse was compared, a strong positive correlation was found between these values on day 2 (Fig. 4*C*, *Upper*), supporting the conclusion that IL-12 plays a critical role in the induction of IFN-γ in mice infected with *T. gondii*. Surprisingly, the close correlation of these cytokines disappeared only 1 d later, when IFN-γ was greatly induced in the DC-*Myd88*$^{-/-}$ mice although the amount of IL-12 was severely lower in these mice (Fig. 4*C*, *Lower*), suggesting that by day 3 other cytokines produced in infected DC-*Myd88*$^{-/-}$ mice were capable of promoting secretion of IFN-γ by NK cells.

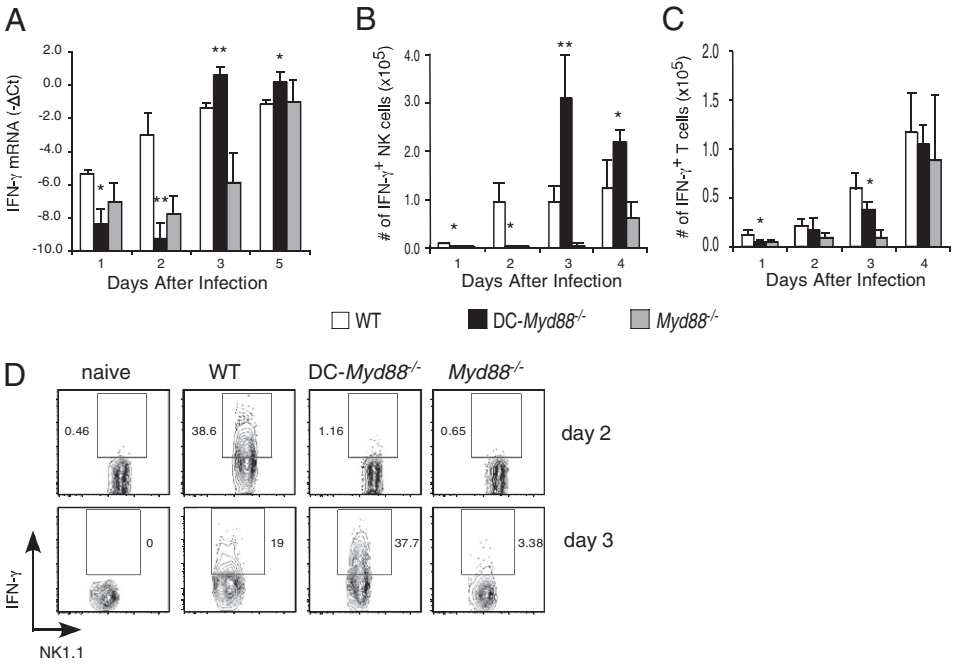


**Fig. 3.** Induction of IFN-γ in the *T. gondii*-infected DC-*Myd88*$^{-/-}$ mice. (*A*) Induction of IFN-γ mRNA in total peritoneal cells as measured by quantitative RT-PCR. Data are expressed as mean plus SD of the normalized threshold cycle number (−ΔCt) of three or four mice and are representative of two or three separate experiments. (*B–D*) Intracellular IFN-γ as analyzed by flow cytometry. (*B*) Numbers of IFN-γ$^+$ NK cells (mean plus SD of three or four mice) in the peritoneum after infection. (*C*) Numbers of IFN-γ$^+$ T cells in the peritoneum after infection. Statistical comparison is between the DC-*Myd88*$^{-/-}$ mice and the WT control mice. **P* < 0.05; ***P* < 0.01. (*D*) Representative FACS plots of intracellular IFN-γ staining on gated NK cells (NK1.1$^+$, TCRβ$^-$) on day 2 and day 3 after infection.

To test whether the loss of IL-12 induction and the transient delay in IFN-γ production in the DC-*Myd88*$^{-/-}$ mice could account for the severely defective immune control of *T. gondii* infection, recombinant IL-12 was administrated into the peritoneum of these mice. After a single treatment on day 1 after infection, NK cells in the DC-*Myd88*$^{-/-}$ mice expressed substantially higher intracellular IFN-γ on day 2 than did the NK cells in the untreated mice, demonstrating that restoration of IL-12 could correct the defect in the induction of

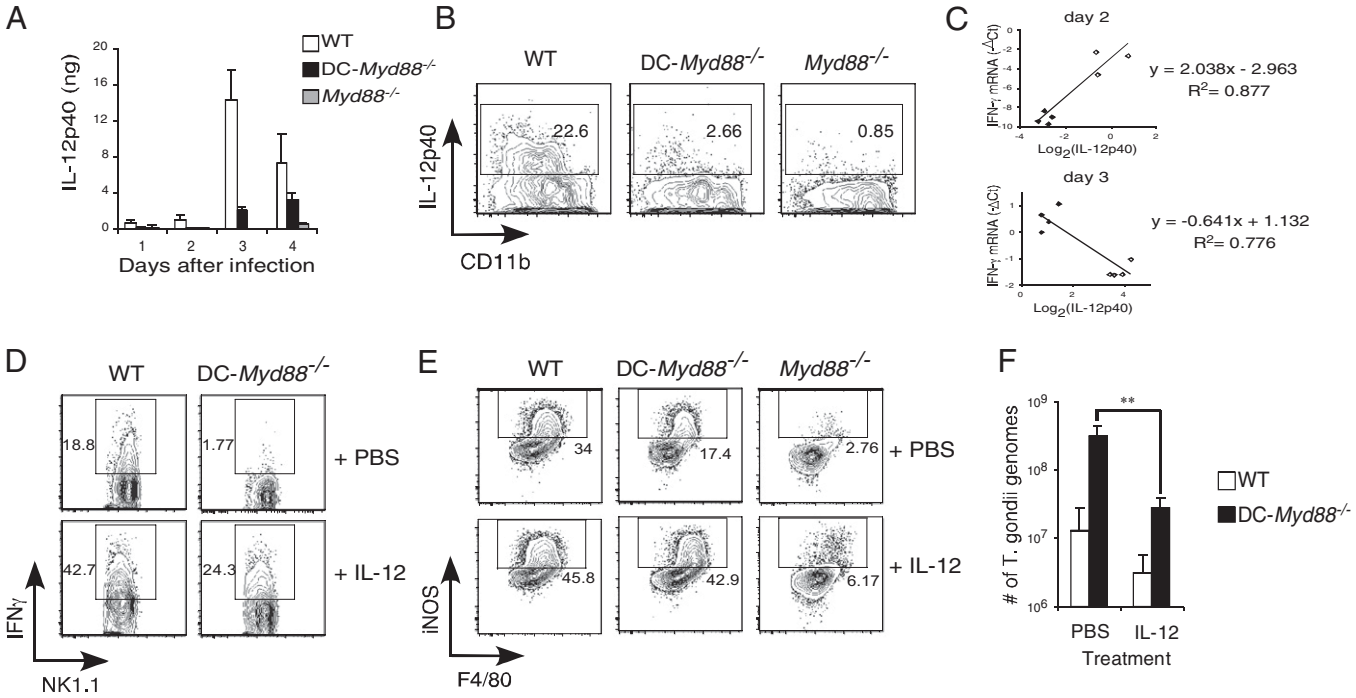


**Fig. 4.** Induction of IL-12 in the *T. gondii*-infected DC-*Myd88*$^{-/-}$ mice. (*A–D*) Induction of IL-12 in peritoneal cells was analyzed after infection with 40 cysts of the Me49 strain. (*A*) Total amount of IL-12p40 (mean plus SD of three or four mice) in the supernatant of peritoneal lavage measured by ELISA. (*B*) Representative flow cytometry plots of intracellular IL-12p40 staining of gated DCs (CD11c$^+$, MHC II$^+$) at day 3 after infection. (*C*) Correlation analysis of the amount of IL-12p40 and the induction of IFN-γ mRNA in the peritoneum at day 2 and day 3 after infection. The amount of IL-12p40 was determined as in *A*, and the level of IFN-γ mRNA was determined as in Fig. 3*A*. ◇, WT mice; ◆, DC-*Myd88*$^{-/-}$ mice. (*D–F*) The effects of IL-12 treatment of mice infected with 40 cysts of the Me49 strain. (*D*) Representative flow cytometry plots of intracellular IFN-γ in gated NK cells 1 d after supplementation with IL-12 or with PBS control on day 1 after infection. (*E*) Representative flow cytometry plots of intracellular iNOS in gated inflammatory monocytes on day 3 after infection. Mice in this experiment received supplementation with IL-12 or with PBS control on day 1 and 2 after infection. (*F*) Total amount of *T. gondii* in the peritoneal cells at day 7 after infection. Four mice of each strain were treated twice with PBS or 150 ng IL-12 daily on day 1 and day 2. Statistical comparison is between the DC-*Myd88*$^{-/-}$ mice treated with or without IL-12. ***P* < 0.01.

IFN-γ by the NK cells of these mice (Fig. 4*D*). Moreover, injection of the DC-*Myd88*$^{-/-}$ mice with IL-12 on the first 2 d after infection also corrected the defective induction of iNOS in inflammatory monocytes in these mice (Fig. 4*E*). Strikingly, this short treatment dramatically alleviated the moribund appearance of these mice and resulted in a >10-fold decrease in the number of tachyzoites present in the peritoneum of these mice on day 7 as compared with the vehicle-treated mice (Fig. 4*F*). These results demonstrate that the early induction of IL-12 by DCs plays a major role in the defense against *T. gondii* infection.

**Partial Defect in Th1 Priming in *T. gondii*-Infected DC-*Myd88*$^{-/-}$ Mice.** Because T-cell–mediated immunity is known to be essential for surviving an acute *T. gondii* infection, we next examined the activation of effector T cells after infection. Eight days after infection, the numbers of effector T cells producing IFN-γ were reduced in DC-*Myd88*$^{-/-}$ mice compared with WT mice, indicating that DC-*Myd88*$^{-/-}$ mice also had a less robust T-cell response to *T. gondii* (Fig. 5 *A* and *B*). However, compared with *Myd88*$^{-/-}$ mice, considerably greater numbers of IFN-γ$^+$ CD4$^+$ and CD8$^+$ T cells were found in the DC-*Myd88*$^{-/-}$ mice, demonstrating that effector T cells can develop despite a deficiency of MyD88 in DCs. The partial activation of T cells may have rescued some of the *T. gondii*-infected DC-*Myd88*$^{-/-}$ mice from acute death, although all these mice died within 5 wk (Fig. 1).

Examination of the brains of infected mice 4 wk after infection revealed that the surviving DC-*Myd88*$^{-/-}$ mice contained 5- to 10-fold more *T. gondii* tissue cysts than did the WT mice (Fig. 5*C*), indicating that they probably experienced more severe toxoplasmic encephalitis. To test whether the failure to control dissemination from the peritoneum and formation of tissue cysts was caused by the defective activation of effector T cells or by increased parasite burden resulting from the defective innate control of parasite replication, we treated the mice with IL-12 for a short time after infection to restrict the expansion of *T. gondii* tachyzoites. We found that this short-term treatment did not correct the number of IFN-γ–producing T cells in the DC-*Myd88*$^{-/-}$ mice (Fig. S10). This result is in agreement with a previous observation indicating that maintenance of Th1 effector function in chronic toxoplasmosis requires persistent IL-12 production (27). To our surprise, the short IL-12 treatment nonetheless substantially reduced the numbers of *T. gondii* tissue cysts in the brains of DC-*Myd88*$^{-/-}$ mice infected with 40 *T. gondii* brain cysts (Fig. 5*C*) and even could rescue mice infected with a higher number of *T. gondii* from acute mortality (Fig. 5*D*), suggesting that the major defect causing chronic death in *T. gondii*-infected DC-*Myd88*$^{-/-}$ mice lies in the failure to contain the early expansion of tachyzoites via innate immunity.

## Discussion

In the work described here, we have examined the role of TLRs on DCs, macrophages, and neutrophils in mediating immunity against *T. gondii*. These experiments revealed that lack of MyD88 signaling only in DCs largely recapitulated the deficiency in innate immunity against *T. gondii* infection observed in complete MyD88$^{-/-}$ mice and also provided considerable insight into the mechanism by which DCs coordinate immune defense against this intracellular pathogen.

Previous studies have established the essential role of T cells in defense against *T. gondii* infection, so we initially were surprised that DC-*Myd88*$^{-/-}$ mice were highly susceptible to acute *T. gondii* infection even though the Th1 responses in these mice were attenuated only slightly. Our subsequent results revealed that TLR recognition by DCs is critical for mounting a rapid type 1 innate immune response to *T. gondii* and that this innate response is essential to prevent acute mortality. Additionally, the innate defect of DC-*Myd88*$^{-/-}$ mice predetermined the outcome of chronic infection by affecting the parasite burden and dissemination to other sites such as the brain. The early immune defects in infected DC-*Myd88*$^{-/-}$ mice included an ablated early IL-12 response by DCs, a delayed IFN-γ response by NK cells, a defective activation of the recruited inflammatory monocytes, and a failure to kill *T. gondii* parasites. Short-term treatment with IL-12 early after infection largely corrected these innate defects but did not correct the attenuated Th1 response, indicating that the defective innate response in these mice is responsible for their increased susceptibility and that the primary cause of this susceptibility is the defective IL-12 response by MyD88-deficient DCs. Previous studies depleting DCs (5) or NK cells (4) or examining IL-12–deficient (25, 26), IFN-γ–deficient (21), or MyD88-deficient mice (12–14) failed to distinguish between early innate defect and defects in generating Th1 effector cells, and it generally was assumed that susceptibility to infection largely reflects a defect in adaptive immunity. Instead, our results demonstrate that a more crucial function of TLR/MyD88 signaling in DCs in defense against *T. gondii* infection is to activate NK cells and inflammatory monocytes. The efficiency of DCs in activating those cells probably arises from their ability to organize the early clustering of those

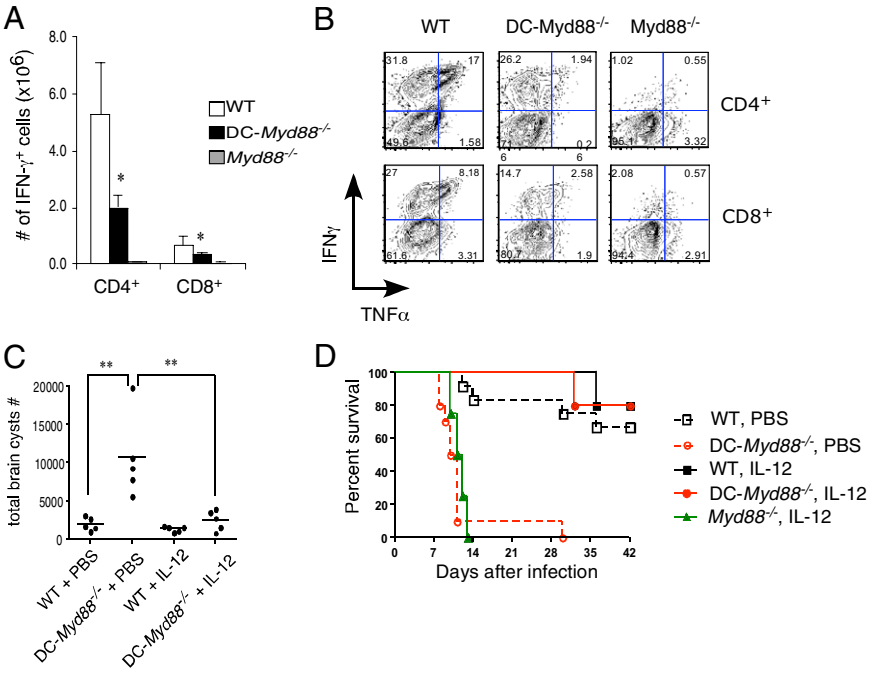


**Fig. 5.** Activation of T cells in the DC-*Myd88*$^{-/-}$ mice. Mice were infected with 40 cysts (*A*–*C*) or 100 cysts (*D*) of the Me49 strain. (*A* and *B*) Splenocytes were analyzed for intracellular IFN-γ and TNF 8 d later after in vitro stimulation with anti-CD3 for 4 h in the presence of brefeldin A. (*A*) The total number of IFN-γ$^+$ CD4$^+$ and CD8$^+$ T cells in the spleens (mean plus SD of four or five mice). (*B*) Representative flow cytometry plots of intracellular IFN-γ and TNF staining of gated CD4$^+$ and CD8$^+$ T cells. (*C* and *D*) Mice were treated with either PBS or 150 ng IL-12 daily from day 1–3. *T. gondii* tissue cysts were enumerated in brain homogenates of surviving mice at day 28 after infection. Statistical comparison is between WT mice and the DC-*Myd88*$^{-/-}$ mice or the DC-*Myd88*$^{-/-}$ mice treated with or without IL-12. *$P < 0.05$. **$P < 0.01$. (*D*) The survival 7- to 8-wk-old female mice was monitored daily up to 6 wk after infection. Results of PBS-treated WT mice ($n = 12$), DC-*Myd88*$^{-/-}$ mice ($n = 10$), IL-12-treated WT mice ($n = 5$), DC-*Myd88*$^{-/-}$ mice ($n = 5$), and *Myd88*$^{-/-}$ mice ($n = 4$) are shown. A significant difference was found between DC-*Myd88*$^{-/-}$ mice treated with or without IL-12 (log-rank test; $P < 0.01$).

innate immune cells around the loci of infection, as recently observed in mice infected with *Listeria monocytogenes* (28), another intracellular pathogen. Surprisingly, among peritoneal-resident cells, only DCs could respond to parasite TLR ligand and coordinate the early type 1 immune defense against *T. gondii*.

We previously found DC-intrinsic MyD88 to be essential in activating Th1 effector cells in response to immunization with soluble *T. gondii* antigen (29). In the case of infection with live *T. gondii* parasites, however, there evidently were additional innate stimuli that promoted a substantial, if somewhat attenuated, Th1 effector response. This finding agrees with our previous observations that aggregated TLR ligands can activate TLR/MyD88 signaling in other myeloid cell types and that in this context cytokines can promote the function of DCs and Th1 development (16). In the case of *T. gondii* infection, we demonstrated that T cells activated by this extrinsic pathway were sufficient to mediate a high level of protection against *T. gondii* provided the innate immune defect was corrected by injection of IL-12.

In summary, our results demonstrate a critical role of TLR/MyD88 signaling in DCs for initiating IL-12– and IFN-γ–dependent innate immune responses to the intracellular pathogen *T. gondii*. Although deficiency of MyD88 signaling in DCs somewhat compromised the activation of effector T cells, the most important defect for host defense was an impaired and delayed activation of innate effector cells, including NK cells and monocytes. Because type 1 immunity similarly is essential for defense against other intracellular pathogens, examining how MyD88 signaling in DCs and other innate immune cells contribute to their defense would add to our understanding of the mechanisms used by the immune system to defend against highly evolved intracellular pathogens.

## Materials and Methods

Full details of methods and associated references are available in *SI Materials and Methods*.

**Mice.** *Myd88*$^{fl}$ mice (B6.129P2-*Myd88*$^{tm1Defr}$) and *Myd88*$^{fl/fl}$ CD11c-Cre (DC-*Myd88*$^{-/-}$) mice have been described previously (16). All animals were housed in a specific pathogen-free animal facility, were used at 7–10 wk of age, and were sex and age matched.

***T. gondii* Infection, Quantitative PCR, and Flow Cytometry.** Two protocols were used to study the immune responses to *T. gondii* infection. In the first protocol, mice were infected i.p. with tissue cysts of the avirulent Me49 strain. In the second protocol, mice were injected with 100 tachyzoites of the GFP- and firefly luciferase (FLUC)-expressing PruΔ*hpt* strain (30). To quantify *T. gondii* organisms in vivo, genomic DNA was extracted using the Allprep DNA/RNA kit or the DNeasy Blood & Tissue kit (Qiagen) from peritoneal cells or organs of the infected mice, and the numbers of *T. gondii* genomes (B1 gene) were quantified by PCR using iTaq SYBR Green Supermix. All flow cytometry data were collected on a LSRII flow cytometer (Becton Dickinson) and were analyzed with FlowJo software (TreeStar).

**Statistical Analysis.** Statistical significance was calculated with the log-rank test, the unpaired Student's *t* test, or the Mann–Whitney *U* test. All *P* values $\leq 0.05$ were considered significant.

**ACKNOWLEDGMENTS.** We thank J. C. Boothroyd and M. Lodoen (Stanford University, Stanford, CA) for providing the PruΔ*hpt* strain and helping with its culture, C. McArthur and Z. Wang for assistance with cell purification by flow cytometry, M. Ji for assistance with the mouse colonies, and D. Sibley for advice. This research was supported by National Institutes of Health Grants R01 AI072058 (to A.L.D.), R01 AI085263 (to F.Y.), and R21 AI079371 (to F.Y.).

1. Boothroyd JC, Dubremetz JF (2008) Kiss and spit: The dual roles of *Toxoplasma rhoptries*. *Nat Rev Microbiol* 6:79–88.
2. Hill DE, Chirukandoth S, Dubey JP (2005) Biology and epidemiology of *Toxoplasma gondii* in man and animals. *Anim Health Res Rev* 6:41–61.
3. Hunter CA, Villarino A, Artis D, Scott P (2004) The role of IL-27 in the development of T-cell responses during parasitic infections. *Immunol Rev* 202:106–114.
4. Goldszmid RS, et al. (2007) TAP-1 indirectly regulates CD4+ T cell priming in *Toxoplasma gondii* infection by controlling NK cell IFN-gamma production. *J Exp Med* 204:2591–2602.
5. Liu CH, et al. (2006) Cutting edge: Dendritic cells are essential for in vivo IL-12 production and development of resistance against *Toxoplasma gondii* infection in mice. *J Immunol* 177:31–35.
6. Bliss SK, Gavrilescu LC, Alcaraz A, Denkers EY (2001) Neutrophil depletion during *Toxoplasma gondii* infection leads to impaired immunity and lethal systemic pathology. *Infect Immun* 69:4898–4905.
7. Yarovinsky F, et al. (2005) TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 308:1626–1629.
8. Debierre-Grockiego F, et al. (2003) Roles of glycosylphosphatidylinositols of *Toxoplasma gondii*. Induction of tumor necrosis factor-alpha production in macrophages. *J Biol Chem* 278:32987–32993.
9. Debierre-Grockiego F, et al. (2007) Activation of TLR2 and TLR4 by glycosylphosphatidylinositols derived from *Toxoplasma gondii*. *J Immunol* 179: 1129–1137.
10. Mun HS, et al. (2003) TLR2 as an essential molecule for protective immunity against *Toxoplasma gondii* infection. *Int Immunol* 15:1081–1087.
11. Minns LA, et al. (2006) TLR9 is required for the gut-associated lymphoid tissue response following oral infection of *Toxoplasma gondii*. *J Immunol* 176:7589–7597.
12. Scanga CA, et al. (2002) Cutting edge: MyD88 is required for resistance to *Toxoplasma gondii* infection and regulates parasite-induced IL-12 production by dendritic cells. *J Immunol* 168:5997–6001.
13. Chen M, et al. (2002) Involvement of MyD88 in host defense and the down-regulation of anti-heat shock protein 70 autoantibody formation by MyD88 in *Toxoplasma gondii*-infected mice. *J Parasitol* 88:1017–1019.
14. Sukhumavasi W, et al. (2008) TLR adaptor MyD88 is essential for pathogen control during oral *Toxoplasma gondii* infection but not adaptive immunity induced by a vaccine strain of the parasite. *J Immunol* 181:3464–3473.
15. Beutler B (2009) Microbe sensing, positive feedback loops, and the pathogenesis of inflammatory diseases. *Immunol Rev* 227:248–263.
16. Hou B, Reizis B, DeFranco AL (2008) Toll-like receptors activate innate and adaptive immunity by using dendritic cell-intrinsic and -extrinsic mechanisms. *Immunity* 29: 272–282.
17. Miller CM, Boulter NR, Ikin RJ, Smith NC (2009) The immunobiology of the innate response to *Toxoplasma gondii*. *Int J Parasitol* 39:23–39.
18. Denkers EY, Butcher BA, Del Rio L, Bennouna S (2004) Neutrophils, dendritic cells and *Toxoplasma*. *Int J Parasitol* 34:411–421.
19. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Förster I (1999) Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res* 8: 265–277.
20. LaRosa DF, et al. (2008) T cell expression of MyD88 is required for resistance to *Toxoplasma gondii*. *Proc Natl Acad Sci USA* 105:3855–3860.
21. Yap GS, Sher A (1999) Effector cells of both nonhemopoietic and hemopoietic origin are required for interferon (IFN)-gamma- and tumor necrosis factor (TNF)-alpha-dependent host resistance to the intracellular pathogen, *Toxoplasma gondii*. *J Exp Med* 189:1083–1092.
22. Martens S, Howard J (2006) The interferon-inducible GTPases. *Annu Rev Cell Dev Biol* 22:559–589.
23. Hunter CA, Reiner SL (2000) Cytokines and T cells in host defense. *Curr Opin Immunol* 12:413–418.
24. Hunter CA, et al. (1997) Comparison of the effects of interleukin-1 alpha, interleukin-1 beta and interferon-gamma-inducing factor on the production of interferon-gamma by natural killer. *Eur J Immunol* 27:2787–2792.
25. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A (1993) Interleukin 12 is required for the T-lymphocyte–independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell–deficient hosts. *Proc Natl Acad Sci USA* 90: 6115–6119.
26. Lieberman LA, et al. (2004) IL-23 provides a limited mechanism of resistance to acute toxoplasmosis in the absence of IL-12. *J Immunol* 173:1887–1893.
27. Yap G, Pesin M, Sher A (2000) Cutting edge: IL-12 is required for the maintenance of IFN-gamma production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J Immunol* 165:628–631.
28. Kang SJ, Liang HE, Reizis B, Locksley RM (2008) Regulation of hierarchical clustering and activation of innate immune cells by dendritic cells. *Immunity* 29:819–833.
29. Yarovinsky F, Kanzler H, Hieny S, Coffman RL, Sher A (2006) Toll-like receptor recognition regulates immunodominance in an antimicrobial CD4+ T cell response. *Immunity* 25:655–664.
30. Kim SK, Karasov A, Boothroyd JC (2007) Bradyzoite-specific surface antigen SRS9 plays a role in maintaining *Toxoplasma gondii* persistence in the brain and in host control of parasite replication in the intestine. *Infect Immun* 75:1626–1634.