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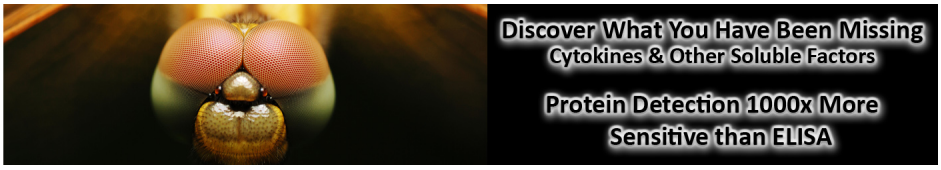


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# IL-23 Provides a Limited Mechanism of Resistance to Acute Toxoplasmosis in the Absence of IL-12[1]

**Linda A. Lieberman,* Fabiola Cardillo,† Alexander M. Owyang,‡ Donna M. Rennick,‡ Daniel J. Cua,‡ Robert A. Kastelein,‡ and Christopher A. Hunter[2]***

**IL-23 and IL-12 are heterodimeric cytokines which share the p40 subunit, but which have unique second subunits, IL-23p19 and IL-12p35. Since p40 is required for the development of the Th1 type response necessary for resistance to *Toxoplasma gondii*, studies were performed to assess the role of IL-23 in resistance to this pathogen. Increased levels of IL-23 were detected in mice infected with *T. gondii* and in vitro stimulation of dendritic cells with this pathogen resulted in increased levels of mRNA for this cytokine. To address the role of IL-23 in resistance to *T. gondii*, mice lacking the p40 subunit (common to IL-12 and IL-23) and mice that lack IL-12 p35 (specific for IL-12) were infected and their responses were compared. These studies revealed that p40$^{-/-}$ mice rapidly succumbed to toxoplasmosis, while p35$^{-/-}$ mice displayed enhanced resistance though they eventually succumbed to this infection. In addition, the administration of IL-23 to p40$^{-/-}$ mice infected with *T. gondii* resulted in a decreased parasite burden and enhanced resistance. However, the enhanced resistance of p35$^{-/-}$ mice or p40$^{-/-}$ mice treated with IL-23 was not associated with increased production of IFN-$\gamma$. When IL-23p19$^{-/-}$ mice were infected with *T. gondii* these mice developed normal T cell responses and controlled parasite replication to the same extent as wild-type mice. Together, these studies indicate that IL-12, not IL-23, plays a dominant role in resistance to toxoplasmosis but, in the absence of IL-12, IL-23 can provide a limited mechanism of resistance to this infection. *The Journal of Immunology*, 2004, 173: 1887–1893.**

Interleukin-23 is a member of the IL-6 family of cytokines and is closely related in structure to IL-12. IL-23 and IL-12 are heterodimeric cytokines that are both composed of an identical p40 subunit and a second smaller subunit, IL-23p19 and IL-12p35, respectively. In addition to the close structural relationship between IL-23 and IL-12, their heterodimeric receptors share the IL-12R$\beta$1 chain and these cytokines have been shown to have similar properties (1, 2). For example, both cytokines are produced by macrophages and dendritic cells in response to LPS, and both can enhance T cell proliferation and the production of IFN-$\gamma$ (1). One of the main physiological functions of IL-12 is to stimulate NK and T cell production of IFN-$\gamma$, which is required for resistance to intracellular infections (3–6) and which is involved in many inflammatory processes (7). Given the structural and functional similarities between IL-12 and IL-23, it seems likely that IL-23 would also play a role in resistance to infection and the development of inflammation. This hypothesis is supported by recent studies that have established that IL-23 plays a role in the development of experimental autoimmune encephalomyelitis (8) and collagen-induced arthritis (9). However, much less is known about the role of IL-23 in resistance to infection although several studies have identified a p40-dependent, IL-12-independent mechanism of resistance to *Cryptococcus neoformans*, *Salmonella enteridis*, *Mycobacterium spp.*, and *Francisella tularensis* (10–13). While these studies have implicated a role for IL-23 in immunity to these pathogens, definitive studies using IL-23-deficient mice are lacking and many questions remain about the relationship between IL-12 and IL-23 in the regulation of innate and adaptive immune responses during infection.

*Toxoplasma gondii* is an important opportunistic pathogen in patients with primary or acquired T cell deficiencies (14–18) and studies with murine models have demonstrated that p40 has a critical role in the development of Th1 type responses and in the production of IFN-$\gamma$ required for resistance to this parasite (4, 17–20). Because p40 is a shared component of IL-12 and IL-23, those primary in vivo studies did not distinguish between the effects of these cytokines during toxoplamsosis. To address the role of IL-23 in resistance to *T. gondii*, a series of in vitro and in vivo studies were performed that revealed that this infection was accompanied by increased expression of IL-23 and IL-23R mRNA transcripts. Initial studies that compared mice that lack IL-12 alone (p35$^{-/-}$) or both IL-12 and IL-23 (p40$^{-/-}$) revealed that p35$^{-/-}$ mice were better able to control parasite replication and survived longer than the p40$^{-/-}$ mice. Moreover, administration of IL-23 to p40$^{-/-}$ mice resulted in enhanced resistance to *T. gondii*, which was associated with decreased parasite burden. However, infection of IL-23p19$^{-/-}$ mice with *T. gondii* demonstrated that IL-23 is not essential for the development of resistance during the acute stage of infection. Together, these studies provide the first evidence that IL-12, not IL-23, plays a dominant role in protective immunity to *T. gondii*; however, in the absence of IL-12, IL-23 can enhance resistance to toxoplasmosis.

## Materials and Methods

### *Mice, infection*

Male BALB/c, IFN-$\gamma^{-/-}$ (GKO), p35$^{-/-}$, and p40$^{-/-}$ mice on a BALB/c background (The Jackson Laboratory, Bar Harbor, ME) were used at 4–8

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wk of age. Mice were maintained in Thoren caging units (Thoren Caging System, Hazelton, PA) within the University Laboratory Animal Research facilities at the University of Pennsylvania (Philadelphia, PA). Female $p19^{-/-}$ mice on a C57BL/6 background or a B6/129 background were developed and maintained at DNAX Research Institute (Palo Alto, CA) (8). Female C57BL/6 mice, $p35^{-/-}$, and $p40^{-/-}$ (on C57BL/6 mice background; The Jackson Laboratory) were maintained at DNAX Research Institute. Age/sex/strain-matched mice were infected i.p. with 1000 tachyzoites of the ts4 strain of *T. gondii* and sacrificed 7 days following infection to assess parasite burden, recall response, cytokine production, and activation state of splenocytes. Tachyzoites of the RH strain of *T. gondii* were used for in vitro infection (6:1). Ts4 and RH parasites were maintained in HS27 cells (American Type Culture Collection, Manassas, VA) in vitro as previously described (21). Me49 tissue cysts used in vivo were prepared from brain homogenates of chronically infected CBA mice (The Jackson Laboratory) and injected i.p. at a dose of 20 cysts per mouse into age/sex/strain-matched mice.

### *Dendritic cells, RT-PCR*

Bone marrow-derived dendritic cells were prepared as previously described (22). A total of $5 \times 10^6$ dendritic cells were stimulated for 4 h at 37°C in 5% $CO_2$ with 20 $\mu$g/ml LPS (Sigma-Aldrich, St. Louis, MO), 20 $\mu$g/ml soluble *Toxoplasma* Ag (STAg[3]; prepared as described (23)), and the RH strain of *T. gondii* (6:1), in the presence or absence of 200 U of IFN-$\gamma$ (BD Pharmingen, San Diego, CA). mRNA was extracted from tissues/cells using Tri Reagent (Sigma-Aldrich). cDNA was prepared using SuperscriptII reverse transcriptase (Invitrogen Life Technology, Auckland, New Zealand) as described by the manufacturer. RT-PCR was used to amplify p19, $\beta$-actin (24), p35, p40 (4), IL-23R (2), and IL-12R$\beta$1. Oligonucleotides were synthesized in the University of Pennsylvania School of Veterinary Medicine DNA facility. PCR products were separated on 2% agarose gels and photographed on a Bio-Rad GelDoc system (Bio-Rad, Hercules, CA).

### *ELISA, cytokines, in vivo treatment with IL-23*

Splenocytes from infected animals were stimulated in vitro with 2 ng/ml IL-12 (Genetics Institute, Cambridge, MA), 20 $\mu$g/ml STAg, 5 ng/ml IL-23 (hyperlink construct provided by DNAX Research Institute (1)) for 48 h and supernatants were assayed for IFN-$\gamma$ by ELISA. IFN-$\gamma$ and IL-12p40 were measured from serum 7 days postinfection (dpi) by ELISA. IL-23 was given i.p. at 500 ng daily starting at day $-1$ before infection (with 20 cysts of Me49 i.p.) and every day thereafter.

### *Flow cytometric analysis*

To assess T cell activation, splenocytes were resuspended in FACS buffer ($1\times$ PBS, 0.2% BSA, 4 mM $NaN_3$) and incubated with Fc block for 30 min on ice. Cells were then stained for various surface markers (CD44-PE, CD62L-allophycocyanin, CD4-PerCP, CD8-FITC; BD Pharmingen) for 20 min on ice. Cells were then washed twice with FACS buffer and resuspended in 2% paraformaldehyde. Stained cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed with FloJo software (Treestar, San Carlos, CA).

### *Cytological analysis for parasite burden*

Cells were collected at the site of infection by peritoneal lavage with 5 ml cold PBS and cytospins were prepared with $5 \times 10^4$ cells/100 $\mu$l. Slides were stained with Protocol Hema3 stain (Biochemical Sciences, Swedesboro, NJ) as recommended by the manufacturer, and mounted and sealed with Cytoseal (Stephens Scientific, Kalamazoo, MI).

### *Statistical analysis*

Unpaired two-tailed Student *t* tests were calculated using INSTAT software (GraphPad, San Diego, CA). Survival curves were plotted using the Kaplan-Meier survival scale and analyzed by the logrank test using PRISM software (GraphPad). A $p$ value of $<0.05$ was considered significant.

## Results

### T. gondii *stimulates expression of IL-23*

IL-23 consists of two subunits, p19 and p40, and its heterodimeric receptor is formed by IL-12R$\beta$1 and IL-23R (2). To assess whether infection with *T. gondii* led to expression of IL-23 and its receptor, mice were infected with *T. gondii* and peritoneal exudate cells were collected 7 dpi, and RNA was isolated for RT-PCR. These studies revealed that infection led to increased levels of both IL-23 and IL-23R mRNA (Fig. 1*A*). However, when an ELISA was used to assay IL-23 in serum or peritoneal lavage, the protein was below the level of detection. Interestingly, similar samples from infected IFN-$\gamma^{-/-}$ (GKO) or Stat1$^{-/-}$ mice (both of which fail to control parasite replication) contained low levels of IL-23 protein (80–600 pg/ml).

Since *T. gondii* is a potent activator of dendritic cell (DC) IL-12 production and these cells have been reported to be a major source of IL-23 (1), in vitro studies were performed to determine whether infection would also stimulate the production of IL-23 by these cells. Bone marrow-derived DCs were stimulated with LPS (as a positive control), live tachyzoites of the RH strain of *T. gondii*, or STAg, and the levels of mRNA for p40 and p19 were measured by RT-PCR (Fig. 1*B*). As has been previously reported, stimulation of DCs with LPS resulted in increased levels of IL-23 mRNA (1). Moreover, whereas STAg was unable to induce a significant increase in IL-23 mRNA expression by DCs, infection with live parasites resulted in an up-regulation of p19 mRNA that was comparable to that produced following LPS stimulation. In addition to producing IL-23, it has been reported that DCs express the IL-23R in response to LPS (2). Therefore the expression of IL-23R mRNA by DCs was assessed following in vitro infection. Analysis of unstimulated DCs revealed constitutive expression of IL-23R mRNA by these cells, while mRNA for the IL-12R$\beta$1 chain was not detected. Stimulation with STAg did not up-regulate IL-12R$\beta$1 expression, but LPS or in vitro infection with *T. gondii* induced expression of both receptor chains. These studies demonstrate that whereas STAg alone failed to up-regulate p19 or the IL-12R$\beta$1, in vivo or in vitro infection with *T. gondii* leads to up-regulation of IL-23 mRNA as well as enhanced expression of IL-23R transcripts, suggesting a possible role for this cytokine in resistance to toxoplasmosis. The detection of IL-23 protein in mice with high parasite burden suggests that infection itself leads to the production of IL-23, which is consistent with the effect of in vitro infection of DCs with live parasite.

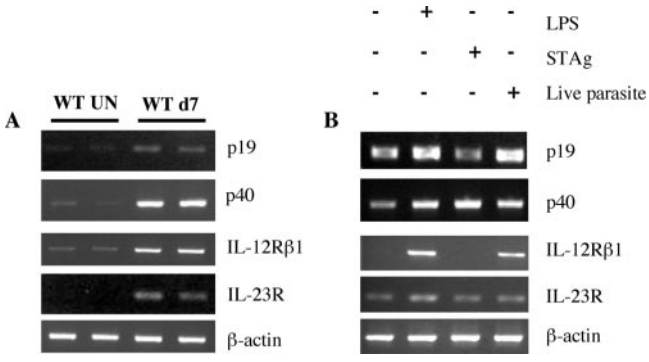


**FIGURE 1.** IL-23/IL-23R expression is up-regulated following infection with *T. gondii*. *A,* Mice were infected i.p. with 20 cysts of the Me49 strain of *T. gondii* and mRNA was extracted from peritoneal cells of naive or *T. gondii*-infected mice 7 dpi. IL-23 and IL-23R transcripts were amplified by RT-PCR and visualized on a 2% agarose gel. $\beta$-Actin was used as a loading control. *B,* Bone marrow-derived dendritic cells were either treated with LPS (20 $\mu$g/ml), STAg (20 $\mu$g/ml) or infected in vitro at a ratio of six parasites per cell for 4 h, then RNA was extracted and transcripts for IL-23, IL-23R and $\beta$-actin were amplified by RT-PCR. Data shown is representative of at least two independent experiments.

[3] Abbreviations used in this paper: STAg, soluble *Toxoplasma* Ag; dpi, days postinfection.

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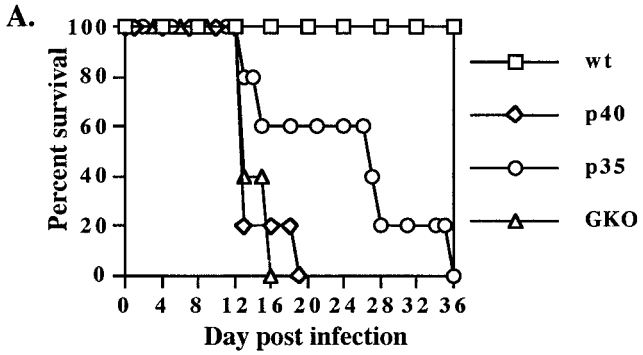


B.

| Strain | Percent infected PECs | |
|---|---|---|
| | Ts4 | Me49 |
| p35-/- | 7.1 ±1.92 | 9 ± 2 |
| p40-/- | 19.78 ± 6.36 | 16.72 ± 4.92 |

**FIGURE 2.** A p40-dependent, p35-independent mechanism of resistance to *T. gondii*. *A*, WT BALB/c (□), p35 (○), p40 (◇), and GKO (△) mice were infected with 1000 tachyzoites of ts4 i.p. and survival was monitored ($n = 5$). Data shown is representative of four independent experiments. *B*, Parasite burden is reduced in p35$^{-/-}$ mice. Peritoneal exudates cells were collected 5 dpi (with either 1000 tachyzoites of ts4 or 20 cysts of Me49) by lavage, and cytospins were prepared. At least 100 cells were counted per slide to determine parasite burden. Data shown is representative of at least two independent experiments.

### *Identification of an IL-12-independent, p40-dependent mechanism of resistance to* T. gondii

The experiments described above indicate that IL-23 is produced in response to infection with *T. gondii*; hence, studies were performed to determine the functional significance of IL-23 production during this infection. Therefore, mice that lack IFN-γ, p35, or p40 were infected with *T. gondii*, and their ability to control infection was assessed. While neither the p35$^{-/-}$ or p40$^{-/-}$ mice produce functional IL-12, the p40$^{-/-}$ mice also lack IL-23. For these experiments two strains of *T. gondii* were used, the temperature-sensitive mutant ts4 strain of *T. gondii*, as well as the more virulent Me49 strain. Since ts4 fails to form cysts and cannot be given orally, all infections were administered i.p. to allow direct comparison between parasite strains as well as to allow quantification of the numbers of cells infected at a local site. It is important to note that similar results were observed with both strains and any instances where differences were found are highlighted. Infection of IFN-γ$^{-/-}$ or p40$^{-/-}$ mice with the ts4 strain of *T. gondii* resulted in 100% mortality by day 18 postinfection whereas p35$^{-/-}$ mice survived for as long as 36 dpi (Fig. 2*A*), which represents a significant difference in survival ($p = 0.0229$). When mice were infected with the more virulent Me49 strain, the differences in survival were not statistically significant. However, analysis of parasite burden following infection with either of these strains revealed that the p35$^{-/-}$ mice have significantly lower burden at the site of infection as compared with p40$^{-/-}$ mice 5 days following infection (Fig. 2*B*). At later time points this difference in parasite burden in the peritoneum was not apparent, consistent with the failure to generate protective T cell responses required for long term resistance to *T. gondii* (data not shown). Nevertheless, these data establish a critical role for p35 and p40 in protective immunity to *T. gondii*, but also identify a p40-dependent, IL-12-independent mechanism of resistance to this pathogen.

### *T cell responses are not enhanced by IL-23*

To understand the basis for the enhanced resistance of p35$^{-/-}$ mice as compared with p40$^{-/-}$ mice, studies were performed to determine whether there were differences in the T cell responses of these mice. Wild-type, p40$^{-/-}$, and p35$^{-/-}$ mice were infected with the ts4 strain of *T. gondii*, and IFN-γ production and T cell activation were assessed. The survival of wild-type mice was associated with elevated serum levels of IFN-γ, whereas p35$^{-/-}$ and p40$^{-/-}$ mice had low levels of serum IFN-γ at 7 dpi that were not significantly different (Fig. 3*A*). Serum levels of IFN-γ were also assessed 5 dpi, but they were below the level of detection. To further assess the difference between p35$^{-/-}$ and p40$^{-/-}$ mice, the ability of splenocytes from infected animals to produce Ag specific IFN-γ (in response to STAg) was measured. Whereas splenocytes from wild-type mice produced low levels of IFN-γ in response to STAg, there was a marked defect in the ability of splenocytes from p40$^{-/-}$ or p35$^{-/-}$ mice to produce IFN-γ (Fig. 3*B*). Since splenocytes from p40$^{-/-}$ or p35$^{-/-}$ mice lack IL-12 and/or IL-23, studies were performed to determine how the addition of these cytokines would affect the production of parasite-specific IFN-γ. The addition of IL-12 to these cultures resulted in enhanced production of IFN-γ by cells from all experimental groups although this effect was most marked for cells from the p35$^{-/-}$ mice (Fig. 3*B*). When IL-23 was added to these cultures, it had no effect on the production of IFN-γ suggesting that the primary role of IL-23 during toxoplasmosis is not to enhance IFN-γ production.

The observation that exogenous IL-12 (but not IL-23) enhanced the production of IFN-γ by splenocytes from infected p35$^{-/-}$ but not p40$^{-/-}$ mice suggested that IL-23 may play a role in the expansion of parasite-specific T cells. This finding is consistent with studies that demonstrated that IL-23 enhances proliferation of activated T cells (1) and may indicate that IL-23 can enhance the

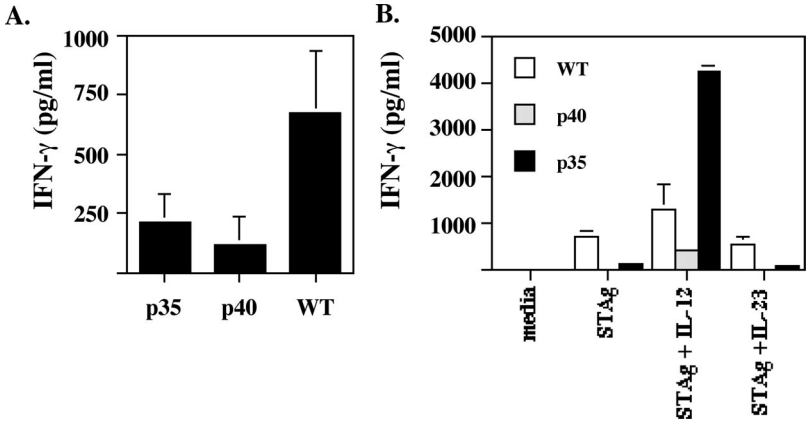


**FIGURE 3.** *A*, Circulating IFN-γ levels are comparable in the KO mice. Serum was taken from mice (BALB/c background) 7 dpi and assayed for IFN-γ by ELISA. No significant difference was found in circulating IFN-γ levels ($n = 8$). *B*, Splenocytes from mice infected 7 days were plated at $4 \times 10^5$ cells/well and stimulated 2 days with STAg (20 μg/ml), STAg + IL-12 (2 ng/ml), or STAg + IL-23 (5 ng/ml). IFN-γ production was assessed by ELISA. IL-23 does not enhance IFN-γ production in response to Ag. Data shown is representative of four independent experiments.

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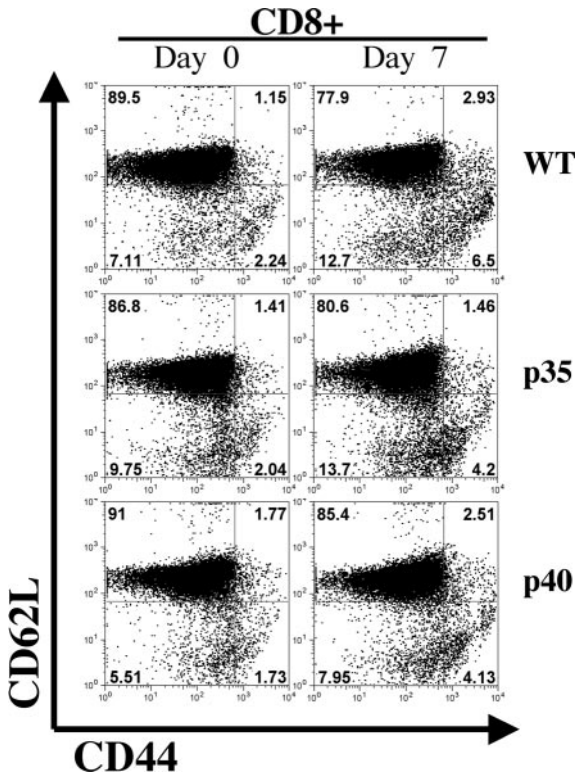


**FIGURE 4.** T cell activation is comparable between $p35^{-/-}$ and $p40^{-/-}$ mice. Splenocytes of WT (BALB/c), $p35^{-/-}$, and $p40^{-/-}$ mice were surface stained for CD4, CD8, CD44, and CD62L. Cells gated on the $CD8^+$ cell surface marker cells displayed an increase in activation following infection. Data shown is representative of four independent experiments.

ability of T cells to respond to IL-12. Therefore, FACS analysis was performed to compare levels of T cell activation in $p35^{-/-}$ and $p40^{-/-}$ mice following infection with the ts4 strain. At 7 dpi neither wild-type, $p35^{-/-}$, or $p40^{-/-}$ mice showed increased activation of $CD4^+$ T cells, where $CD44^{high}CD62L^{low}$ cells were considered activated (data not shown). In contrast, all three strains of mice up-regulated expression of activation markers on $CD8^+$ T cells (Fig. 4), indicating the importance of $CD8^+$ T cells in the immune response to this strain of *T. gondii*. Nevertheless, there was no significant difference in activation between the $p35^{-/-}$ and $p40^{-/-}$ mice suggesting that T cell activation does not explain the differences observed in survival. Infection with Me49 resulted in comparable activation of WT, $p35^{-/-}$, and $p40^{-/-}$ $CD4^+$ T cells, but revealed a defect in CD8 activation in $p35^{-/-}$ and $p40^{-/-}$ mice compared with wild-type mice, though there was no significant difference in the activation levels between the $p40^{-/-}$ and $p35^{-/-}$ mice (data not shown). These data suggest that differences in the levels of T cell activation in $p40^{-/-}$ and $p35^{-/-}$ mice do not provide a basis for the enhanced resistance of $p35^{-/-}$ mice.

### *Administration of IL-23 has a protective effect in vivo*

The studies described above suggest a role for endogenous IL-23 in resistance to *T. gondii*. To directly assess the protective effect of IL-23 in vivo, $p40^{-/-}$ mice were treated with rIL-23 beginning 24 h before i.p. infection and every day thereafter. This treatment regime resulted in a striking reduction in parasite burden at 7 dpi as compared with untreated mice ($p = 0.0018$; Fig. 5, *A* and *B*) and led to a significant increase in the survival of $p40^{-/-}$ mice ($p = 0.0010$) though they eventually succumbed to infection (Fig. 5*C*). In these studies, treatment of $p40^{-/-}$ mice with IL-23 did not enhance serum levels of IFN-$\gamma$ nor the levels of IFN-$\gamma$ produced during recall responses from these mice (data not shown). Nevertheless, these findings demonstrate that exogenous IL-23 can enhance resistance to *T. gondii* independently of IL-12, but this protective effect is not sufficient for long-term resistance to infection.

### *$p19^{-/-}$ mice are resistant to* T. gondii

Thus far, the data presented above indicates that in the absence of IL-12, IL-23 provides a limited mechanism of resistance to *T. gondii*. However these studies do not clarify whether IL-23 contributes to the development of protective immunity in the presence of IL-12. The availability of IL-23$p19^{-/-}$ mice allowed experiments to assess whether the protective effects of IL-12 are dependent on IL-23. Therefore, $p19^{-/-}$ mice were infected with *T. gondii* and their ability to mount a protective immune response was assessed.

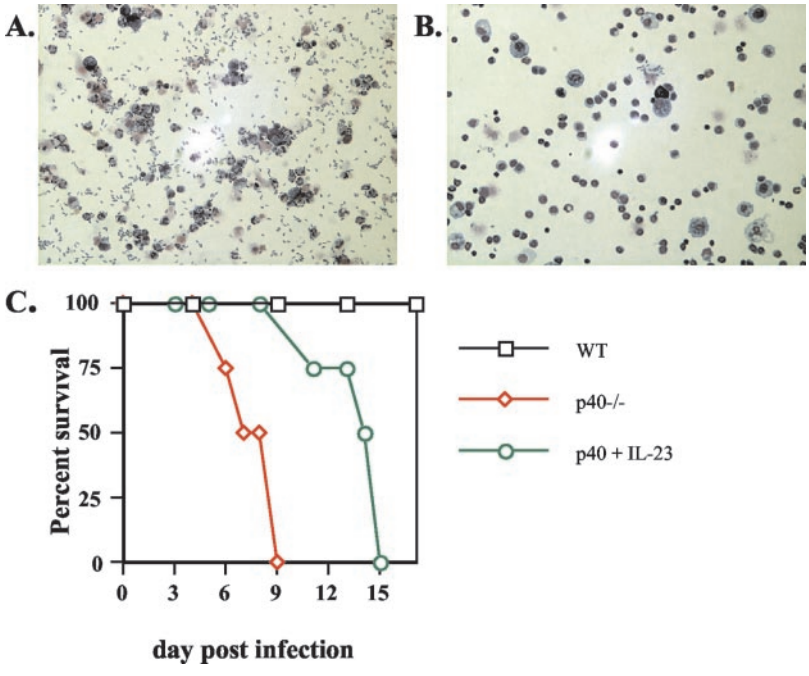


**FIGURE 5.** IL-23 has a protective effect in vivo. $p40^{-/-}$ mice were treated daily with 500 ng IL-23 ip from day −1. Mice were infected with 20 cysts of Me49 i.p. and some mice were sacrificed at 7 dpi. *A*, $p40^{-/-}$ mice have a high parasite burden following infection. *B*, $p40^{-/-}$ mice treated daily with IL-23 have a very low parasite burden. *C*, IL-23 treatment enhances survival of $p40^{-/-}$ mice due to decreased parasite burden ($n = 4$). Data shown is representative of at least two independent experiments.

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Infection with either ts4 (data not shown) or Me49 strains of *T. gondii* resulted in the acute susceptibility of p40$^{-/-}$ and p35$^{-/-}$ mice, whereas wild-type and p19$^{-/-}$ mice survived beyond the acute stage of infection (Fig. 6*A*). Furthermore, analysis of cells from the site of infection at 7 dpi revealed the presence of large numbers of parasites in the p35$^{-/-}$ and p40$^{-/-}$ mice, while wild-type and p19$^{-/-}$ mice had a low parasite burden (<1%). The ability of wild-type and p19$^{-/-}$ mice to control parasite replication was associated with similar levels of infection-induced IL-12 and IFN-$\gamma$ in the serum of these mice (Fig. 6*B*). Similarly, IFN-$\gamma$ production by splenocytes from infected wild-type and p19$^{-/-}$ mice in response to STAg was not significantly different (Fig. 6*C*). These data reveal that the production of IFN-$\gamma$ during toxoplasmosis is independent of IL-23. In addition, FACS analysis revealed that there was no defect in the infection-induced activation of CD4$^+$ (Fig. 7*A*) and CD8$^+$ (Fig. 7*B*) T cells in the p19$^{-/-}$ mice. As shown in Fig. 1, wild-type mice have increased expression of IL-23 at 7 dpi, though the p19$^{-/-}$ mice reveal that IL-23 is not necessary for the control of parasite burden. Together, these studies provide the first evidence that IL-12, not IL-23, plays a dominant role in protective immunity to *T. gondii*.

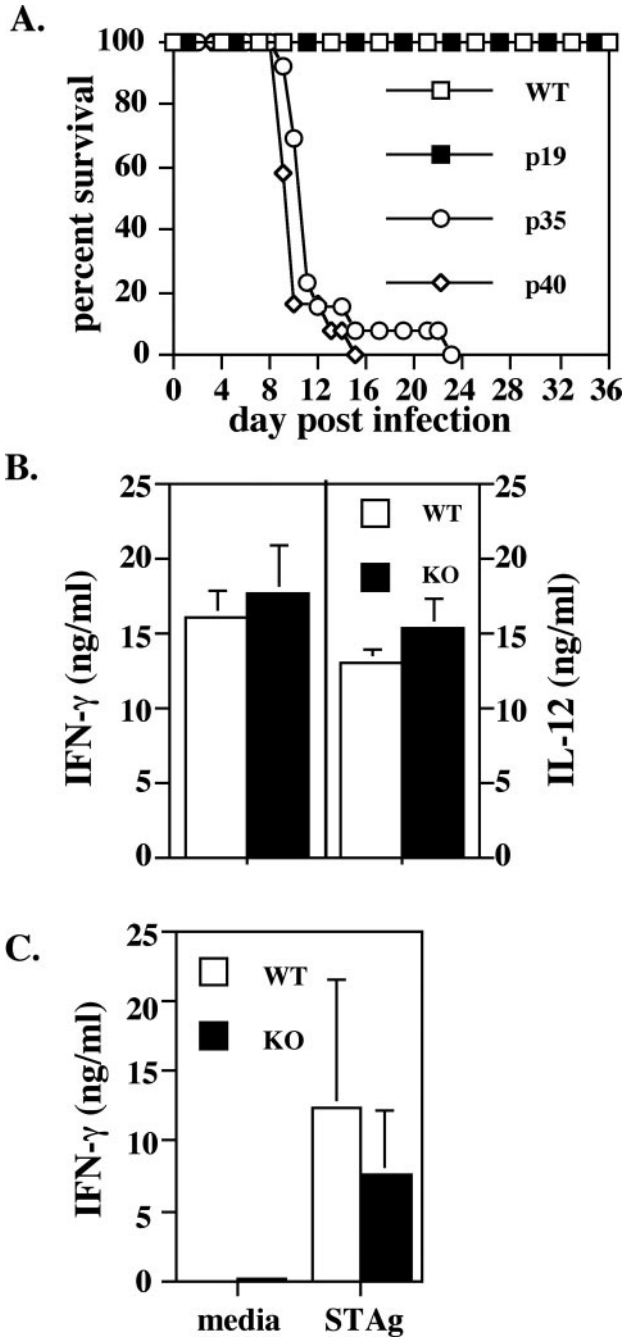


**FIGURE 6.** IL-23 protection is not necessary in the presence of IL-12. *A*, WT C57BL/6, p19$^{-/-}$, p35$^{-/-}$, and p40$^{-/-}$ mice were infected with 20 Me49 ip and survival was assessed. p35$^{-/-}$ and p40$^{-/-}$ mice succumb to infection acutely while p19$^{-/-}$ mice are resistant to acute toxoplasmosis ($n$ = 12). *B*, Serum levels of IFN-$\gamma$ and IL-12 were measured from WT (BL/6) and p19$^{-/-}$ mice by ELISA. Data shown is representative of three individual experiments. *C*, Splenocytes from WT (129/B6) and p19$^{-/-}$ mice infected 7 days were plated at 4 $\times$ 10$^5$ cells/well and stimulated 2 days with STAg (20 $\mu$g/ml). IFN-$\gamma$ production was assessed by ELISA and no significant difference was found in IFN-$\gamma$ production. Data shown is representative of three individual experiments.

## Discussion

While previous studies have demonstrated that p40 is essential for the development of protective immunity to *T. gondii* (4, 17–20), they have not distinguished between the role of IL-12 and IL-23 during toxoplasmosis. The data presented in this manuscript defines a need for IL-12 in the development of protective immunity to *T. gondii*, and has also identified an IL-12-independent, p40-dependent mechanism of resistance to acute toxoplasmosis in p35$^{-/-}$ mice. Given what is currently known about the biology of IL-12 and IL-23 it seems likely that in the absence of IL-12, this mechanism of resistance is mediated by IL-23. This conclusion is supported by the data presented here, which reveal for the first time that administration of IL-23 to p40$^{-/-}$ mice can enhance resistance against an intracellular pathogen. However, these results do not exclude the possibility that molecules other than IL-23 play a role in resistance in the p35$^{-/-}$ mice. For instance, p40 homodimers are produced at higher levels than IL-12 p70, but the role of these homodimers is unclear. The majority of studies indicate that these homodimers are antagonists of IL-12 mediated signaling (25, 26) although there are data that support the idea that they can act as agonists (24, 27). Alternatively, p40 may have other undiscovered binding partners besides p19, which may contribute to some of the differences observed between p40$^{-/-}$ and p35$^{-/-}$ mice. Evidence for this hypothesis is supported by the phenotype of patients with defects in their IL-12R$\beta$1 chain or in p40. Although IL-12R$\beta$1 is a shared receptor component for IL-12 and IL-23, it has been reported that patients with p40 deficiency have an increased mortality rate compared with patients with IL-12R$\beta$1 deficiency (28). These observations suggest that p40 may bind other subunits forming novel cytokine receptor interactions that are important in resistance to infection.

Based on previous studies that demonstrated that IL-23 could enhance the production of IFN-$\gamma$ by human NK (2) and T cells (1), the obvious explanation for the increased resistance of p35$^{-/-}$ mice, or p40$^{-/-}$ mice treated with IL-23, would be that they produced more IFN-$\gamma$ than p40$^{-/-}$ mice. The only data presented here that support this hypothesis is that splenocytes from infected p35$^{-/-}$ mice produced higher levels of IFN-$\gamma$ in response to IL-12 plus STAg than did splenocytes from infected p40$^{-/-}$ mice. This may indicate a role for IL-23 in the expansion or priming of Th1 cells, or the regulation of IL-12 responsiveness. However, the majority of data from these studies found no evidence that p35$^{-/-}$ mice produced more IFN-$\gamma$ than p40$^{-/-}$ mice, and p19$^{-/-}$ mice did not exhibit a defect in IFN-$\gamma$ production. Additionally, exogenous IL-23 did not enhance the production of IFN-$\gamma$ in STAg-specific recall responses from WT, p40$^{-/-}$, or p35$^{-/-}$ mice. These studies raise a fundamental question of how IL-23 can mediate an IL-12-independent mechanism of resistance to *T. gondii*. Reports

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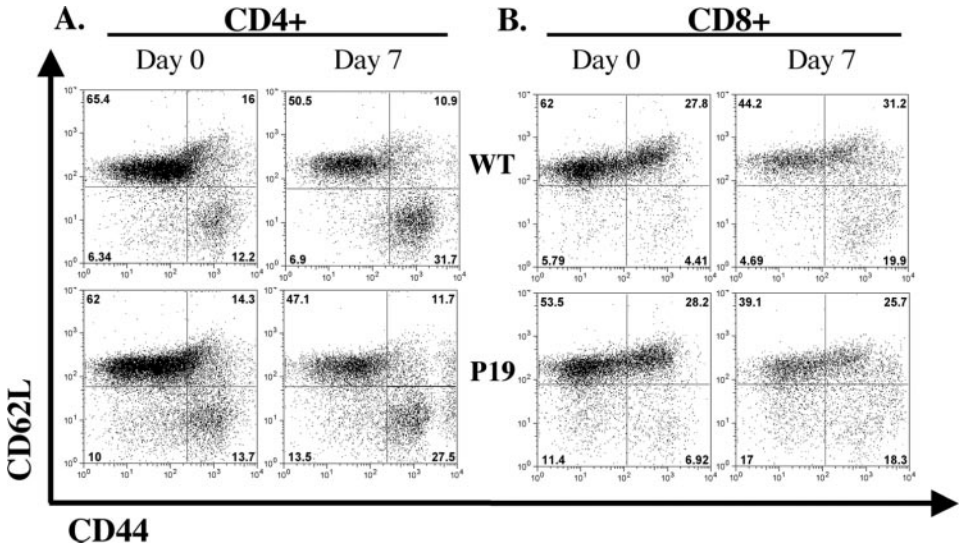


**FIGURE 7.** T cell activation is not deficient in p19$^{-/-}$ mice. Splenocytes of WT (129/B6) and p19 $^{-/-}$ mice were surface stained for CD4, CD8, CD44, and CD62L. Splenocytes gated on CD4$^{+}$ (*A*) T cells or CD8$^{+}$ (*B*) T cells display comparable activation between WT and p19$^{-/-}$ cells. Data shown is representative of three individual experiments.

that dendritic cells and macrophages express a functional IL-23R and that IL-23 can activate STAT1 (2, 29), a transcription factor that regulates the expression of proteins involved in the control of *T. gondii* (inducible NO synthase, inducibly expressed GTPase, T cell-specific GTP) (30–32), led to the hypothesis that IL-23 could directly activate the anti-microbial activity of macrophages. However, initial studies in which different populations of resting and inflammatory macrophages were stimulated with IL-23 (alone or in combination with other cytokines) did not result in increased expression of inducibly expressed GTPase, T cell-specific GTP, or increased production of NO, nor an inhibition of parasite replication (data not shown). While these studies do not rule out a role for IL-23 in the direct regulation of anti-microbial activity in vivo, it seems likely that the anti-toxoplasma effects of IL-23 in vivo may be mediated through other molecules. For example, IL-23 has been shown to induce the production of IL-17 by CD4$^{+}$ T cells (33), a cytokine associated with chronic inflammatory disease (34, 35) and which is required for resistance to *Klebsiella pneumoniae* (36, 37). In addition, IL-23 has been implicated in the induction of TNF-$\alpha$ and IL-1 (8, 38), two cytokines involved in resistance to *T. gondii* (39–43). Whether the IL-23 mediated protective effects during toxoplasmosis are dependent on these cytokines is under investigation.

It is important to note that despite the likely role for IL-23 in resistance to *T. gondii* in p35$^{-/-}$ mice, they still succumbed to acute toxoplasmosis and while administration of IL-23 to p40$^{-/-}$ mice enhanced resistance to *T. gondii*, it was not sufficient for long term survival. Thus, in the absence of IL-12, IL-23 was not sufficient for the development of T cell responses required for complete protection. Though the cause of death of the IL-23 treated p40$^{-/-}$ mice has not been examined, the treated mice display higher parasite burden (Fig. 5*B*) than that expected in similarly infected wild-type mice, which suggests that ultimately these mice cannot control parasite burden associated with a defect in the development of protective T cell responses. The experiments using the p35$^{-/-}$ and p40$^{-/-}$ mice allowed for the study of IL-23 in the absence of IL-12, but the recent availability of IL-23p19$^{-/-}$ mice allowed us to assess the relative contribution of IL-12 and IL-23 in resistance to toxoplasmosis. These studies revealed that IL-23 is not required for the development of a protective immune response to *T. gondii* and indicates that any protective effects of IL-23 observed in the p35$^{-/-}$ mice are secondary to the dominant effects of IL-12. This conclusion is in marked contrast to recent reports that used models of experimental autoimmune encephalomyelitis and collagen-induced arthritis to demonstrate that IL-23, not IL-12, plays the dominant role in the induction of autoimmunity (8, 9).

At present it is unclear what determines the dominance of IL-12 vs IL-23 in different experimental models of infection or autoimmunity, but it is unlikely to be simply a consequence of the preferential induction of one cytokine. Rather, the context and type of stimulus is likely to determine the relative role of these cytokines. It is possible that the observation that the protective effects of IL-23 are secondary to those of IL-12 during toxoplasmosis will also be the case with other intracellular infections. However, close analysis of the responses of p35$^{-/-}$ and p40$^{-/-}$ mice infected with different intracellular pathogens reveals differences that indicate IL-23 may be more important in some infections than others. For example, p35$^{-/-}$ mice infected with *S. enteritidis* (11) or *Mycobacterium tuberculosis* (12) produce higher levels of IFN-$\gamma$ than p40$^{-/-}$ mice, whereas this is not seen following infection with *T. gondii*, *C. neoformans* (10) or *F. tularensis* (13). Similarly, p35$^{-/-}$ mice can clear infection with *F. tularensis* (13) or *Mycobacterium bovis* (24), whereas these same mice cannot maintain long-term resistance to *T. gondii*. Given that the differences between p35$^{-/-}$ and p40$^{-/-}$ mice are dependent on the virulence and dose of the pathogens being used, it seems likely that these subtleties may determine the relative contribution of IL-23 to the overall immune response. At present, it seems unlikely that the primary role of IL-23 is to promote autoimmunity, but its natural function in anti-microbial immunity remains unknown. The comparison of the responses of p35$^{-/-}$ and p40$^{-/-}$ mice did not allow us to study the role of IL-23 during chronic toxoplasmosis, and additional studies with the p19$^{-/-}$ mice will be required to evaluate the role of IL-23 during chronic toxoplasmosis.

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