Interferon (IFN)-γ is an absolute requirement for resistance against acute acquired infection with *Toxoplasma gondii* and development of toxoplasmic encephalitis (TE) during the late stage of infection. Multiple populations of both T and non–T cells are important sources of IFN-γ in resistance. In the absence of IFN-γ–producing non–T cells, T cells cannot prevent TE. Interleukin-12, Bcl-3, NF-κB(2), and CD40-CD40L ligand interaction are important for up-regulation of IFN-γ production. *T. gondii* infects a variety of host cells, and IFN-γ–mediated immune responses control the parasite in both phagocytic and nonphagocytic cells through at least five different mechanisms, most likely depending on the types of cells responding to IFN-γ. Such effector functions involve production of NO by iNOS, tryptophan degradation by the enzyme IDO (indolamine 2,3-dioxygenase), unidentified mechanism(s) mediated by 47- to 48-kDa proteins encoded by an IFN-γ responsive gene family, limiting the availability of intracellular iron to the parasite, and production of reactive oxygen intermediates.

*Toxoplasma gondii* is a ubiquitous obligate intracellular protozoan parasite in humans and animals. Chronic (latent) infection with this parasite is likely one of the most common human infections. During the acute stage of the infection, tachyzoites quickly proliferate within a variety of nucleated cells and spread throughout host tissues. The acute infection/disease is caused by infection with this form of the parasite. Following the acute stage, the parasite forms cysts (latent stage) in various organs, especially the brain, heart, and skeletal muscle, establishing chronic infection. Infection in immunocompetent persons usually is unnoticed or is a benign self-limiting illness and results in chronic infection. Immunosuppression in the chronically infected person may result in reactivation of a latent infection that is initiated by disruption of cysts and followed by proliferation of tachyzoites. Such reactivation of *T. gondii* infection usually presents as toxoplasmic encephalitis (TE). TE has emerged as a major opportunistic infectious disease in the central nervous system in AIDS patients. Since immunocompetent persons usually have no apparent untoward effects, including development of encephalitis, it is clear that the immune response is critical for prevention of TE.

Murine models are the primary means used to analyze the mechanisms of host resistance to acute infection and development of TE during the late stage of infection. Resistance of mice to chronic infection is under genetic control. Resistant mouse strains (e.g., BALB/c) establish a latent chronic infection, as do immunocompetent humans. In contrast, susceptible strains (e.g., C57BL/6) spontaneously develop necrotizing TE during late stage of infection. It is unclear whether the encephalitis in these mice is due to reactivation of infection. It may be caused by a continuous acute infection. Thus, resistant strains of mice are more suitable than susceptible strains for analyzing the mechanism for maintaining chronic (latent) infection and preventing TE. However, most available information on the resistance mechanism has been obtained with the use of susceptible mouse strains. Interferon (IFN)-γ–dependent cell-mediated immunity plays a major role in the resistance to *T. gondii* in both resistant and susceptible mouse strains, although humoral immunity is also involved. Many different types of cells are involved in the resistance as producers of IFN-γ and responders to IFN-γ for controlling the parasite. Multiple cell types are also important as producers of interleukin (IL)-12, which is required for induction of IFN-γ production.

**IL-12 Producers**

**Dendritic cells.** IL-12 is the most important inducer of IFN-γ synthesis. Neutralization of IL-12 with anti–IL-12 antibodies results in 100% mortality in mice infected with an avirulent strain of *T. gondii* in association with decreased IFN-γ production [1], and dendritic cells were identified as the source of IL-12 in the spleen in response to *T. gondii* [2]. All IL-12–positive cells in spleens of *T. gondii*-stimulated mice were found in T cell areas and were CD8α+, CD11c+, DEC205+ dendritic cells. CCR5 expressed on the surface of dendritic cells is responsible for their migration into splenic T cell areas after
stimulation [2]. CCR5 signaling also plays an important role in activation of dendritic cells to produce IL-12 [3]. CCR5-deficient mice are impaired in IL-12 production by dendritic cells and are highly susceptible to T. gondii infection.

During the chronic stage of infection, cells bearing dendritic cell markers (e.g., CD11c and 33D1) are located at the inflammatory site in the mouse brain [4]. These brain dendritic cells are mature as indicated by high-level expression of major histocompatibility complex (MHC) class II, CD40, CD54, CD80, and CD86 and can trigger antigen-specific T cell responses in vitro. The dendritic cells were the major producers of IL-12 among mononuclear cells isolated from brains of infected animals [4]. Granulocyte-macrophage colony-stimulating factor is suggested to be important for induction of the dendritic cells in primary brain cell cultures with T. gondii [4]. Because IL-12 is important for the maintenance of IFN-γ production in T cells mediating resistance to chronic infection [5], dendritic cells in the brains of infected mice might play a role in maintaining IFN-γ production by T cells in the brain.

**Neutrophils.** Neutrophils rapidly infiltrate into the peritoneal cavity of mice following intraperitoneal infection with T. gondii. About 85% of the neutrophils displayed intracellular storage of IL-12 [6]. Depletion of neutrophils during the first 6 days of infection results in increased mortality of mice in mice in association with decreased production of IL-12 and IFN-γ by splenocytes [7]. Rapid infiltration of neutrophils into the site of infection appears to play an important role in induction of the protective Th1-type immune responses against T. gondii during the early stage of infection.

**IFN-γ Producers**

**Involvement of “Innate Immunity”**

**γδ T cells.** During the acute stage of infection with T. gondii, increased numbers of T cells expressing the γδ T cell receptor (TCR) have been observed in the spleen and peritoneal cavity of mice and in peripheral blood of humans. γδ T cells are cytotoxic to infected target cells and produce IFN-γ and tumor necrosis factor (TNF)-α in vitro in response to the parasite. Involvement of γδ T cells in resistance against acute infection with T. gondii has been shown in mice. Mice deficient in γδ T cells due to treatment with anti-TCR δ monoclonal antibody (MAb) [8] or lack of the functional TCR δ gene [9] die earlier than control mice during the acute stage of infection.

γδ T cells, which are detectable in brains of chronically infected mice and rats, may also be involved in prevention of TE during the late stage of infection. Of interest, the relative percentages of γδ T cells in lymphocyte preparations isolated from brains of infected mice are significantly higher than in the spleen [10]. This suggests that γδ T cells preferentially infiltrate the brain of T. gondii–infected mice. A human with a CD40L defect (hyper-IgM syndrome) who developed TE had a marked increase in γδ T cells in his peripheral blood [11]. He responded well to antitoxoplasmic chemotherapy and to high-dose immunoglobulin replacement therapy. γδ T cells may play a partial but important role against T. gondii in the brain although their protective activity is not sufficient by itself to prevent TE. Lepage et al. [12] suggested a possible role for γδ T cells to enhance the protective activity of CD8 αβ T cells in their studies that used adoptive transfer of the lymphocyte populations.

**NK cells.** NK cells are an important source of IFN-γ in resistance against T. gondii during early infection. Depletion of NK cells results in early or increased mortality in SCID and wild type mice. In contrast to the early stage of infection, NK cells do not appear to be crucial for prevention of TE during the late stage of infection. Our recent studies revealed that depletion of NK cells did not abolish resistance of SCID mice that had received adoptive transfer of immune T cells against development of TE [13]. In the depleted mice, NK cells were undetectable in brain and spleen by flow cytometry.

Unidentified IFN-γ–producing non–T cells. We recently found that IFN-γ–producing non–T cells are required to prevent reactivation of T. gondii infection in mouse brains [13]. Athymic nude, SCID, and IFN-γ–deficient mice were infected with T. gondii and treated with sulfadiazine to establish chronic infection. After discontinuation of sulfadiazine, each animal developed severe TE due to reactivation of the chronic infection and died. When animals received adoptive transfer of immune spleen or T cells before discontinuation of sulfadiazine, infected athymic nude and SCID mice did not develop TE and survived. However, the infected IFN-γ–deficient mice still developed TE and died even after the cell transfer [13]. Before cell transfer, IFN-γ mRNA was detected in brains of nude and SCID mice but not in brains of the IFN-γ–deficient mice. IFN-γ mRNA was also detected in brains of infected SCID mice depleted of NK cells and these animals did not develop TE after receiving immune T cells [13]. Thus, IFN-γ production by non–T cells, in addition to T cells, is required for prevention of reactivation of T. gondii infection in the brain. The IFN-γ–producing non–T cells do not appear to be NK cells.

**Importance of “Acquired Immunity”**

**CD4 and CD8 αβ T cells.** It is clear that αβ T cells are essential for resistance against T. gondii since athymic nude and SCID mice that lack T cells succumb to the acute infection and their mortality is associated with proliferation of large numbers of tachyzoites in various organs including the brain. CD8 T cells are the major effector limb of the protective cellular immunity against acute infection although CD4 T cells are also involved. The protective activity of CD8 T cells is predominantly mediated by IFN-γ and these cells appear to be a major source of IFN-γ during the acute stage of the infection [14]. However, both CD8 and CD4 T cells from spleens of infected mice can produce this cytokine in vitro after stimulation with tachyzoite antigens.
IFN-γ also plays a critical role in prevention of TE during the late stage of infection in mice [15, 16]. Neutralization of the activity of IFN-γ in chronically infected mice by treatment with anti–IFN-γ MAb resulted in severe acute inflammation and development of large areas of necrosis in the brain [15]. In areas of acute inflammation and necrosis, tachyzoites and T. gondii antigens were detected, indicating that such inflammatory responses were caused by proliferation of tachyzoites. Reverse transcriptase polymerase chain reaction also detected increased amounts of tachyzoite-specific SAG1 and SAG2 mRNA, indicating a marked increase in numbers of tachyzoites in mouse brains after treatment with anti–IFN-γ MAb [17].

Thus, IFN-γ plays a critical role in prevention of tachyzoites in mouse brains. The same appears to be true in humans, since AIDS patients have an impaired activity to produce IFN-γ and frequently develop TE.

Both CD4 and CD8 T cells infiltrate the brain of mice following infection. Gazzinelli et al. [16] reported that CD4 and CD8 T cells act additively or synergistically to prevent development of TE, probably through production of IFN-γ. Brown and McLeod [18] reported that CD8 T cells are involved in the resistance by regulating the numbers of T. gondii cysts in the brains of mice.

Bcl-3 oncoprotein, a distinct member of the I-κB family, which functions as a positive regulator of nuclear factor (NF) κB activity, plays a critical role in mounting a protective Th1 immune response to T. gondii [19]. Bcl-3-deficient mice survive the early acute stage of the infection, but most die 3–5 weeks after infection. The ability of spleen cells to produce IFN-γ in response to T. gondii antigens is normal in the early stage (7 days after infection) but impaired in the later stage (12–31 days after infection). Cytotoxic activity of T cells but not of NK cells is also defective in these mice. These results suggest a critical role for Bcl-3 in antigen-specific priming of the long-term protective Th1-type T cells following T. gondii infection.

In regard to NF-κB, Caamano et al. [20] recently showed the importance of NF-κB(2) for T cell responses in resistance to TE. NF-κB(2)–deficient mice have no defect in their ability to produce IL-12 and IFN-γ during the acute stage of infection, but during the chronic stage of infection, succumb to TE in association with a reduced capacity of production of IFN-γ by splenocytes. T cell apoptosis appears to be involved in the reduced production of this cytokine.

Subauste et al. [21] recently reported that CD40-CD40L signaling is required for optimal T cell production of IFN-γ in response to T. gondii in humans. However, the role of the CD40-CD40L interaction in the T cell response appears to differ in mice. CD40L-deficient mice produce comparable levels of IFN-γ to control animals following infection, although CD40L is important for resistance to TE [22].

In addition to production of IFN-γ, both human and mouse CD4 and CD8 T cells are capable of killing T. gondii–infected target cells in vitro in a major histocompatibility complex (MHC)-restricted manner. Denkers et al. [23] reported an accelerated mortality beginning 75 days after infection in perforin-deficient mice. In their studies, about half of the perforin-deficient animals survived until day 150 after infection whereas all CD8 T cell–deficient mice died by day 50 after infection [23]. Thus, perforin-mediated cytolysis by T cells appears to play a limited role in resistance against T. gondii. Since perforin-deficient mice die during the late stage of the infection, the cytotoxic activity of T cells might play a role in prevention of TE. This point needs clarification.

Effector Mechanisms in IFN-γ–Mediated Resistance

NO produced by inducible NO synthase (iNOS). As mentioned, IFN-γ is the central cytokine in resistance against acute acquired infection with T. gondii and recrudescence of chronic infection (TE). Macrophages become quickly activated to kill intracellular tachyzoites following infection. This activation is mediated by IFN-γ since neutralization of the activity of this cytokine by treatment with anti–IFN-γ MAb blocks the activation [24]. In the absence of activity of endogenous IFN-γ, mice die within 1 week after intraperitoneal infection and their mortality is associated with numerous tachyzoites in the peritoneal cavities [24]. Thus, IFN-γ–mediated activation of macrophages is critical for resistance against acute infection with this parasite.

Murine peritoneal macrophages become activated after treatment with a combination of IFN-γ and TNF-α in vitro and the activated cells inhibit intracellular replication of tachyzoites through generation of NO by iNOS. However, the situation appears to differ in vivo. Mice lacking TNF receptor types 1 (R1) and 2 (R2) [25] and those lacking iNOS [26] can control parasite growth in the peritoneal cavity following intraperitoneal infection, indicating that TNF-α and iNOS are not essential for control of acute infection in mice. Consistent with these findings are findings in experiments in mice that lack IFN-γ regulatory factor 1 (IRF-1), which is essential for iNOS induction by IFN-γ [27]. Although IRF-1–deficient animals are more susceptible to infection with T. gondii than control animals, they survive the acute stage of the infection through iNOS-independent mechanism(s). These results indicate that the protective mechanism(s), which requires IFN-γ but not TNF-α or iNOS, is sufficient for mice to control parasite growth during the acute stage of infection. These results do not exclude the possibility that TNF-α and iNOS play a partial role in resistance to T. gondii in this stage of infection. The iNOS-independent mechanisms described in the sections below (e.g., tryptophan degradation by IDO [indolamine 2,3-dioxygenase], IGTP) may be involved in this resistance.

In contrast to the acute stage of infection, mice deficient in TNF R1/R2 [25] or iNOS [26] succumb to necrotizing TE during the late stage of infection. These results are consistent with those of earlier studies; treatments of chronically infected wild
type mice with anti–TNF-α MAb or aminoguanidine (an iNOS inhibitor) resulted in development of TE [17, 28]. Thus, TNF-α and iNOS are critical for prevention of proliferation of tachyzoites in the brain, although there is a possibility that in deficient mice the absence of TNF-α– and iNOS-mediated resistance during the acute stage of infection may have resulted in increased cyst burden in organs and may have partially contributed to increased mortality during the late stage of infection. As mentioned, IFN-γ plays a central role in resistance of the brain against this parasite [15, 16]. Since neutralization of IFN-γ or TNF-α results in decreased iNOS expression and development of severe TE [17], activation of iNOS mediated by IFN-γ and TNF-α appears to play a key role in prevention of TE.

Microglia are likely important effector cells involved in iNOS-mediated protective mechanisms in mouse brains. Murine microglia become activated in vitro to inhibit intracellular proliferation of tachyzoites following treatment with IFN-γ plus lipopolysaccharide (LPS) [29]. NO mediates the inhibitory effect of activated murine microglia on intracellular replication of tachyzoites; treatment of these cells with N G-monomethyl-

in increased cyst burden in organs and may have partially contributed to increased mortality during the late stage of infection. As mentioned, IFN-γ plays a central role in resistance of the brain against this parasite [15, 16]. Since neutralization of IFN-γ or TNF-α results in decreased iNOS expression and development of severe TE [17], activation of iNOS mediated by IFN-γ

in later stages of the infection. Yap et al. [36] demonstrated an involvement of iNOS-independent mechanisms in prevention of mortality in T. gondii–infected mice in an elegant study that used bone marrow chimera. In order to address the mechanisms for restricting the growth of T. gondii within cells of nonhematopoietic origin, they developed chimeric mice that expressed IFN-γ receptors, TNF R1/R2, or iNOS on hematopoietic or nonhematopoietic cells. Resistance to acute and persistent infection was displayed only by mice in which IFN-γ receptors and TNF R1/R2 were expressed in both hematopoietic and nonhematopoietic cells. In contrast, expression of iNOS by only hematopoietic cells was sufficient for host resistance. These results suggest that in concert with bone marrow–derived effector cells, nonhematopoietic cells can directly mediate IFN-γ– and TNF-α–dependent resistance to the parasite. This resistance does not require expression of iNOS in nonhematopoietic cells. Requirement of these multiple mechanisms for resistance to T. gondii appears to be due to its infection of not only mononuclear phagocyte lineage but also of a wide variety of host cells.

Resistance to development of TE is under genetic control in both humans and mice. Our recent studies suggest a crucial role for IFN-γ–dependent, but iNOS-independent, mechanism in the genetic resistance of BALB/c mice to this disease. BALB/c-background IFN-γ–deficient mice infected and treated with sulfadiazine developed severe TE after discontinuation of sulfadiazine treatment even though these animals expressed large amounts of mRNA for TNF-α and iNOS and in their brains. The amounts of the mRNA expressed were equivalent to those expressed in the brains of infected control mice, which prevented development of TE [37]. Thus, expression of TNF-α and iNOS is insufficient for prevention of TE in the absence of IFN-γ.

Tryptophan degradation by IDO. IDO is an enzyme that catalyzes the initial rate-limiting step of tryptophan catabolism to N-formylkynurenine and kynurenine. The depletion of intracellular tryptophan pools by IDO is an important mechanism by which IFN-γ–mediated toxoplasmostasis, indicating that the protective activity of the activated glioblastoma cells [35]. Daubener et al. [38] found that stimulation of human brain microvascular endothelial cells (HBMEC) with IFN-γ resulted in the induction of toxoplasmastosis. The capacity of HBMEC to restrict Toxoplasma growth after IFN-γ stimulation was enhanced in the presence of TNF-α, and such capability correlated with their IDO activities. Furthermore, the addition of excess amounts of tryptophan to the HBMEC cultures resulted in a complete abrogation of IFN-γ–TNF-α–mediated toxoplasmastosis, indicating that the protective activity is mediated by IDO.

In contrast to human models, the role of IDO in resistance to T. gondii is unclear in mice. IFN-γ does not induce IDO
and toxoplasmatidal activity in mouse fibroblasts [39] and is not important for controlling *T. gondii* in murine astrocytes [40]. However, two groups recently reported IFN-γ–dependent expression of IDO in the brains and lungs of mice during the acute stage of infection [41, 42]. More studies are needed to assess a possible involvement of IDO in resistance to *T. gondii* in mice.

**IGTP**  
*IGTP*, a recently identified IFN-γ responsive gene, is representative of a family of at least 6 genes encoding 47- to 48-kDa proteins that contain a GTP-binding sequence and that are expressed at high levels in immune and nonimmune cells after exposure to IFN-γ. Several of these proteins, including IGTP, localize to the endoplasmic reticulum of cells, suggesting they may be involved in the processing or trafficking of immunologically relevant proteins, such as antigens or cytokines. Taylor et al. [43] recently generated IGTP-deficient mice and found that despite normal immune cell development and normal clearance of *Listeria monocytogenes* and cytomegalovirus, the mice displayed a profound loss of host resistance to acute infection with *T. gondii*. Thus, IGTP is an essential mediator of specialized antimicrobial activities of IFN-γ.

In vitro studies demonstrated an importance of IGTP for prevention of *T. gondii* replication in murine astrocytes. After pretreatment with IFN-γ or a combination of this cytokine with either TNF-α, IL-1, or IL-6, murine astrocytes can inhibit proliferation of tachyzoites in vitro. The inhibitory effect of activated astrocytes is not mediated by IDO, NO, reactive oxygen intermediates, or iron deprivation. Halonen et al. [40] recently reported that astrocytes from IGTP-deficient mice did not cause a significant inhibition of *T. gondii* growth following treatment with IFN-γ, whereas wild type astrocytes inhibited the growth. Therefore, IGTP plays a central role in the IFN-γ–induced inhibition of the parasite in murine astrocytes.

In relation to *IGTP*, the roles of other members of the gene family in resistance against *T. gondii* were studied in knockout mice that lacked expression of the genes LRG-47 and IRG-47 [44]. LRG-47-deficient mice succumbed uniformly and rapidly during the acute stage of the infection; in contrast, IRG-47-deficient mice displayed only partially decreased resistance that was not manifested until the chronic phase of infection. Thus, LRG-47 and IRG-47 have vital but distinct roles in immune defense against *T. gondii*.

**Limiting the availability of intracellular iron to the parasite.** Dimier and Bout [45] reported that activation of rat enterocytes with rat recombinant IFN-γ resulted in an inhibition of intracellular replication of *T. gondii*. Neither nitrogen and oxygen derivatives nor tryptophan starvation are involved in the inhibitory effect. Experiments that used Fe2⁺ salt as well as carrier and chelator suggested that IFN-γ–treated enterocytes inhibit *T. gondii* replication by limiting the availability of intracellular iron to the parasite [45]. It is not known whether this iron-dependent mechanism is also involved in resistance of other types of cells activated by IFN-γ.

**Reactive oxygen intermediates (ROI).** ROI have been implicated in the toxoplasmacidal activity of normal human monocytes and IFN-γ–activated human macrophages in vitro [46, 47]. Involvement of ROI in their antimicrobial activity was suggested since impairing the ability of the cells to generate oxygen intermediates (by glucose deprivation or treatment with superoxide dismutase, catalase, or mannitol) inhibited toxoplasmacidal activity by greater than 80% [47] and because killing of *T. gondii* by monocytes obtained from chronic granulomatous disease patients was impaired [47]. However, the physiologic significance of the ROI pathway remains unclear, especially in mice. The parasites are resistant to the oxygen metabolites produced in murine macrophages [48]. Recently, p47 phox-deficient mice, which lack an inducible oxidative burst, were reported to control both the acute and chronic stages of *T. gondii* infection [26].

**Conclusions**  
Resistance against *T. gondii* requires a network that involves 4 different types of IFN-γ–producing cells and many types of IFN-γ–responding effector cells that prevent proliferation of the parasite through at least 5 different mechanisms. For controlling *T. gondii* in the brain during the late stage of infection, at least 3 types of IFN-γ–producing cells and 3 effector mecha-

### Table 1. Interferon (IFN)-γ–mediated resistance against *Toxoplasma gondii* in the brain.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function</th>
<th>Cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12</td>
<td>Maintaining IFN-γ production by T cells</td>
<td>Dendritic</td>
<td>[4, 5]</td>
</tr>
<tr>
<td>Bcl-3</td>
<td>Maintaining IFN-γ production by T cells</td>
<td>T</td>
<td>[19]</td>
</tr>
<tr>
<td>NF-κB(2)</td>
<td>Maintaining IFN-γ production by T cells</td>
<td>T</td>
<td>[20]</td>
</tr>
<tr>
<td>CD40L</td>
<td>Up-regulating IFN-γ production by T cells (human)</td>
<td>γδ T (?), Non-T (non-NK)</td>
<td>[11, 21]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Activation of effector cells to control <em>T. gondii</em></td>
<td>ad T</td>
<td>[13, 16, 18]</td>
</tr>
<tr>
<td>iNOS</td>
<td>Production of NO to control <em>T. gondii</em> in effector cells</td>
<td>Microglia (mouse), Astrocytes (human)</td>
<td>[29, 30]</td>
</tr>
<tr>
<td>IDO</td>
<td>Tryptophan degradation to control <em>T. gondii</em> in effector cells</td>
<td>Astrocytes (human)</td>
<td>[35]</td>
</tr>
<tr>
<td>IGTP</td>
<td>Unidentified mechanism to control <em>T. gondii</em> in effector cells</td>
<td>Astrocytes (mouse)</td>
<td>[40]</td>
</tr>
</tbody>
</table>

**NOTE:** IDO, indolamine 2,3-dioxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; NF, nuclear factor.
anisms in IFN-γ–responding effector cells appear to be involved (table 1). IL-12, Bcl-3, NF-κB(2), and CD40-CD40L ligand interaction are important for up-regulating and maintaining the IFN-γ–mediated resistance for prevention of TE (table 1). However, IL-10 plays a crucial role in down-regulating the responses for preventing development of immune response-mediated pathology during the acute stages of infection. Lipoxin A4 may play an important down-regulatory role during the late stage of infection [49]. Further studies will elucidate more details of the resistance mechanisms and provide better understanding of how this resistance network functions and how the system is regulated.

References


