Toxoplasma gondii encephalitis is a common opportunistic infection of the central nervous system in AIDS patients. Gamma interferon (IFN-γ) alone or in combination with interleukin-1 (IL-1), IL-6, or tumor necrosis factor alpha significantly inhibits the growth of T. gondii in murine astrocytes, suggesting these are important nonimmune effector cells in the brain. Inhibition was found to be independent of a nitric oxide-mediated or tryptophan starvation mechanism. Both reactive oxygen intermediates and iron deprivation are IFN-γ-mediated mechanisms known to operate against intracellular parasites in other cell types. Astrocytes generated from mice genetically deficient in the production of reactive oxygen intermediates (phox−/− mice) were found to inhibit growth of T. gondii when stimulated with IFN-γ alone or in combination with other cytokines. The reactive oxygen inhibitor catalase and the reactive oxygen scavengers mannitol and thiourea failed to reverse the IFN-γ-induced inhibition of T. gondii in astrocytes. These data indicate that IFN-γ-induced inhibition in astrocytes is independent of reactive oxygen intermediates. IFN-γ-induced inhibition could not be reversed by the addition of iron salts, ferric citrate, ferric nitrate, or ferric transferrin. Pretreatment of astrocytes with desferrioxamine also did not induce the inhibition of T. gondii. These data indicate that the mechanism of IFN-γ inhibition was not due to iron deprivation. IFN-γ had no effect on T. gondii invasion of astrocytes, but inhibition of growth and loss of tachyzoite vacuoles were evident in IFN-γ-treated astrocytes by 24 h after invasion. Overall, these data suggest that IFN-γ-activated astrocytes inhibit T. gondii by an as-yet-unknown mechanism.
TABLE 1. Effect of cytokine treatments in *phox*−/− murine astrocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Infection (mean ± SD)</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.6 ± 4.1</td>
<td>100</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>11.4 ± 1.0*</td>
<td>53.6</td>
</tr>
<tr>
<td>IFN-γ + TNF-α</td>
<td>8.9 ± 1.9*</td>
<td>43.2</td>
</tr>
<tr>
<td>IFN-γ + IL-6</td>
<td>7.0 ± 0.5*</td>
<td>34.0</td>
</tr>
<tr>
<td>IFN-γ + TNF-α + IL-6</td>
<td>8.8 ± 2.2*</td>
<td>42.7</td>
</tr>
</tbody>
</table>

*Cells were incubated with cytokines for 72 h prior to infection; all cytokines were added at 100 U/ml, and cells were fixed 48 h after infection. *: significance at the *P* < 0.05 level versus the control.

TABLE 2. Effect of reactive oxygen intermediate on IFN-γ in murine astrocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Infected cells (mean ± SD)</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.8 ± 2.0</td>
<td>100</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.9 ± 0.2*</td>
<td>16.1</td>
</tr>
<tr>
<td>Catalase</td>
<td>12.9 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>IFN-γ + catalase</td>
<td>1.5 ± 0.2*</td>
<td>11.6</td>
</tr>
<tr>
<td>Thiourea</td>
<td>10.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>IFN-γ + thiourea</td>
<td>1.2 ± 0.4*</td>
<td>11.8</td>
</tr>
<tr>
<td>Mannitol</td>
<td>12.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>IFN-γ + mannitol</td>
<td>0.9 ± 0.1*</td>
<td>7.3</td>
</tr>
</tbody>
</table>

*Cells were pretreated with IFN-γ (100 U/ml) for 72 h prior to infection; catalase, thiourea, and mannitol were added 2 h after infection, and cells were fixed 48 h postinfection. *: significance at the *P* < 0.05 level versus the control. There was no significant difference between the IFN-γ, IFN-γ + catalase, IFN-γ + thiourea, and IFN-γ + mannitol values.

**Effect of oxygen scavengers on IFN-γ-induced inhibition of *T. gondii***

To further test the role of an oxygen-dependent mechanism in the anti-*Toxoplasma* activity of IFN-γ in astrocytes, various inhibitors or scavengers were added to murine astrocyte cultures. Neither catalase, which converts hydrogen peroxide to water and oxygen, nor mannitol or thiourea, which are scavengers of hydroxyl radicals, reversed the inhibitory effect induced by IFN-γ (Table 2).

**Effect of iron(III) on the IFN-γ-induced inhibition of *T. gondii***

To further test whether the IFN-γ-induced inhibition of *T. gondii* in astrocytes is iron dependent, cells were incubated with the siderophore desferrioxamine (DFO). DFO (50 μM) did not induce inhibition of *T. gondii* in astrocytes; the addition of the iron salts, ferric citrate, or ferric transferrin to DFO did, however, cause a significant increase (two- to threefold) in the growth of *T. gondii* in astrocytes (Table 3). The role of iron in the IFN-γ-induced anti-*Toxoplasma* effect was further tested by the addition of ferric citrate at 5, 50, and 100 μM to IFN-γ-treated cultures. Ferric citrate did not reverse the IFN-γ-induced inhibition of *T. gondii* in astrocytes at any of the concentrations used (Table 4). These results indicate that the IFN-γ-induced anti-*Toxoplasma* effect is iron independent in astrocytes.

**Effect of IFN-γ on invasion and growth of *T. gondii***

The effect of IFN-γ pretreatment of astrocytes on invasion and growth of *T. gondii* was also tested by counting the percent infected cells and the number of tachyzoites per vacuole at 2 and 24 h postinvasion, respectively. No significant difference was observed in the percent infected cells at 2 h between control and IFN-γ-treated cells (Table 5), indicating that IFN-γ pretreatment of astrocytes has no effect on the invasion of host cells. By 24 h, however, both the percent infected cells and the number of tachyzoites per vacuole were significantly less in IFN-γ-treated cervals versus control cells (Table 5). The decrease...
in the percentage of infected cells indicates that IFN-γ induces a microbicidal effect, while the decrease in the number of parasites per vacuole suggests that a microbicstatic effect occurs by 24 h postinvasion.

**DISCUSSION**

IFN-γ is the main cytokine controlling *T. gondii* in the brain (35). Previous studies demonstrated that IFN-γ significantly inhibits *T. gondii* in astrocytes via a nitric oxide- and tryptophan-independent mechanism (19). In this study the mechanism of IFN-γ-induced inhibition of *T. gondii* in astrocytes was further investigated. IFN-γ-induced inhibition was found to be independent of reactive oxygen intermediates, as evidenced by the inability of oxygen radical scavengers to reverse the inhibition and the fact that IFN-γ could also induce inhibition in astrocytes incapable of producing the reactive oxygen intermediates. The role of iron deprivation in IFN-γ-induced inhibition was also addressed. The inability of DFO to induce the inhibition of the growth of *T. gondii* and the inability of ferric salts to reverse the IFN-γ-mediated growth inhibition indicate that the IFN-γ-induced inhibition of *T. gondii* in murine astrocytes is independent of iron deprivation. IFN-γ was found not to affect invasion by *T. gondii* of astrocytes but was found to have a microbicstatic and microbicidal effect that was evident by 24 h after invasion.

The mechanisms of IFN-γ-induced inhibition of *T. gondii* which have been demonstrated in other cell types include reactive oxygen intermediates, induction of nitric oxide production, tryptophan starvation, and iron deprivation. In human mononuclear phagocytes, IFN-γ induces toxoplasmal activity via reactive oxygen intermediates (28). In murine macrophages and microglia, IFN-γ activates inhibition of *T. gondii* via l-arginine-dependent production of nitric oxide (1, 5). In nonmyeloid cells, IFN-γ-induced inhibition of *T. gondii* was found to occur via tryptophan degradation in human fibroblasts and retinal pigment cells (29, 32), while in enterocytes inhibition occurred via iron deprivation (37).

In murine astrocytes, we have previously shown that IFN-γ-induced inhibition of *T. gondii* was independent of nitric oxide intermediates and tryptophan degradation (19). While the mechanism of IFN-γ-induced inhibition of *T. gondii* in astrocytes was also independent of reactive oxygen derivatives and iron deprivation. Astrocytes have been shown to produce superoxide via a neutrophil-type NADPH oxidase during recovery from hypoxia (21, 36). The respiratory burst as an antitoxoplasmatic mechanism in astrocytes has not previously been investigated. The finding that reactive oxygen intermediates do not play a role in the antitoxoplasmatic activity of astrocytes is consistent with studies that have found that p47 phox−/− mice, which lack an inducible oxidative burst, are able to control both the acute and chronic stages of *T. gondii* infection (2). Iron deprivation, a common antimicrobial mechanism, was also not found to be the mechanism of IFN-γ-induced inhibition of *T. gondii* in astrocytes. These data indicate that the IFN-γ-induced inhibition of *T. gondii* in astrocytes occurs via an unknown mechanism.

IFN-γ is known to induce a diverse array of effects on cells (5, 6). IFN-γ is a 34-kDa glycoprotein that binds to a membrane receptor. The IFN-γ receptor is ubiquitously expressed on all nucleated cells at modest levels (6). Binding of IFN-γ to the membrane receptor transmits signals to the cytoplasm and nucleus by the Jak-STAT pathway which mediate the transcription of IFN-γ-specific genes (5, 6). Several primary response genes are themselves transcription factors and are required for the induction of other secondary components of the cellular response to IFN-γ. More than 200 IFN-γ-regulated genes have been identified (6). The function of many of these genes is known, and they have been identified as being involved in a diverse range of distinct cellular programs which collectively orchestrate the immune response. For example, IFN-γ induces the expression of major histocompatibility complex (MHC) I and II molecules, which are involved in antigen presentation; the induction of enzymes, resulting in the respiratory burst; nitric oxide and tryptophan degradation, which have antimicrobial effects; and the induction of expression of ICAM molecules and chemokines, which are involved in leukocyte-endothelium interactions. The function of many of the other known IFN-γ response genes, however, is not understood.

While the mechanism of IFN-γ-induced inhibition of *T. gondii* in astrocytes is not understood, it was found that IFN-γ resulted in a microbicstatic and microbicidal effect that was evident by 24 h after invasion. IFN-γ has a wide variety of effects on the physiology of cells, including cell shape changes, an antiproliferative effect, and the induction of mitogen-activated protein kinases, which may regulate some of these effects (4, 5, 26). One possible mode of action of IFN-γ in *T. gondii* may be through disruption of the intracellular organization of the cytokoskeleton or other host cell organelles, which may in turn affect the parasitophorous vacuole, an organelle essential for the intracellular survival of *T. gondii*. The acquisition of host cell cytokoskeleton, endoplasmic reticulum, and mitochondria around the parasitophorous vacuole of *T. gondii* is well documented, and inhibition of lysosomal fusion with the parasitophorous vacuole is also known to be essential for intracellular survival (24, 34). In support of this, IFN-γ was found to interfere with the intracellular development and survival of the parasite in astrocytes, and it is possible that this effect of IFN-γ is due to the disruption of interactions of the parasitophorous vacuole with the host cell organelles.

Whatever the mechanism of IFN-γ-induced inhibition of *T.
CD8+ presenting cells in the brain (3). IFN-γ-activated astrocytes, for example, could serve to stimulate MHC class I-restricted CD8+ cells, which are cytolytic for infected cells and thought to play a major role in host immunity against T. gondii (17). Our studies indicate that IFN-γ-activated astrocytes also have direct antimicrobial effects on T. gondii. It is well established that IFN-γ-activated macrophages and microglia, cells of hemopoietic origin, have direct antimicrobial effects in T. gondii and other intracellular pathogens through toxic reactive nitrogen and oxygen intermediates (7–9, 14). Until recently, the role of IFN-γ-activated microbicidal mechanisms in nonhemopoietic cells has been unclear. Yap and Sher (38) addressed this question recently in a study in which susceptibility to T. gondii infection was tested in chimeric mice in which IFN-γ receptors were expressed on both hemopoietic and nonhemopoietic cells or on hemopoietic cells only. Yap and Sher found that resistance to both acute and chronic infections by T. gondii required the expression of IFN-γ receptors in both the hemopoietic and nonhemopoietic compartments (38). These results indicate that nonhemopoietic cells are necessary for host resistance to T. gondii. Since T. gondii infects a number of nonhemopoietic cells, including cells of epithelial, mesodermal, and neuronal origin, IFN-γ-activated nonhemopoietic cells may be of particular importance to host resistance to T. gondii. For instance, it has been suggested that IFN-γ-activated enterocytes and endothelial cells are important effector cells controlling parasite dissemination during an acute infection and in congenital toxoplasmosis, respectively (11, 37). Likewise, our studies indicate that IFN-γ-activated astrocytes may be important effector cells controlling replication of T. gondii in the central nervous system and are possibly involved in the prevention of reactivated toxoplasmonic encephalitis. Further studies investigating the role of these nonhemopoietic cells in acute infection, congenital toxoplasmosis, and toxoplasmonic encephalitis may yield important insights into the pathogenesis of T. gondii.

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REFERENCES


25. Pollock, J. D., D. A. Williams, M. A. C. Gifford, L. L. Li, D. A. Du, J. Fisherman,


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