independent samples of recA-DNA complexes and always obtained images of similar quality. Our results prove that relief data at macromolecular level can be achieved directly without averaging. In case of recA-DNA complexes, the STM showed directly the number of recA monomers per helical repeat of the complex, whereas successful determination of this number with TEM required averaging (*9*). In TEM, the third dimension must be calculated from averaged different projections (*17*). When averaging is difficult, STM seems to be a straightforward, reliable way to reveal a three-dimensional structure, particularly for small relief corrugations that are difficult to reconstruct from projections.

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Interferon-γ: The Major Mediator of Resistance Against *Toxoplasma gondii*

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Mice were injected with a monoclonal antibody to interferon-γ to examine the importance of endogenous production of this lymphokine in resistance against infection with the sporozoan parasite *Toxoplasma gondii*. Mice with intraperitoneal infections of *T. gondii* that received no antibody survived and developed chronic *T. gondii* infection, whereas the infected mice that received the monoclonal antibody died of toxoplasmosis. The activation of macrophages, which kill *T. gondii* in vivo, was inhibited by administration of the monoclonal antibody, but the production of antibodies to *T. gondii* was not suppressed. The fact that an antibody to interferon-γ can eliminate resistance to acute *Toxoplasma* infection in mice suggests that this lymphokine is an important mediator of host resistance to this parasite.

DATA FROM STUDIES PERFORMED in vitro and in vivo suggest that the major mechanism of resistance against *Toxoplasma gondii* is cell-mediated (*1*). Adoptive transfer of lymphocytes confers strong resistance against challenge infection with *T. gondii* in laboratory animals (*2*), whereas passive transfer of antibodies does not (*3*). Recently, the high prevalence of toxoplasmic encephalitis reported in patients with acquired immunodeficiency syndrome (AIDS) and the fact that almost all such cases occur in individuals who have serologic evidence of prior infection with *T. gondii* have underscored the importance of T cell–mediated immunity against this organism. The major immunological defect in patients with AIDS is the decreased numbers and function of CD4 lymphocytes (*4*).

On the basis of studies in vitro, we (*5*) and others (*6, 7*) have postulated that the activated macrophage is the effector arm of cell-mediated immunity against *T. gondii*. Interferon-γ (IFN-γ) has the capacity to activate mouse macrophages in vitro (*7, 8*) and in vivo (*9*), and human macrophages in vitro inhibit or kill this parasite (*8, 10*). When IFN-γ was administered in vivo, significant protection against *T. gondii* was observed in a mouse model of toxoplasmosis (*9*). These observations suggest that IFN-γ is of primary importance in host resistance against *T. gondii*. In all of these studies, exogenous IFN-γ was used as the macrophage-activating factor. Thus, the relative importance of IFN-γ to resistance against this parasite has not been evaluated during the natural host response to infection with *T. gondii*. Our experiments reveal that IFN-γ is essential for resistance to acute *T. gondii* infection in vivo.

All control mice that received saline or normal hamster immunoglobulin G (IgG) survived intraperitoneal or oral infection with an avirulent strain of *T. gondii* (strain ME49). In contrast, seven out of seven mice injected with a monoclonal antibody to IFN-γ (H22.1) died after intraperitoneal inoculation with the parasite, and four of five died after peroral infection (Table 1). This H22.1 antibody (100 or 200 μg) caused no apparent illness or mortality in noninfected BALB/c mice. The effect of H22.1 on host resistance to infection with *T. gondii* was dose-dependent (Fig. 1). Whereas all mice that received normal hamster IgG survived the infection, a dosage of the monoclonal antibody as small as 7.4 μg resulted in death due to toxoplasmosis in 83% (5/6) of the mice. All mice that received 2.5 μg of the antibody survived, but all mice that received 67 μg of the monoclonal antibody died of the infection. Buchmeier and Schreiber (*11*) reported that this same monoclonal antibody, H22.1, significantly reduced resistance against *Listeria*

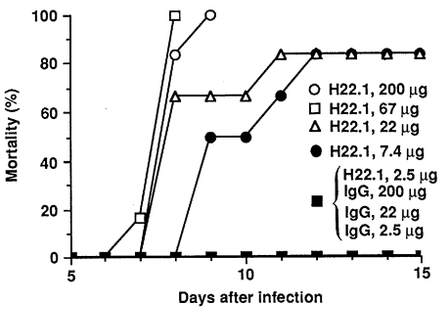

Fig. 1. Dose effect of monoclonal antibody to IFN-γ on resistance to infection with *T. gondii*. Mice were injected intraperitoneally with various doses of H22.1 or normal hamster IgG diluted in saline. There were six mice in each group. One day later, mice were infected intraperitoneally with 18 cysts of *T. gondii* strain ME49 prepared as described (Table 1). No deaths occurred in the group treated with 2.5 μg of H22.1 alone or in any of the three groups treated with IgG alone. The latter results are depicted as solid squares on the abscissa.

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monocytogenes infection in a mouse model. The H22.1 antibody inhibits binding of IFN-γ to the interferon receptor, neutralizes the ability of IFN-γ to activate macrophages to kill *Leishmania major* and tumor target cells, and inhibits the expression of macrophage Ia induced by IFN-γ (*12*).

Because the activated macrophage is considered to be the major effector arm in resistance against *T. gondii*, we examined whether the activation of macrophages that regularly occurs when mice are infected with *T. gondii* (*13*) is inhibited by administration of H22.1. Peritoneal macrophages were harvested from H22.1-treated mice after they were infected with the ME49 strain of *T. gondii*. Macrophage monolayers were challenged in vitro with tachyzoites of the RH strain to examine their ability to kill *T. gondii* (*14, 15*). Controls were macrophages from nontreated, noninfected mice and from infected mice that had been treated with normal hamster IgG. Peritoneal macrophages from 3-day infected control mice were activated to effectively kill *T. gondii* (Table 2). In contrast, macrophages from infected mice that had been treated with H22.1 did not kill the parasite; organisms proliferated within these macrophages as well as they did in macrophages from noninfected control mice. On day 7 of infection, 70% of macrophages collected from the peritoneal cavities of the H22.1-treated mice were observed microscopically to be infected with tachyzoites of the ME49 strain. These macrophages were destroyed by proliferation of the tachyzoites during overnight incubation in the absence of challenge with RH tachyzoites. In contrast, at this same time after infection, neither macrophages nor peritoneal fluids from ME49-infected control mice contained tachyzoites. Thus, treatment of the mice with the monoclonal antibody to IFN-γ prevented the activation of peritoneal macrophages, which therefore could not kill *T. gondii*. We found that the 7-day infected mice that had been treated with the H22.1 had peritoneal fluids containing large numbers of extracellular *T. gondii* in addition to the intracellular organisms described above. We have never before observed such large numbers of extracellular parasites of an avirulent strain with any form of immunosuppression, including treatment with cortisone or antibody to the L3T4 subset of T lymphocytes (*16*). These peritoneal fluids resembled those seen in mice infected with the highly virulent RH strain (*17*).

To determine the specificity of the effect of H22.1, we attempted to reverse its action in vivo by administering recombinant IFN-γ (rIFN-γ) (*18*). The effect of rIFN-γ in these experiments was dose-dependent. Seven mice were injected with 100 μg of H22.1 1 day before infection with *T. gondii* and were injected with 5×10^3, 5×10^4, or 5×10^5 U of rIFN-γ every other day beginning 1 day before infection (experimental mice). Another group of seven mice received the monoclonal antibody but no rIFN-γ (control mice). Mice that received both H22.1 and 5×10^3 U of rIFN-γ died at the same time as control mice. By day 9 when all of the seven control mice had died, 57% (four of seven) of experimental mice injected with 5×10^4 U of rIFN-γ ($P < 0.05$; χ^2 test) and 86% (six of seven) of mice injected with 5×10^5 U rIFN-γ were still alive ($P < 0.005$). All of the former group ultimately died of toxoplasmosis, whereas three of seven (43%) in the latter group survived. The reasons rIFN-γ did not completely ablate the effect of the monoclonal antibody might have been due to delivery of too low a dose of the rIFN-γ and to differences between the half-lives of the rIFN-γ (10 minutes) and the monoclonal antibodies (14 days).

These results demonstrate the importance of endogenous IFN-γ production and activity in resistance against *T. gondii* infection. Infection with the ME49 strain of *T. gondii* is usually not lethal for mice, but cysts are formed in multiple tissues and persist for the entire life-span of the animals. Our results reveal that endogenous IFN-γ is the mediator that allows mice to survive this infection.

The formation of antibodies to *T. gondii* was enhanced by injection of H22.1 (Table 3), indicating that the antibody response is not the major protective factor in host resistance to *T. gondii* infection. However, *Toxoplasma* antibodies do play a role in protection against this parasite (*3*). The reason for higher antibody titers in the monoclonal antibody–treated mice may have been the greater availability of *T. gondii* antigen in the treated mice, since the numbers of tachyzoites were far greater (for example, in the peritoneal cavity) in monoclonal antibody–treated mice than in control mice.

Functions of IFN-γ other than macrophage activation include degradation of

Table 1. Effect of monoclonal antibody H22.1 on natural resistance to *T. gondii*. BALB/c female mice (18 g; Simonsen) were injected intraperitoneally with 200 μg of H22.1 (hamster-mouse hybridoma) once each week beginning 1 day before intraperitoneal or peroral infection with *T. gondii* strain ME49. Control mice were injected with saline or 200 μg of normal hamster IgG (Cooper Biochemical). The inoculum of *T. gondii* consisted of the cyst form obtained from brains of chronically infected mice. Mice were killed by CO_2 narcosis, and their brains were removed and triturated in phosphate-buffered saline (*p*H 7.2). A portion of the brain suspension was examined for the number of cysts, and after appropriate dilution in phosphate-buffered saline each mouse received either 18 cysts intraperitoneally or 15 cysts by gavage. Mice were examined daily at the same times and followed until time of death. Mice that died during the experiment were examined for the presence of *T. gondii* in their peritoneal fluid.

Injection	Infection route	Mice dead/total mice (*n*)
Saline	Intraperitoneal injection	0/7
	By mouth	0/5
IgG	Intraperitoneal injection	0/7
	By mouth	0/5
H22.1	Intraperitoneal injection	7/7
	By mouth	4/5

Table 2. In vitro effect of peritoneal macrophages from mice treated with monoclonal antibody H22.1 on intracellular *T. gondii*. Mice were injected with 100 μg of H22.1 or normal hamster IgG 1 day before intraperitoneal infection with 18 cysts of *T. gondii*. Three days later, three mice of each group were killed and macrophages were harvested from the peritoneal cavities as described (*15*). Macrophage monolayers were challenged with tachyzoites of the RH strain of *T. gondii*, and inhibition and killing of the parasite were evaluated (*14, 15*). Results were reproducible in three separate experiments performed on different days.

Group	Number of cells infected (%)		Parasites per 100 cells	
	0 hour	18 hours	0 hour	18 hours
Nontreated, noninfected	27	35	59	277
IgG, infected	24	0.3	32	0.3
H22.1, infected	27	32	54	248

Table 3. Production of antibody to *T. gondii* in mice treated with H22.1 during infection. Mice that had received a single dose of 100 μg of H22.1 or normal hamster IgG were inoculated intraperitoneally with 18 cysts of *T. gondii* strain ME49. Three and 7 days later, sera of three mice from each group were tested for *Toxoplasma* antibody in the agglutination test with the use of Formalin-fixed tachyzoites (*24*). Results obtained by two persons who had no prior knowledge of the experimental design were identical.

Treatment	*Toxoplasma* antibody titer	
	Day 3	Day 7
IgG	20, 20, 20	1,280, 2,560, 5,120
H22.1	40, 40, 40	20,480, 20,480, 20,480

tryptophan in human fibroblasts, which results in inhibition of growth of intracellular *T. gondii* (*19*). In contrast, IFN-γ did not induce tryptophan degradation in some mouse fibroblast cell lines and primary cultures of embryo fibroblasts of BALB/c mice (*20*). Therefore, in resistance to *T. gondii* in the mouse model, the primary effect of IFN-γ in vivo appears to be its ability to activate macrophages. Critical to this point is that rIFN-γ can activate macrophages to inhibit or kill *T. gondii* without collaboration of any other lymphokine (*7, 8, 10*). Our results along with those reported by Murray and his colleagues on the importance of IFN-γ in resistance to *T. gondii* (*21*) and the impaired production of this lymphokine in patients with AIDS (*22*) support the suggestion that the IFN-γ should be evaluated for treatment of toxoplasmosis in patients with AIDS (*22*).

Note added in proof: Since submission of this report, Schofield *et al.* (*23*) demonstrated that anti–IFN-γ reversed immunity to sporozoite (malaria) challenge in mice and rats.

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Guanosine Triphosphatase Activating Protein (GAP) Interacts with the p21 *ras* Effector Binding Domain

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A cytoplasmic protein that greatly enhances the guanosine triphosphatase (GTPase) activity of N-*ras* protein but does not affect the activity of oncogenic *ras* mutants has been recently described. This protein (GAP) is shown here to be ubiquitous in higher eukaryotes and to interact with H-*ras* as well as with N-*ras* proteins. To identify the region of *ras* p21 with which GAP interacts, 21 H-*ras* mutant proteins were purified and tested for their ability to undergo stimulation of GTPase activity by GAP. Mutations in nonessential regions of H-*ras* p21 as well as mutations in its carboxyl-terminal domain (residues 165–185) and purine binding region (residues 117 and 119) did not decrease the ability of the protein to respond to GAP. In addition, an antibody against the carboxyl-terminal domain did not block GAP activity, supporting the conclusion that GAP does not interact with this region. Transforming mutations at positions 12, 59, and 61 (the phosphoryl binding region) abolished GTPase stimulation by GAP. Point mutations in the putative effector region of *ras* p21 (amino acids 35, 36, and 38) were also insensitive to GAP. However, a point mutation at position 39, shown previously not to impair effector function, did not alter GAP-p21 interaction. These results indicate that GAP interaction may be essential for *ras* p21 biological activity and that it may be a *ras* effector protein.

Ras GENES ENCODE MEMBRANE-ASsociated proteins that are involved in the control of cell proliferation (*1*). Members of this gene family are widely expressed in the cells of both lower and higher eukaryotes. In *Saccharomyces cerevisiae*, *ras* genes have been shown to regulate adenylate cyclase activity (*2*), however, the function of mammalian *ras* gene products remains unclear. All *ras* proteins bind guanine nucleotides with high affinity and possess an intrinsic GTPase activity (*1, 3*). They resemble other guanine binding proteins (such as the signal-transducing G proteins) in that they are biologically active when in the guanosine triphosphate (GTP)–bound form and inactive when bound to guanosine diphosphate (GDP) (*4, 5*).

Each of the three closely related mammalian *ras* genes (H-*ras*, K-*ras*, and N-*ras*) encode a 21-kD protein (p21) possessing a low intrinsic GTPase activity. This activity is reduced by three- to tenfold in many oncogenic mutants of p21 (*1, 3, 4*). However, these differences in intrinsic GTPase activity are insufficient to explain the greatly increased biological potency of the oncogenic mutants of p21. A cytoplasmic protein has recently been detected that can accelerate the GTPase activity of the wild-type human N-*ras* protein more than 100-fold (*4*). As a result of interaction with this protein (referred to as GAP, GTPase activating protein), N-*ras* p21 injected into *Xenopus* oocytes was shown to be maintained in the inactive, GDP-bound state. By contrast, two N-*ras* oncogenic mutants (Val 12 and Asp 12) were not responsive to GAP, remaining in the active (GTP-bound) form.

GAP appears to be widely expressed in the cells of higher eukaryotes. We have detected GAP-like activity (ability to stimulate intrinsic N-*ras* p21 GTPase activity) in cell extracts from human and mouse (i) normal tissues (brain, liver, placenta, B cells,

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