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Resistance to *Cryptococcus neoformans* Is Associated with an Inflammatory Response to *Toxoplasma gondii* in the Central Nervous System of Mice

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We have studied the resistance of *Toxoplasma gondii*-infected mice to subsequent infection with *Cryptococcus neoformans*. Mice infected with the moderately virulent ME49 strain of *T. gondii* are resistant to proliferation of yeast cells in their brains after intravenous inoculation of the serotype A *C. neoformans* strain 184. The resistance serves to limit proliferation of yeast cells that colonize the brain. Maximal levels of resistance correlate not with maximal systemic specific anti-*Toxoplasma* resistance but rather with high levels of inflammatory response, presumably to parasites released from cysts in the brain. Resistance is localized, as mice infected with ME49 show only limited resistance in their lungs after intratracheal instillation of yeast cells, but there is substantial protection against development of cerebral cryptococcosis.

It has been known for some time that infection with one pathogen may offer protection against a second, unrelated organism (4, 7, 12, 16). For example, Gentry and Remington (7) reported that mice infected with the protozoan *Toxoplasma gondii* and then challenged with a lethal inoculum of the yeast *Cryptococcus neoformans* lived twice as long as non-*Toxoplasma*-infected controls.

C. neoformans is the most common life-threatening fungal infection among patients with AIDS (11). Typically, the yeast is inhaled and establishes a pulmonary infection that, even in immunocompromised individuals, is asymptomatic or mild. However, yeast cells which evade pulmonary defenses of immunocompromised individuals and enter the vasculature frequently invade the brain, where they cause a cryptococcal meningoencephalitis that is uniformly fatal if untreated. In immunodeficient individuals, chemotherapy often fails and relapse is frequent (15).

However, we have observed that immunocompetent mice are capable of surviving the seeding of *C. neoformans* in their brains that occurs when the yeast cells escape their pulmonary defenses. At early time points after an intratracheal instillation of yeast cells, greater than 50% of the animals have detectable yeast cells in their brains (1). Yet few of these animals go on to develop severe cryptococcal meningoencephalitis. The fact that immunocompetent mice possess defense mechanisms that are effective against yeast cells within the central nervous system (CNS) suggests that comparable defense mechanisms exist in the human host.

Mechanisms of anticryptococcal resistance that are effective against yeast cells within the CNS and are acquired as a result of prior exposure to *C. neoformans* or other organisms deserve study, as elucidation of such mechanisms might yield information leading to more effective therapy for those at risk for cryptococcal meningoencephalitis.

The present study was performed to determine whether effective *T. gondii*-mediated resistance to *C. neoformans* is demonstrable inside the CNS and to characterize the mechanism of this acquired response. If a sustained inflammatory response associated with sporadic cyst rupture in *Toxoplasma*-infected brains serves to limit proliferation of *C. neoformans* within the brain, then it might be possible to design a prophylactic treatment for individuals at risk for infection with *C. neoformans* and other CNS-tropic pathogens.

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MATERIALS AND METHODS

C. neoformans. *C. neoformans* 184, a thinly encapsulated serotype A strain (13), was maintained by passage every two weeks on Sabouraud dextrose (Sabdex) agar slants. For inoculation into mice, a sample from a slant was grown overnight on a fresh slant and subsequently seeded into Sabdex broth and incubated at 37°C overnight on a rotary shaker. Organisms were harvested by centrifugation, washed, and suspended to the desired concentration in phosphate-buffered saline (PBS). Inocula were always plated on Sabdex agar and routinely consisted of greater than 85% viable yeast cells. Mice were inoculated intratracheally with 10^6 yeast cells in 0.1 ml of PBS while under halothane-oxygen anesthesia (8). Pulmonary infections are established at essentially 100% efficiency by this method. Intravenous challenges were performed with 2×10^4 organisms delivered in 0.2-ml volumes via the retro-orbital sinus. We have determined experimentally that infection via the retro-orbital sinus results in 0.1 to 1.0% deposition of yeast cells in the brains following intravenous infusion of 2×10^4 strain 184 yeast cells, regardless of whether organisms are delivered via the lateral tail vein or the retro-orbital sinus. Injections in this study were consistently delivered via the retro-orbital sinus.

T. gondii. Cysts of the mildly virulent strain ME49 were prepared from suspensions of brain tissue of chronically infected B6 source mice (9). Cysts were enumerated by direct microscopic counts, suspended in volumes of Hanks balanced salt solution to yield concentrations of 200 cysts per ml, and administered intraperitoneally (i.p.). Tachyzoites of the vaccine strain ts-4 (14) and the highly virulent RH strain were maintained in vitro in HS-68 human foreskin fibroblasts (ATCC CRL 1635) at 33° and 37°C, respectively. Immunity to challenge with the RH strain of *T. gondii* was assessed by survival following i.p. inoculation of 10^3 organisms. Ordinarily, deaths occur within 7 to 10 days of such a dose.

The virulence of the immunizing *T. gondii* ME49 suspensions varies somewhat from experiment to experiment in our hands, and it is not unusual for some B6 mice to die during the 6-week period of establishment of immunity, i.e., before the mice receive the challenge infection. Therefore, at some time points, four rather than five mice were killed.

To determine whether any ts-4 organisms persisted in the brains of mice at 6 weeks after they were inoculated, five C57BL/6J mice were inoculated i.p. with 2×10^4 ts-4 organisms. After 6 weeks their brains were homogenized in 2.0 ml of PBS. One-half milliliter of each suspension was administered i.p. to each of three lymphodeficient SCID mice to detect viable ts-4 tachyzoites in the suspension. As few as 20 viable tachyzoites kill a SCID mouse at approximately 3 weeks postinfection (unpublished observation). It was determined that mouse brains contain fewer than 80 ts-4 tachyzoites (the limit of detection using this procedure) by 6 weeks after i.p. inoculation with 2×10^4 ts-4 organisms.

Mice. Eight- to twelve-week-old male C57BL/6J (B6) and (BALB/cBy ×

C57BL/6J)F_1 (CByB6F1) mice were used. In addition, 10-week-old male C.B-17 *scid/scid* (SCID) mice were used to determine the persistence of *T. gondii* ts-4. Mice were purchased from the Animal Breeding Facility of the Trudeau Institute and maintained under standard husbandry conditions. Serum samples from sentinel mice were periodically screened by an independent laboratory to determine that mice were free of infection by any of a large panel of pathogens. Immunodeficient mice were maintained in autoclaved cages and received sterilized food and water. Air was supplied to SCID mice through sterile filters.

When noted, mice were maintained for a portion of the experiment on drinking water containing 400 mg of sulfadiazine per liter. This drug protects them against nonencysted *T. gondii* and allows long-term survival (6). The severity and kinetics of infection with strain 184 yeast cells alone were identical in mice maintained on sulfadiazine-treated or untreated water.

Collection of tissue samples and yeast-cell enumeration. Mice were killed by CO_2 asphyxiation, and brains and lungs were placed in 3.0 ml of cold PBS. Organs were homogenized with Teflon pestles and then serially diluted, plated on Sabdex agar plates, and incubated at 26°C. Colonies were counted 48 h later.

In one experiment (see Fig. 2), hematoxylin- and eosin-stained paraffin sections of brains were examined histologically. Brain sections were selected at random from each of three mice per group (B6 and CByB6F1) to illustrate the different levels of inflammation seen in B6 and CByB6F1 mice.

For the histopathologic observations scored in Table 1, mice were exsanguinated and perfused with PBS and brains were harvested and treated as described above. Four or five serial sagittal sections were taken adjacent to the midline and examined for each animal. Sections were evaluated without knowledge of the treatment group and were scored separately for cellular infiltration of the meninges; presence of perivascular inflammatory cuffs; frequency, size, and cellular composition of glial nodules; number of foci of host necrosis; and number of tissue cysts. Scores were assigned according to a four-point scale, where 1 was the most minimal lesion observable, and 4 corresponded to a marked inflammatory response.

Statistics. Experiments involving comparison of the numbers of organ CFU were analyzed by Student's *t* test. Survival data in the experiment involving irradiation of CByB6F1 mice was analyzed by the Mann-Whitney test.

RESULTS

C57BL/6J mice infected with *T. gondii* are resistant to yeast cells in their brains after intravenous challenge. Groups of 20 B6 mice were inoculated i.p. with either 20 ME49 cysts in a mouse brain suspension or an equivalent volume of homogenized uninfected mouse brain in experiment 1. In a subsequent experiment, mice were infected with cysts or were left untreated. Six weeks later, mice were inoculated intravenously with 2×10^4 *C. neoformans* 184 yeast cells. Five mice from each group were killed at 24 h, 3 days, and 10 days after inoculation. In addition, five ME49-infected mice were killed 22 days after inoculation in experiment 1. Only three of five ME49-infected mice survived until the 22-day time point in experiment 2. It is likely that death resulted from *Toxoplasma* infection (see Materials and Methods), since $\log_{10}$ CFU of *C. neoformans* in the brain typically reach 8.0 before mice die, and we have not observed yeast burdens in the brain that high in ME49-infected animals at any point after infection. All sham-infected mice died between 19 and 21 days of infection in experiment 1. Log_{10} CFU of yeast cells in brains in a sample of moribund mice ranged from 7.4 to 7.8. In the subsequent experiment, all five naive mice were alive at 22 days, but quite sick. Brain burdens of yeast cells in these mice were similar to those of moribund sham-infected mice in the first experiment.

At each time point, yeast cells in lungs and brains were enumerated. Curves for brain yeast burdens were generated in two separate experiments and are shown in Fig. 1. Seeding of yeast at 24 h after inoculation was equivalent in the sham-infected and untreated mice and in *Toxoplasma*-infected mice. However, at all subsequent time points, the brains of *Toxoplasma*-infected animals contained significantly fewer yeast cells than sham-infected or untreated controls. No significant differences in yeast cell numbers were found in the lungs of *Toxoplasma*- and sham-infected mice at any time. We conclude that mice infected with *T. gondii* limit the proliferation of intravenously inoculated *C. neoformans* cells which colonize their brains.

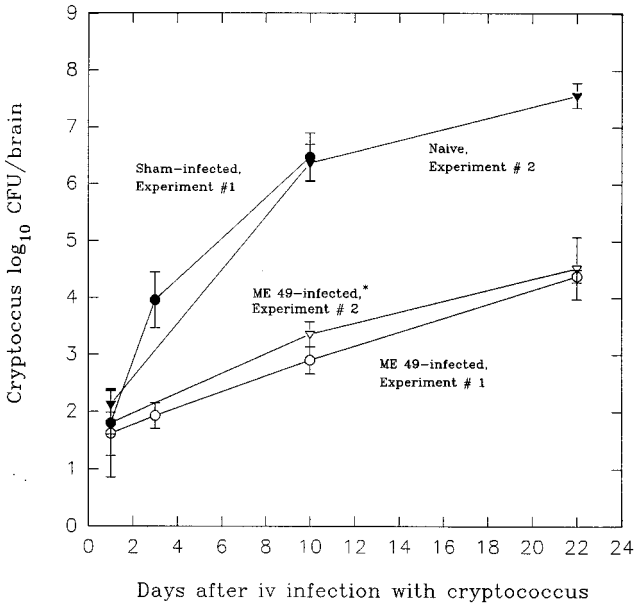

FIG. 1. *C. neoformans* infections in brains of *T. gondii*-infected, sham-infected, and naive B6 mice. Groups of B6 mice were infected with 20 ME49 toxoplasma cysts in a brain suspension i.p. in experiments 1 and 2 or treated with an equal volume of uninfected brain suspension in experiment 1 or left untreated in experiment 2. After 6 weeks, they were challenged with 2×10^4 strain 184 yeast cells intravenously (iv). Four or five mice from each group were killed at the time points indicated, and yeast CFU in their brains were enumerated. Bars indicate standard deviations. Mean $\log_{10}$ CFU are deemed significantly different when $P \leq 0.05$, and these points are identified in the text. *, in experiment 2, two of five mice died in the interval between days 10 and 22. The datum point given is for the remaining three mice, who were killed and analyzed at day 22.

CByB6F1 mice infected with *T. gondii* exhibit only limited resistance to subsequent infection with *C. neoformans*. Mouse strains differ in their response to infection with ME49. CByB6F1 mice, unlike B6 mice, are resistant to ME49 (9). That resistance is correlated with a relatively small number of cysts in their brains and a lower level of inflammation in brain tissue. B6 and CByB6F1 brains were harvested from three mice of each strain 6 weeks after i.p. inoculation with 20 ME49 cysts. Sections are shown in Fig. 2. B6 mice had from 1,000 to 5,000 cysts in their brains, while CByB6F1 mice had $\leq$200 cysts. It was difficult to find instances of inflammation in CByB6F1 brains. Histological examination revealed that B6 brains had consistently greater inflammation than did CByB6F1 brains. Meninges of B6 mice were more heavily infiltrated with mononuclear inflammatory cells, and the inflammation extended along perivascular spaces in the brain. Glial nodules were more common and larger in B6 mice.

We hypothesized that the resistance being studied involves an inflammatory response to *T. gondii*. If the inflammation present in the brain as a result of *Toxoplasma* infection has as its by-product rapidly expressed early resistance to cryptococcal brain infection, then that nonspecific resistance should be directly proportional to the severity of the *Toxoplasma* infection in the brain. Therefore, since brains of CByB6F1 mice are less affected by *Toxoplasma* infection than are those of B6 mice, CByB6F1 mice should be less resistant than B6 mice to secondary infection with *C. neoformans*.

To test this hypothesis, CByB6F1 mice were inoculated i.p. with 20 ME49 cysts or uninfected mouse brain in an experiment identical in design to that for which results are reported in Fig. 1. Both groups of CByB6F1 mice had similar numbers

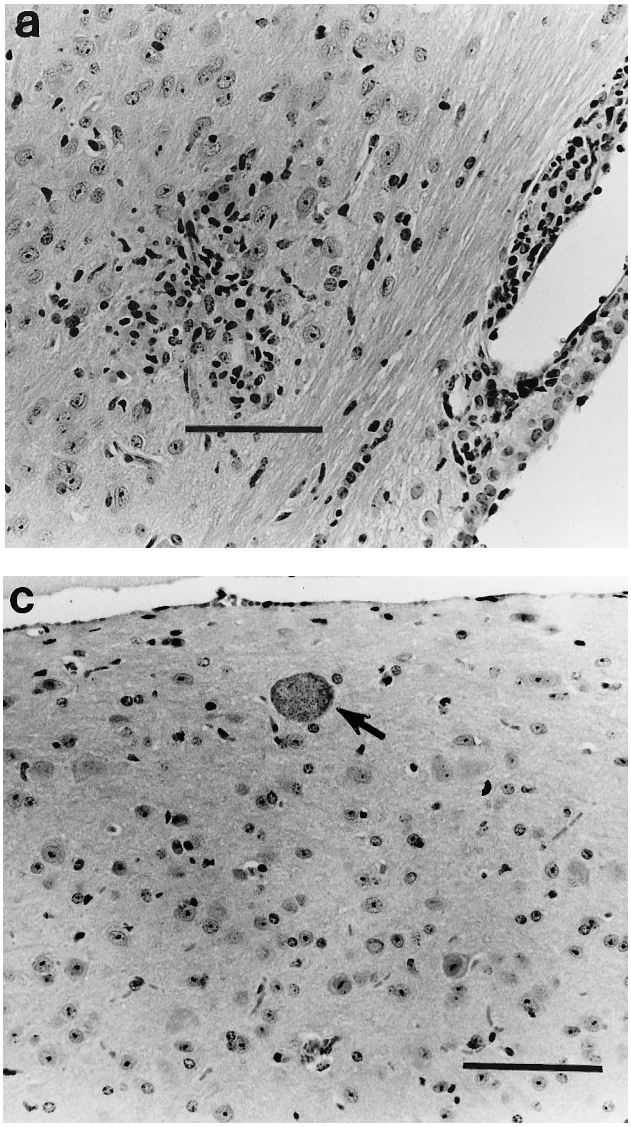

FIG. 2. B6 and CByB6F1 mouse brains after *Toxoplasma* infection. Three B6 and three CByB6F1 mice were infected with 20 ME49 toxoplasma cysts in a brain suspension i.p. After 6 weeks, they were killed and their brains were harvested and treated as described in Materials and Methods. (a) Section of B6 brain, showing meningeal inflammation and infiltration of parenchyma with inflammatory cells. Bar = 100 μm. (b) Section of B6 brain, showing perivascular cuffing. Bar = 200 μm. (c) Section of CByB6F1 brain, showing absence of an inflammatory response. The arrow indicates the presence of a rare *Toxoplasma* cyst. Bar = 100 μm.

of yeast cells in their brains 3 days after inoculation (Fig. 3). At subsequent time points, however, ME49-infected mice had significantly fewer yeast cells than control sham-infected mice. By day 22, two of five sham-infected control mice were dead. The $\log_{10}$ CFU of yeast cells in the brains of the remaining three mice averaged 7.5. ME49-immunized mice had 10-fold fewer yeast cells. As with B6 mice, there were no significant differences in lung burdens of yeast cells between groups A and B. Note that inherent strain-to-strain variation in response to *C. neoformans* results in only 5- to 10-fold differences in brain burdens in the two strains of sham-infected mice at any sampling time. However, the difference between yeast CFU in *T. gondii*-infected versus sham-infected mice within each strain while only 10-fold in CByB6F1 mice was 10,000-fold in B6 at 10 days after infection, when the resistance observed was maximal. Thus, although CByB6F1 mice exhibit significant resistance to yeast in the CNS, the resistance was less strong than that exhibited by B6 mice (Fig. 1). These data are consistent with the hypothesis that *T. gondii*-mediated resistance to *C. neoformans* infection in the brain is correlated with the severity of the inflammatory response to the primary pathogen.

Irradiation of mice prior to infection with *Toxoplasma* cysts increases the inflammatory response to *T. gondii* along with resistance to subsequent Cryptococcal infection. It is difficult to find cysts in the brains of CByB6F1 mice 6 weeks after inoculation of *T. gondii*. However, if at 24 h prior to ME49 inoculation mice are given 500 rads of whole-body gamma irradiation (which temporarily depletes lymphocytes), the in-

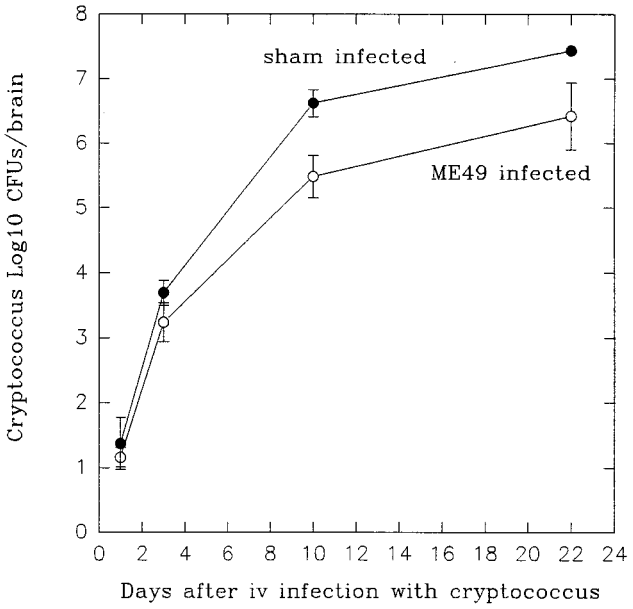

FIG. 3. *Cryptococcus* infections in brains of *T. gondii*-infected CByB6F1 mice. Experimental protocol and analysis were identical to those described for Fig. 1, experiment 1, but utilized CByB6F1 rather than B6 mice. iv, intravenous.

TABLE 1. After *T. gondii* infection, irradiated CByB6F1 mice show more intense inflammation than nonirradiated CByB6F1 mice[a]

Group	Histological findings				
	Meningeal inflammation	Perivascular inflammation	Glial nodules	Necrosis	Cysts
A	2	2	3	1.5	2
B	1	1	1	1	0

[a] Sections of brains 6 weeks after *T. gondii* ME49 inoculation of irradiated (group A) or unirradiated (group B) CByB6F1 mice. Tissues were evaluated blind for the degree of inflammatory change according to a 4-point scale: 1, the most minimal lesion observable; 2, mild; 3, moderate; 4, marked inflammatory response. The number of tissue cysts present was assessed by the same scoring system. Scores shown are medians of four (group A) and five (group B) mice.

fection is exacerbated (10), such that it is possible to detect about 300 to 400 cysts or more per brain, with some attendant inflammation. In contrast, very little inflammation is evident in ME49-infected, nonirradiated CByB6F1 control brains at 6 weeks.

To test more exhaustively the hypothesis that the host inflammatory response is responsible for acquired resistance to *C. neoformans* in this system, the following experiment was performed. First, inflammatory responses in brain tissue were scored in two groups of *Toxoplasma*-infected mice that were killed at 6 weeks after *T. gondii* infection, just prior to the yeast challenge of the other animals in this experiment (see below). These scores are compiled in Table 1. On histologic examination, irradiated CByB6F1 mice scored higher for every parameter of inflammation evaluated than did the nonirradiated mice, including meningeal infiltration, perivascular response, and number of glial nodules. The response in irradiated mice consistently involved a small number of polymorphonuclear leukocytes, which were observed in only one of five nonirradiated mice. Next, we challenged irradiated and nonirradiated *Toxoplasma*-infected mice along with irradiated and nonirradiated uninfected CByB6F1 mice intravenously with 2×10^4 184 yeast cells. Numbers of yeast cells in brains 11 days after inoculation are shown in Table 2, in which it can be seen that irradiated, *T. gondii*-infected mice had nearly 100-fold fewer brain yeast cells than did unirradiated, infected controls.

The experiment described above was repeated and yielded similar results and the following additional information regarding the effect of *T. gondii* infection on long-term survival of *Cryptococcus*-challenged mice. Among *T. gondii*-immunized mice, most (9 of 13) survived *Cryptococcus* challenge for more than 60 days, regardless of whether they were irradiated. In contrast, only 2 of 11 mice never infected with *T. gondii* survived this long. The difference in survival times was significant ($P < 0.01$, Mann-Whitney test).

TABLE 2. Irradiated *T. gondii*-infected CByB6F1 mice exhibit increased resistance to *C. neoformans* in their brains[a]

Group	Irradiation	ME49	Log_{10} yeast CFU in brain, day 11
A	Yes	Yes	$3.27 \pm 0.62^{a,b}$
B	No	Yes	5.00 ± 0.25^{a}
C	Yes	No	6.78 ± 0.16
D	No	No	6.84 ± 0.22

[a] Groups of five CByB6F1 mice were given 500 rads of whole-body radiation or left untreated. Twenty-four hours later, one group of irradiated and one group of unirradiated mice were given 20 ME49 cysts i.p. The other two groups were sham immunized. Six weeks later, all animals received 2×10^4 184 yeast cells intravenously. Mice were killed at 11 days, and yeast cells in their brains were enumerated. Results shown are means for five mice ± standard deviations. [a] $P \leq 0.01$ compared with group C. [b] $P \leq 0.01$ compared with group B.

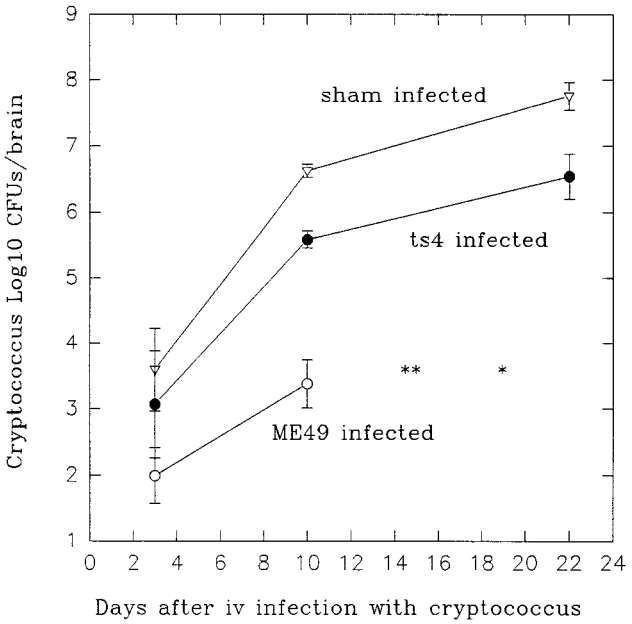

FIG. 4. *Cryptococcus* infections in the brains of ts-4-immunized B6 mice. Groups of B6 mice were infected i.p. with 20 ME49 toxoplasma cysts in a brain suspension, an equal volume of uninfected brain suspension, or 10^4 ts-4 organisms. After 6 weeks, all mice were challenged with 2×10^4 strain 184 yeast cells intravenously (iv). Five mice from each group were killed at the time points indicated, and yeast CFU were enumerated. Bars indicate standard deviations. Asterisks indicate mice that died in the intervals between time points. Statistical significance is discussed in the text, and mean $\log_{10}$ CFU are deemed significantly different when $P \leq 0.05$.

Taken together, these data further support our contention that a heightened inflammatory response to *T. gondii* in the brain results in heightened resistance to *C. neoformans* at that site.

Infection with non-cyst-forming *T. gondii* is associated with only limited resistance to *C. neoformans*. We sought to determine whether *Toxoplasma*-dependent resistance to *C. neoformans* is a direct result of specific immunity to *T. gondii* or instead is an indirect effect of *Toxoplasma*-induced brain inflammation. For this purpose we used ts-4 tachyzoites, which do not form brain cysts and are avirulent for immunocompetent mice but engender a strong immunity to subsequent challenge with ME49 cysts, or with tachyzoites of the RH strain of *T. gondii*, which are lethal for unimmunized mice.

Groups of 20 B6 mice were inoculated i.p. with (i) 20 ME49 cysts, as described above, (ii) uninfected brain, or (iii) 2×10^4 ts-4 tachyzoites. At 6 weeks postinoculation, all mice were challenged with 2×10^4 184 yeast cells intravenously, and yeast cells in brains and lungs were enumerated 3, 10, and 22 days later.

Three days after cryptococcal challenge ts-4-infected animals were not different from sham-infected animals (Fig. 4). Although ts-4-infected animals expressed significant resistance to *C. neoformans* in their brains at subsequent time points, the resistance was far less than that expressed by the ME49-infected animals. By day 22, all ME49-immunized mice had died, presumably of *Toxoplasma* infection, as discussed previously. Four remaining ts-4-infected animals were killed 70 days after intravenous infection. Three had no detectable yeast cells in their brains, and one had about 10^4 organisms.

TABLE 3. *Toxoplasma*-infected mice challenged with intratracheal *C. neoformans* exhibit limited resistance to yeast cells in their lungs but strong resistance to yeast cells in their brains[a]

Toxoplasma infected	Log_{10} yeast CFU/lung		Log_{10} yeast CFU/brain	
	6 wk	8 wk	6 wk	8 wk
Yes	6.2 ± 0.9	4.46 ± 1.9	2.2 ± 1.3	1.92 ± 0.9
No	6.4 ± 0.8	5.00 ± 1.6	4.8 ± 0.6	3.67 ± 1.5

[a] One group of 15 B6 mice were inoculated with 20 ME49 cysts i.p. A control group was left untreated. After 28 days, all mice were given sulfadiazine in their drinking water. Six weeks later, all animals were inoculated intratracheally with 10^6 strain 184 yeast cells. Five mice from each group were killed at each time point, and yeast cells in their lungs and brains were enumerated. Results shown are the means for five mice ± standard deviations. Differences are statistically significant for brain values only, at both time points examined, with $P \leq 0.01$.

As in the previous experiments, no significant differences were noted in lung yeast burdens at 3 days. However, lungs of ts-4-immunized animals at 10 and 22 days had about 10-fold fewer yeast cells than sham-immunized control animals.

These data suggest that substantial resistance to *C. neoformans* in the brain occurs in the presence of cerebral toxoplasma cysts and the inflammation that results from their sporadic rupture. There is, however, a limited resistance that is at comparable levels in the lungs and brains of ts-4-infected animals, which allows them to slow yeast proliferation somewhat at both sites.

Strong anticryptococcal resistance is observed in the brains but not the lungs of *Toxoplasma*-infected mice after intratracheal instillation of yeast cells. In the experiments described so far, only small differences were noted in lung yeast burdens between *Toxoplasma*-immunized and nonimmunized mice challenged intravenously with *C. neoformans*. However, 3 weeks after intravenous inoculation of 2×10^4 184 yeast cells, there were usually fewer than 1,000 yeast cells in the lungs. We were interested in observing the effect of prior exposure to *T. gondii* on severe pulmonary infections.

Groups of 12 B6 mice received either 20 ME49 cysts or uninfected brain i.p. Seven days later, both groups of mice were given sulfadiazine in their drinking water. This chemotherapy protects them against nonencysted *T. gondii* but does not affect the severity of the cryptococcal infection (see Materials and Methods). Sulfadiazine therapy allows long-term survival of ME49 infection by B6 mice, yet treated mice harbor encysted organisms, which represent potential chronic stimuli for inflammation. Six weeks after *Toxoplasma* inoculation, mice were challenged with 10^6 strain 184 yeast cells delivered intratracheally. Four mice from each group were killed at 6 days, 2 weeks, and 4 weeks after intratracheal infection, and yeast cells in the lungs and brains were enumerated.

Up to 4 weeks after intratracheal infection, lung yeast cell burdens were not significantly different between the two groups. The brains of 10 of the 12 sham-immunized animals contained a detectable number of yeast cells, ranging from approximately 30 (the limit of detection for this experiment) to approximately 100,000. The same numerical range of yeast cells was found in the 6 (of 12) *Toxoplasma*-infected mice in which yeast cells were detected.

To extend the previous observation to later time points, a second set of mice were treated as described above, but five mice per group were killed at 6 and 8 weeks after intratracheal inoculation. The results are shown in Table 3. At neither time point were significant differences in lung burdens detected. At 6 weeks, yeast cells were detected in only two of three brains from ME49-infected mice, each having approximately 100 organisms. In contrast, all five of the sham-infected mice had severe yeast infections in the brain (log_{10} CFU: 5.12, 5.16, 5.22, 4.75, and 3.78). At 8 weeks, three of four ME49-infected mice had no yeast cells in their brains, and one had a log_{10} CFU of 3.25. Four of five sham-infected mice, however, had significant levels of brain yeast cells (log_{10} CFU: 4.65, 3.62, 3.26, and 5.35).

These data are consistent with the earlier finding that acquired resistance to *C. neoformans* is localized in the cyst-bearing brains of *T. gondii*-infected mice, when that organ is compared with the lungs of the same animal.

DISCUSSION

Mice carrying a sufficient number of *Toxoplasma* cysts in their brains are resistant to subsequent infection with *C. neoformans*. The resistance acts to limit proliferation of yeast cells that colonize the brain. Increased levels of *T. gondii*-induced anticryptococcal resistance correlate with increased levels of inflammatory response to *T. gondii*. The *T. gondii*-induced anticryptococcal effect is localized to the brain, regardless of the route of cryptococcal inoculation.

When an immunocompetent individual is infected with *T. gondii*, the infection is typically asymptomatic. After an initial stage of tachyzoite proliferation, parasites encyst as bradyzoites. Cysts are found throughout the body, but exist in especially high concentration in the brain. Immunocompetent individuals appear, however, to have a protective mechanism which acts in the brain against the parasites which are released intermittently from ruptured cysts (5), since the incidence of toxplasmic encephalitis in immunologically normal persons is low. Histological and immunocytochemical studies have shown (3, 5) that cyst rupture is followed by a rapid influx of inflammatory cells, predominantly macrophages, and the development of microglial nodules. The phagocytic cells engulf and destroy the debris and parasites associated with the ruptured cyst.

Several lines of evidence demonstrate that maximal expression of *Toxoplasma*-induced anticryptococcal resistance correlates not with maximal expression of systemic specific anti-*Toxoplasma* resistance per se but rather with high levels of inflammatory response to parasites released from cysts in the *T. gondii*-infected brain. (i) *T. gondii*-infected B6 mice, carrying more cysts in their brains and possessing higher levels of inflammation than *T. gondii*-infected CByB6F1 mice, express far greater resistance to *C. neoformans* in their brains (Fig. 1 to 3). (ii) Irradiated, *T. gondii*-infected CByB6F1 mice, which have more brain inflammation than unirradiated controls, expressed far greater resistance to *C. neoformans*. (iii) ts-4-infected mice, which have no brain parasites to act as an inflammatory stimulus, exhibit only slight resistance to brain yeast cells, despite their strong *Toxoplasma*-specific immunity.

Toxoplasma-induced anticryptococcal resistance is differentially localized to the brain, judged by the small difference between lung burdens of previously *Toxoplasma*-infected and sham-infected mice that were inoculated intratracheally with large numbers of yeast cells. On the other hand, brain infections arising from yeast cells that escaped from the lungs were much better controlled by *Toxoplasma*-immunized than sham-immunized mice (Table 3). The far greater resistance in the brain than in the lung further supports the contention that the resistance is a direct result of inflammatory response to the *Toxoplasma* organisms in the brain, since it is widely accepted that lung burdens of *T. gondii* and attendant inflammation are far lower than those in the brain at late time points after inoculation (2, 17).

Brain tissues from *T. gondii*-infected, *C. neoformans*-challenged B6 mice and irradiated *T. gondii*-infected CByB6F1 mice were examined in an attempt to determine if an inverse spatial correlation exists between inflamed areas and cryptococcal foci. These attempts were unsuccessful, since in both cases anticryptococcal resistance held yeast cell proliferation to levels at which it is technically difficult to detect yeast cells in brain tissue, even with yeast-specific stains.

It is conceivable that any persistent irritant harbored within the brain would cause an inflammatory response sufficient to provide nonspecific protection against proliferation of yeast cells subsequently encountered. Manipulation of such effects might serve as feasible prophylaxis for individuals at risk for infection with *C. neoformans*.

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