Short Communication

Removal of *Toxoplasma gondii* Cysts from the Brain by Perforin-Mediated Activity of CD8⁺ T Cells

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Chronic infection with *Toxoplasma gondii* is one of the most common parasitic infections in humans. Formation of tissue cysts is the basis of persistence of the parasite in infected hosts, and this cyst stage has generally been regarded as untouchable. Here we provide the first evidence that the immune system can eliminate *T. gondii* cysts from the brains of infected hosts when immune T cells are transferred into infected immunodeficient animals that have already developed large numbers of cysts. This T cell-mediated immune process was associated with accumulation of microglia and macrophages around tissue cysts. CD8⁺ immune T cells possess a potent activity to remove the cysts. The initiation of this process by CD8⁺ T cells does not require their production of interferon- γ , the major mediator to prevent proliferation of tachyzoites during acute infection, but does require perforin. These results suggest that CD8⁺ T cells induce elimination of *T. gondii* cysts through their perforin-mediated cytotoxic activity. Our findings provide a new mechanism of the immune system to fight against chronic infection with *T. gondii* and suggest a possibility of developing a novel vaccine to eliminate cysts from patients with chronic infection and to prevent the establishment of chronic infection after a newly acquired infection. (Am J Pathol 2010, 176:1607–1613; DOI: 10.2353/ajpath.2010.090825)

Toxoplasma gondii is an obligate intracellular protozoan parasite capable of infecting many warm-blooded mam-

mals including humans. Acute infection is characterized by proliferation of tachyzoites and is known to cause various diseases including lymphadenitis and congenital infection of fetuses.¹ Interferon (IFN)- γ -mediated immune responses limit proliferation of tachyzoites, but the parasite establishes a chronic infection by forming cysts, which can contain hundreds to thousands of bradyzoites, primarily in the brain. Chronic infection with *T. gondii* is one of the most common parasitic infections in humans. It is estimated that 5×10^8 people worldwide are chronically infected with this parasite.² The tissue cysts remain largely quiescent for the life of the host, but can reactivate and cause life-threatening toxoplasmic encephalitis in immunocompromised patients, such as those with AIDS, neoplastic diseases and organ transplants.^{3,4} In immunocompetent individuals, recent studies suggested that *T. gondii* is an important cause of cryptogenic epilepsy,^{5,6} and is likely involved in the etiology of schizophrenia.^{7,8} The tissue cyst is not affected by any of the current drug treatments and it has been generally regarded as untouchable. However, the immune responses against *T. gondii* cysts remain largely unexplored.

Resistance to *T. gondii* is under genetic control in humans^{9,10} and mice.^{11,12} BALB/c mice are genetically resistant and have only small numbers of cysts in their brains at 2 to 3 months after infection.^{11,12} These mice may be able to prevent formation of cysts by efficiently controlling proliferation of tachyzoites during the acute stage of infection. However, it is also possible that the immune system of these animals has the capability to recognize *T. gondii* cysts and eliminate them from their brains. To examine whether immune cells have an activity to remove cysts that have already been formed in the brain, we adoptively transferred immune cells obtained

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from chronically infected BALB/c mice into infected, sulfadiazine-treated athymic nude or severe combined immunodeficient (SCID) mice, both of which lack T cells and developed large numbers of cysts in their brains. We present evidence for a potent capability of CD8⁺ immune T cells to eliminate *T. gondii* cysts from the brains through their perforin-mediated activity.

Materials and Methods

Mice

BALB/c-background IFN- γ -deficient (IFN- γ ^{-/-}), athymic nude, SCID, and wild-type BALB/c mice were obtained from The Jackson Laboratories (Bar Harbor, ME). Swiss-Webster mice were from Taconic (Germantown, NY). BALB/c-background perforin-deficient (PO) mice¹³ were kindly provided by John T. Harty (University of Iowa) and bred in our animal facility. Mouse care and experimental procedures were performed under pathogen-free conditions in accordance with established institutional guidance and approved protocols from the Institutional Animal Care and Use Committee. Female mice were used for all studies. There were three to seven mice in each experimental group.

Infection with *T. gondii*

Cysts of the ME49 strain were obtained from brains of chronically infected Swiss-Webster mice.¹⁴ All animals were infected with 10 (for cell donors) or 20 (for cell recipients) cysts perorally by gavage. Mice were treated with sulfadiazine in the drinking water (400 mg/L) beginning at 4 days (for IFN- γ ^{-/-} mice), 7 to 9 days (for nude and SCID mice), or 26 days (PO mice) after infection for the entire period of experiment to control proliferation of tachyzoites and establish a chronic infection. IFN- γ ^{-/-} mice also received pyrimethamine (1 mg/0.2 ml) perorally by gavage once daily for 3 days before sacrifice to help ensure that their spleen cell preparations would not contain tachyzoites.

Purification and Transfer of Immune T Cells

Spleen cells were obtained from chronically infected (infected for more than 6 weeks) IFN- γ ^{-/-}, PO and wild-type mice, suspended in Hanks' balanced salt solution (Irvine Scientific, Santa Ana, CA) with 2% fetal bovine serum (Sigma, St. Louis, MO). CD4⁺ and CD8⁺ T cell populations were purified by treating the spleen cells with magnetic bead-conjugated anti-mouse CD4 (GK1.5) or anti-mouse CD8 (53-6.7) monoclonal antibodies (Mitenyi Biotech, Auburn, CA).¹⁵ Total T cell population was purified by treating the spleen cells with both monoclonal antibodies.¹⁶ The purity of the T cell population in each of the preparations was >95%. A total of 0.7 to 1 × 10⁷ total T cells, or 2.1 to 3.5 × 10⁶ CD4⁺ or CD8⁺ T cells were injected intravenously into recipient nude or SCID mice at 3 and/or 4 weeks after infection in most experiments.

Quantifying Numbers of *T. gondii* Cysts in the Brains of Recipient Mice

Seven days or 1 month (36 to 39 days) after the transfer of T cells, a half brain of each of the recipient nude or SCID mice was triturated in 0.5 or 1 ml of PBS. Numbers of cysts in two or three aliquots (20 μ l each) of the brain suspensions were counted microscopically.

Semiquantitative Reverse Transcription-PCR for Detection of mRNA for Bradyzoite-Specific BAG1

Seven days or 1 month after the transfer of T cells, RNA was isolated from a half brain of each of the infected nude or SCID mice and cDNA was synthesized using the RNA as described previously.^{11,17} In the experiments with a transfer of CD8⁺ immune T cells from IFN- γ ^{-/-}, PO and wild-type BALB/c mice, the RNA were pretreated with DNase I (Invitrogen, Carlsbad, CA) to remove genomic DNA contaminating the RNA preparations, and then applied for cDNA synthesis. PCR for β -actin and BAG1 were performed with 1 to 5 μ l of diluted cDNA with GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) using 30 cycles of 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1 minute and 20 seconds to produce an amount of DNA within a linear range.^{11,17} The dilutions of cDNA were determined in preliminary studies using different amounts of cDNA of the sample. The primers were 5'-GGGGTGGAGTCGGTCTTAAT-3' (sense) and 5'-ATAACGATGGCTCCGTTGTC-3' (antisense) for BAG1, and 5'-AGCCATGTACGTAGCCATCC-3' (sense) and 5'-CTCTCAGCTGTGGTGGTGAA-3' (antisense) for β -actin. In one experiment, another set of primers for β -actin^{11,17} was also used. Ten-microliter aliquots of the final PCR mixtures were electrophoresed at 100 V for 1 hour on a 1.5% agarose gel and stained with ethidium bromide. The quantification of the PCR products was performed by densitometry analysis with Gel Logic 100 (Eastman Kodak, Rochester, NY) and normalized to the β -actin level.

Histopathology

Two days after transfer of 0.7 × 10⁷ normal or immune T cells, the infected recipient nude mice were euthanized and their brains were removed and immediately fixed in a solution containing 10% formalin, 70% ethanol, and 5% acetic acid. Five-micron-thick sagittal sections were stained with H&E. Immunoperoxidase staining using rabbit polyclonal anti-BAG 1 serum (kindly provided by Anthony Sinai of the University of Kentucky) was used for the detection of bradyzoites.¹⁸

Statistical Analysis

Levels of significance for numbers of cysts and the amount of BAG1 mRNA in the brains were determined by Student's *t*, Alternate Welch *t*-test, or Mann-Whitney test. Alternative Welch *t* or Mann-Whitney test was applied

when standard deviations were significantly different between groups tested. Differences which provided $P < 0.05$ were considered significant.

Results

Adoptive Transfer of Immune Spleen Cells Reduces Numbers of *T. gondii* Cysts in the Brains of Chronically Infected Nude Mice

BALB/c-background nude mice were infected and treated with sulfadiazine to establish a chronic infection.¹⁵ Animals received 2×10^7 immune spleen cells obtained from chronically infected BALB/c mice at 3 and 4 weeks after infection. At 1 day before the first cell transfer (Day -1), large numbers of *T. gondii* cysts were detected in their brains (Figure 1, A and B). Each cyst had a typical cyst wall (Figure 1B, arrow), indicating they are the cyst stage of the parasite, rather than bradyzoite-infected cells. At 1 month after the second cell transfer (Day 39), the numbers of cysts in the brain were compared between the mice that had received the immune spleen cells and the control animals that had not received the cells. The numbers of *T. gondii* cysts in the brains of control mice (Day 39) tended to be greater than those at Day -1 (Figure 1A). In contrast, the brain cyst numbers in the animals that had received the immune spleen cells were markedly fewer than those in the control mice at Day -1 and Day 39 ($P < 0.05$; Figure 1A). These results suggest that the immune cells have the capability to remove *T. gondii* cysts that have already formed in the brain of infected mice.

Immune T Cells Remove *T. gondii* Cysts from the Brains of Chronically Infected Mice

To determine the cell population that has an activity to remove *T. gondii* cysts, we examined whether T cells have the protective activity against cysts. Immune T cells purified from spleens of chronically infected BALB/c mice were transferred into infected, sulfadiazine-treated nude mice as described above. At 1 month after the second cell transfer (Day 36), numbers of cysts in the brains of the animals that had received immune T cells were significantly fewer than those observed in the brains of control mice without the cell transfer at the time of one day after the first cell transfer (Day 1) and Day 36 ($P < 0.01$; Figure 1C). In contrast, numbers of cysts in brains did not differ between Days 1 and 36 in the control mice that had not received any immune cells (Figure 1C). Consistent with the marked reduction of cyst numbers in the brains of animals that had received immune T cells, amounts of mRNA for bradyzoite (the stage of the parasite located within tissue cysts)-specific BAG1 were markedly less in the brains of mice that had received the T cells than those that had not received any cells (Figure 1D). The reduction of cyst number did not require 1 month after transferring T cells. Even 7 days after the cell transfer, numbers of cysts in the brains were significantly fewer in mice that

Figure 1. Immune spleen cells and T cells have the ability to reduce the numbers of cysts in the brains of recipient nude mice and the anti-cyst activity of immune T cells is associated with an accumulation of mononuclear cells on *T. gondii* cysts. Athymic nude mice were infected with 20 cysts of the ME49 strain perorally and treated with sulfadiazine¹⁵ beginning at 7 days after infection for the entire period of the observation. The animals received 2×10^7 of immune spleen cells (A) or 0.7 to 1×10^7 of immune T cells (C–G) obtained from chronically infected BALB/c mice at 3 and 4 weeks after infection. **A:** Numbers of cysts in the brains of infected nude mice at 1 day before the first cell transfer (Day -1) and 1 month after the second cell transfer (Day 39). As an additional control, euthymic BALB/c mice were infected, and numbers of cysts in their brains were examined at Day 39 without cell transfer. A half brain of each mouse was suspended in 0.5 ml of PBS and numbers of cysts in $60 \mu\text{l}$ (3 samples of $20 \mu\text{l}$ each) of the brain suspensions were counted microscopically. The data represent means $\pm$ SD. $*P < 0.05$ compared with control nude mice without cell transfer, Day -1 and Day 39. **B:** Representative cyst detected at Day -1. Each cyst detected had a typical cyst wall (arrow). **C:** Numbers of cysts in the brains of infected nude mice at one day after the first cell transfer (Day 1) and 1 month after the second cell transfer (Day 36). The data represent means $\pm$ SD. The data shown are representative of four independent experiments. $**P < 0.01$ compared with control nude mice without cell transfer, Day 1 and Day 36. **D:** Reverse transcription-PCR detecting mRNA for bradyzoite-specific BAG1 at Day 39. **E–G:** Accumulation of mononuclear cells on and within *T. gondii* cysts in the brains of infected nude mice at 2 days after a transfer of immune T cells (0.7×10^7 cells) ($n = 3$). **E** and **F:** H&E stain. Scale bar = $50 \mu\text{m}$. **G:** Immunostaining for BAG1 to detect bradyzoites (some are highlighted with arrows). **H:** A representative of *T. gondii* cysts observed in the brains of nude mice that had received normal T cells ($n = 2$) or those that had not received any T cells ($n = 2$) (H&E stain). At least five sections ($25 \mu\text{m}$ or more distance between sections) were examined on each brain sample.

Table 1. Immune T Cells Can Reduce Numbers of *T. gondii* Cysts within 7 Days in the Brains of the Recipient Nude Mice

Mice	Number of mice	Cells transferred	Number of cysts*
Nude	4	None	9, 8, 77, 12
Nude	4	Normal T cells [†]	10, 2, 8, 2
Nude	5	Immune T cells [‡]	0, 0, 1, 2, 0 ($P < 0.05$) [§]

*A half brain of each mouse was suspended in 1 ml of PBS and 40 μ l of the suspension was examined microscopically.

[†]Purified from uninfected BALB/c mice.

[‡]Purified from chronically infected BALB/c mice.

[§]When compared with the other two groups.

had received immune T cells than those in animals that had not received any T cells or those in animals that had received normal T cells ($P < 0.05$; Table 1). These results indicate that immune T cells have a potent activity to remove *T. gondii* cysts from the brains of mice within a short period of time, 7 days.

Immune T Cells Induce Accumulation of Microglia and Macrophages Around *T. gondii* Cysts in the Brain

Histological studies were performed on the brains of infected, sulfadiazine-treated nude mice at 2 days after receiving normal or immune T cells. An accumulation of mononuclear cells, primarily microglia and macrophages morphologically, around *T. gondii* cysts was observed in the brains of the recipients of immune T cells. We detected cysts partially covered (Figure 1E) or totally covered (Figure 1F) by microglia and macrophages. Immunostaining for bradyzoite-specific BAG1 showed that microglia and macrophages accumulated around the parasite positive for BAG1, indicating that the cyst stage of the parasite is the target of these phagocytes (Figure 1G). The immunostaining also showed that the accumulated phagocytes penetrated within the cyst (Figure 1G). Infiltration of T cells and microglia/macrophages was confirmed by immunohistochemistry detecting both CD3⁺ cells and F4/80⁺ cells adjacent to a *T. gondii* cyst (data not shown). We did not observe such cysts associated with accumulation of inflammatory cells in the brains of control mice that had received normal T cells or no T cells (Figure 1H). These results indicate that immune T cells have the activity to induce an accumulation of microglia and macrophages on *T. gondii* cysts in the brain. Accumulated microglia and macrophages appear to be the main effector cells that destroy *T. gondii* cysts and eliminate them from the brain after initiation of this process by immune T cells.

CD8⁺ Immune T Cells Possess a Potent Activity to Remove *T. gondii* Cysts from the Brain

We next examined whether CD4⁺ or CD8⁺ T cells, or both, have the activity against *T. gondii* cysts. For this purpose, we used SCID mice, which lack both T and B cells, as recipients of cell transfer. In this way, we can

exclude a possible T cell-assisted involvement of B cells in the cyst elimination. Infected and sulfadiazine-treated SCID mice received CD4⁺ or CD8⁺ T cells from either uninfected or infected BALB/c mice at 4 weeks after infection. Numbers of brain cysts in the control animals that had not received any T cells did not differ between one day before and 7 days after the cell transfer (Figure 2A). The brain cyst numbers in mice that had received normal CD8⁺ T cells tended to be a little fewer than those in the control animals without cell transfer but the difference did not reach statistical significance (Figure 2A). In contrast, the cyst numbers in the animals that had received the immune CD8⁺ T cells were markedly fewer (Figure 2A) when compared with the control animals without the cell transfer ($P < 0.005$) and those that had received normal CD8⁺ T cells ($P < 0.05$). Mice that had received either normal or immune CD4⁺ T cells from infected animals had fewer cysts than the control mice ($P < 0.05$), but cyst numbers in mice that had received CD4⁺ immune T cell were three times greater than those of recipients of CD8⁺ immune T cells (data not shown). In addition, there were no differences in the cyst numbers between the recipients of normal and immune CD4⁺ cells. These results indicate that immune CD8⁺ T cells have a potent activity to induce elimination of *T. gondii* cysts, and CD4⁺ T cells seem to possess a similar activity to a lesser extent. Reverse transcription-PCR semi-quantifying amounts of mRNA for bradyzoite-specific BAG1 confirmed a significant reduction of *T. gondii* cysts in the brains of mice that had received immune CD8⁺ T cells (Figure 2B; $P < 0.0005$).

IFN- γ Production by CD8⁺ T Cells Is Not Required for Their Protective Activity to Induce Removal of *T. gondii* Cysts from the Brain

For understanding the mechanism of CD8⁺ T cell-mediated elimination of the cyst stage of *T. gondii*, it is important to determine the function(s) of T cells required for this anti-cyst protective activity. IFN- γ has been shown to play the central role for controlling proliferation of tachyzoites during the acute stage of infection,^{19–21} and for preventing the development of toxoplasmic encephalitis caused by proliferation of tachyzoites in the brain during the later stage of infection.^{22–24} Therefore, this cytokine may also play an important role in the activity of immune CD8⁺ T cells against the cyst stage of the parasite. On the other hand, *in vitro* studies have shown that long-term culturing of *T. gondii* cysts can be accomplished within infected astrocytes in the presence of IFN- γ .²⁵ To examine whether IFN- γ plays an important role in the protective activity of immune CD8⁺ T cells against the cysts, infected, sulfadiazine-treated SCID mice received immune CD8⁺ T cells from either infected IFN- γ ^{−/−} or wild-type BALB/c mice at 3 weeks after infection. At 1 week after the cell transfer, cyst numbers were markedly fewer in the mice that had received immune CD8⁺ T cells from either wild-type or IFN- γ ^{−/−} donors than in the control animals that had not received any T cells (Figure 2C; $P < 0.0001$ and $P = 0.0001$, respectively). There were no differences

Figure 2. CD8⁺ T cells remove *T. gondii* cysts from the brains of recipient nude mice and their protective activity does not require IFN- γ but requires perforin. **A:** Numbers of *T. gondii* cysts and **(B)** the amounts of BAG1 mRNA in the brains of infected, sulfadiazine-treated SCID mice at 1 day before and 7 days after a transfer of normal or immune CD8⁺ T cells (3.5×10^6 cells) from uninfected or chronically infected BALB/c mice. The cell transfer was performed at 3 weeks after infection. **C:** Numbers of *T. gondii* cysts and **(D)** the amounts of BAG1 mRNA in the brains of infected, sulfadiazine-treated SCID mice at 1 and 7 days after a transfer of CD8⁺ immune T cells (2.1×10^6 cells) from infected IFN- $\gamma^{-/-}$ or wild-type BALB/c mice. **E:** Numbers of *T. gondii* cysts and **(F)** the amounts of BAG1 mRNA in the brains of infected, sulfadiazine-treated SCID mice at 1 day before and 7 days after a transfer of CD8⁺ immune T cells (2.1×10^6 cells) from infected perforin-deficient (PO) or wild-type BALB/c mice. All data represent mean $\pm$ SD. The data shown in **(E)** are representative of two independent experiments. * $P < 0.005$, ** $P < 0.0005$, *** $P = 0.0001$, **** $P < 0.0001$ compared with control nude mice without cell transfer, Day 7.

in numbers of brain cysts in the mice that had received wild-type or IFN- $\gamma^{-/-}$ CD8⁺ T cells (Figure 2C). The marked reduction of brain cysts in the recipients of these CD8⁺ T cell populations was confirmed by reverse transcription-PCR detecting BAG1 mRNA (Figure 2D; $P < 0.0005$). These results indicate that IFN- γ production by CD8⁺ T cells is not required for their protective activity to induce elimination of *T. gondii* cysts from the brain.

Perforin Is Required for the Protective Activity of CD8⁺ T Cells to Remove *T. gondii* Cysts from the Brain

Human and mouse CD4⁺ and CD8⁺ T cells are capable of lysing tachyzoite-infected target cells *in vitro* in a major histocompatibility complex-restricted manner.^{26–29} Although two studies have shown that perforin-deficient mice survive acute infection,^{24,30} perforin-mediated cytotoxic activity of CD8⁺ T cells may play a previously unappreciated but important role against tissue cysts during the later stage of infection. To address this possibility, we transferred immune CD8⁺ T cells from infected PO or wild-type mice into infected, sulfadiazine-treated SCID mice. At 1 week after the cell transfer, numbers of cysts were significantly less in the brains of animals that had received immune CD8⁺ T cells from wild-type donors than in control mice that had not received any T cells (Figure 2E, $P < 0.005$). In contrast, brain cyst numbers of animals that had received immune CD8⁺ T cells from PO

mice were equivalent to those of the control animals without cell transfer (Figure 2E). Reverse transcription-PCR detecting mRNA for BAG1 confirmed a significant reduction of brain cysts only in the recipients of wild-type CD8⁺ T cells (Figure 2F; $P < 0.0001$). These results indicate that perforin is required for the protective activity of CD8⁺ T cells to induce elimination of *T. gondii* cysts from the brain of infected mice. Thus, perforin-mediated cytotoxic activity of CD8⁺ T cells appears to be crucial to remove *T. gondii* cysts from the brains.

Discussion

The present study provides the first evidence for the capability of the immune system and CD8⁺ T cells to eliminate *T. gondii* cysts from the brains of infected hosts. Of importance, the anti-cyst activity of CD8⁺ T cells is mediated by perforin, not IFN- γ . Perforin is the major molecule that mediates cytolysis of target cells by CD8⁺ cytotoxic T cells. Since *T. gondii* cysts reside within infected cells,^{31,32} cytolysis of the cyst-containing cells by perforin-mediated cytotoxic activity of CD8⁺ T cells appears to induce removal of the cysts. Kim and Boothroyd³³ previously reported that when mice were infected intraperitoneally with tachyzoites engineered to constitutively express SRS9, a bradyzoite-specific antigen, in both tachyzoite and bradyzoite stages, spleen cells of the infected animals produced IFN- γ in response to SRS9 and that cyst numbers in their brains decreased

in later stage of infection. However, in their studies, it was not clear whether the decrease in cyst numbers was due to the immune responses or a shorter life span of cysts of the genetically engineered parasite. Blackwell et al³⁴ also reported that numbers of cysts in the brains of BALB/c mice gradually increased until 35 days after infection and thereafter decreased at 77 days after infection. These decreases in cyst numbers in the previous studies could be, at least in part, due to the perforin-mediated anti-cyst activity of CD8⁺ T cells observed in the present study. Ferguson et al³⁵ previously noted that brain cysts occasionally rupture in immunocompetent mice during the chronic stage of infection. The observed cyst ruptures may have been mediated by the anti-cyst activity of CD8⁺ T cells.

In resistance against *T. gondii*, the absolute requirement of IFN- γ to control tachyzoite proliferation during the acute stage of infection has been well documented.^{19–21} In contrast, perforin-mediated cytolytic activity of T cells was less appreciated before. Perforin-knockout mice survive acute infection as mentioned earlier.^{24,30} *In vitro* studies demonstrated that the lysis of tachyzoite-infected cells by cytotoxic CD8⁺ T cells results in release of viable parasites.³⁶ However, the situation of the parasite in the cyst stage seems different. Whereas the cyst resides within an infected cell^{31,32} as do tachyzoites, bradyzoites within the cyst are surrounded by a thick cyst wall, which is unique to the cyst. Therefore, cytolysis of cyst-containing cells by perforin-mediated activity of CD8⁺ T cells followed by quick accumulation of large numbers of microglia and macrophages would probably provide the parasite little time to escape from the coordinated attack by the T cells and phagocytes. Denkers et al³⁰ previously reported a three- to fourfold increase in brain cyst numbers in perforin-knockout mice in the later stage of infection. Although the mechanisms underlying the increased cyst numbers were unclear at that time, the absence of perforin-dependent anti-cyst activity of CD8⁺ T cells in these animals may have contributed to their observation.

By infecting C57BL/6 mice with ovalbumin-expressing *T. gondii*, Schaeffer et al³⁷ recently reported that ovalbumin-specific CD8⁺ T cells accumulated in regions containing isolated parasites (tachyzoites) but not around cysts in the brain. C57BL/6 mice are genetically susceptible to chronic infection with the parasite and develop large numbers of cysts in their brains. Therefore, it is possible that CD8⁺ T cells of these animals do not have a potent anti-cyst activity, in contrast to the T cells of genetically resistant BALB/c mice as observed in the present study.

During the T cell-mediated anti-cyst activity, microglia and macrophages accumulated on the cysts, and these phagocytes appear to be the main effector cells that destroy *T. gondii* cysts. IFN- γ has been shown to be the major activator of microglia and macrophages to prevent proliferation of *T. gondii* tachyzoites.^{19,38} Although the present study demonstrated that IFN- γ production by CD8⁺ T cells is not required to induce removal of *T. gondii* cysts from the brain, it is possible that IFN- γ produced by cells other than T cells contributes to activating microglia and macrophages for their anti-cyst activity. We previ-

ously reported that microglia and blood-derived macrophages produce IFN- γ in the brains of nude and SCID mice following *T. gondii* infection.^{39,40} NK cells are also known to produce this cytokine following infection.^{41,42} One cyst can contain hundreds to thousands of bradyzoites. During destruction of tissue cysts by perforin-mediated activity of CD8⁺ T cells and the phagocytes, a leak of bradyzoites could result in their conversion to tachyzoites. Although IFN- γ production by CD8⁺ T cells is not required for destruction of cysts as shown in the present study, IFN- γ production by CD8⁺ T cells might play a role in preventing subsequent proliferation of tachyzoites in surrounding cells (eg, astrocytes).⁴³ In this regard, we measured amounts of mRNA for tachyzoite-specific SAG1 in the brains of SCID mice that had received CD8⁺ T cells from IFN- γ ^{−/−} or wild-type mice at 7 days after cell transfer. We detected slightly higher amounts of SAG1 mRNA in the recipients of IFN- γ ^{−/−} CD8⁺ T cells than in those of wild-type CD8⁺ T cells but the difference was not statistically significant (data not shown). This point may need to be further analyzed.

The results in the present study indicate that perforin-mediated activity of CD8⁺ T cells can induce elimination of the cyst stage of *T. gondii* when the T cells are activated appropriately. This information opens a door for possible development of a novel vaccine to eliminate cysts from chronically infected individuals. This vaccine would also be able to prevent formation of tissue cyst and establishment of chronic infection with *T. gondii* following newly acquired infection. With combination of these two effects, we may be able to eradicate *T. gondii* infection, which is one of the most common parasitic infections of humans worldwide. The vaccine against the cyst stage of *T. gondii* appears to be a powerful strategy to fight against this parasite.

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