HHS Public Access

Author manuscript

Parasitol Int. Author manuscript; available in PMC 2022 April 01.

Published in final edited form as:

Parasitol Int. 2021 April ; 81: 102259. doi:10.1016/j.parint.2020.102259.

A pathway to cure chronic infection with *Toxoplasma gondii* through immunological intervention

Yasuhiro Suzuki[*]

Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, Lexington, KY, USA

Abstract

Toxoplasma gondii, an obligate intracellular protozoan parasite, can establish a chronic infection in the brain by forming tissue cysts. This chronic infection is widespread in humans worldwide including developed countries, with up to one third of the population being estimated to be infected with this parasite. Diagnosis of this chronic infection is usually conducted by serological detection of IgG antibodies against this parasite. Since infected individuals remain positive for these antibodies for years, it has generally been considered that this infection is a lifelong infection. It is also often considered that this chronic infection is "latent" or "quiescent". However, recent discovery of the capability of perforin-dependent, $CD8^+$ T cell-mediated immune responses to eliminate *T. gondii* cysts in collaboration with phagocytes illustrated dynamic interplays between *T. gondii* cysts and host immune system during this chronic infection. Importantly, the cytotoxic T cell-mediated protective immunity is able to remove mature cysts of the parasite. It is now clear that chronic *T. gondii* infection is not "latent" or "quiescent". Elucidating the mechanisms of the dynamic host-pathogen interactions between the anti-cyst protective immunity and *T. gondii* cysts and identifying the pathway to appropriately activate anti-cyst $CD8^+$ cytotoxic T cells would be able to open a door for eradicating *T. gondii* cysts and curing chronic infection with this parasite.

Keywords

Toxoplasma gondii; chronic infection; cyst; persistence; the protective immunity; $CD8^+$ cytotoxic T cells

1. Introduction

Toxoplasma gondii is a protozoan parasite that can infect all mammals including humans. The definitive host of this parasite is felines, and the parasite performs the sexual cycle in

[*]Corresponding author: yasu.suzuki@uky.edu (Y. Suzuki).

Conflict of interest statement

The author declares that he has no conflict of interest.

the intestines of the definitive hosts to generate oocysts that are shed into their feces [1]. Intermediate hosts including humans become infected by consuming food and water contaminated with the oocysts. During the acute stage of infection, tachyzoites proliferate within a variety of nucleated cells in various organs. IFN-γ-mediated immune responses are crucial to control the tachyzoite proliferation (1–4). However, even when the IFN-γ-mediated protective immunity becomes activated during the acute stage of infection, the parasite transforms into tissue cysts in various organs especially in the brain, heart, and skeletal muscle and establish a chronic infection (5, 6). The cyst is also a major form of this parasite that can transmit infection [5]. When animals prey upon meats of infected animals that contain *T. gondii* cysts, they become infected with this parasite. Humans can also be infected through this pathway when consuming undercooked meats of infected meat animals, such as pork and muttons [6]. In addition, tachyzoites can also transmit the infection when previously uninfected women become infected during their pregnancy, in which tachyzoites can go through the placenta and infect their fetuses [6].

The chronic infection with *T. gondii* is widespread in humans worldwide including developed countries [6]. The presence of IgG antibodies in serum is the standard method for diagnosis of this chronic infection [6], and up to one third of human population in the world is estimated to be infected with this parasite [6]. Infected individuals usually remain serologically positive for the IgG antibodies for years [6], and *T. gondii* cysts are detectable in the brains of these individuals [7, 8]. In addition, multiple studies reported *T. gondii* cysts are detected in immunocompetent mice infected for up to 22 months [9–11]. Therefore, it has often been considered that chronic infection with this parasite is a lifelong infection. However, we recently discovered the capability of the immune system to detect and eliminate *T. gondii* cysts using murine models [12, 13]. The advances in understanding the capability of the anti-cyst protective immunity supports the development of an immunological method to potently activate the protective immunity against *T. gondii* cysts and eradicate this chronic stage of the parasite to cure this widespread infection.

2. $CD8^+$ immune T cells possess the capability to promptly remove *T. gondii* cysts *in vivo*

In order to investigate whether the immune system possesses the capability to detect and eliminate *T. gondii* cysts, we performed an adoptive transfer of immune cells obtained from chronically infected immunocompetent mice to infected immunodeficient mice. In this study, athymic nude or SCID mice, which lack T cells, were first infected with *T. gondii* and treated with sulfadiazine to control tachyzoites proliferation and establish the cysts in their brains. Thereafter, these animals received a systemic transfer of the immune spleen cells containing T and B cells from chronically infected wild-type mice [13]. At one month after the spleen cell transfer, the cerebral cyst burden in the recipients became markedly less than control animals that had not received the immune cell transfer. Further studies revealed that the cyst removal occurs as quickly as within seven days after the cell transfer [13]. $CD8^+$ T cells are identified to be the cell population that has a potent capability to eliminate *T. gondii* cysts from the recipients [13]. The cysts are present within infected host cells, mostly in the neurons and astrocytes in the brain of infected hosts [9, 14]. The attack of *T. gondii* cysts in

the brain by $CD8^+$ T cells is supported by a recent study using a laser capture dissection reporting that neurons that directly interact with or harbor the cysts are in close contacts with $CD8^+$ T cells in the brains of infected mice [15].

3. $CD8^+$ immune T cells are able to eliminate *T. gondii* cysts beyond the genotypes of the parasite

The majority of *T. gondii* isolates from infected individuals in North America and Europe are one of three genotypes, types I, II, and III [16], in which type II is predominant and type III is also common in the isolates from immunocompromised individuals [17, 18]. Our studies using infections with types II and III strains revealed that $CD8^+$ immune T cells primed with either a type II or a type III strain are both able to efficiently remove cysts of a type II strain [19]. Similarly, type II-primed $CD8^+$ T cells can efficiently eliminate cysts of a type III strain [19]. Thus, $CD8^+$ T cells are capable of removing *T. gondii* cysts beyond the genotypes of the parasite by recognizing epitopes commonly expressed in types II and III strains or cross-reactive between these two genotypes. The amino-terminus region (amino acids 41–152) of dense granule protein 6 of *T. gondii* is identified to be a key target antigen of the parasite that $CD8^+$ T cells recognize to initiate anti-cysts immune process [20].

4. $CD8^+$ T cells utilize a perforin-mediated mechanism(s) to penetrate into *T. gondii* cysts

T cells secrete IFN-γ to activate both phagocytes and non-phagocytic cells such as fibroblasts to prevent intracellular tachyzoite growth [1–4]. In contrast, the anti-cyst activity of $CD8^+$ T cells does not require their IFN-γ [13]. Notably, the T cells employ perforin to display their anti-cyst activity [12, 13]. $CD8^+$ T cells deficient in perforin fail to reduce cyst numbers in infected SCID mice [13]. *T. gondii* forms tissue cysts within host cells. $CD8^+$ immune T cells recognize the cells harboring cysts and attach on the surface of these cells [12]. Importantly, immunohistological studies revealed the T cells located a half way through cyst wall, which composes the outer layer of the cysts, as well as the T cells that are located totally within the cysts. The presence of the T cells within the cyst was confirmed by Z-stack 3-dimensional images [12]. There were usually no other cells, or a few if any, detected on the surface of those cysts attached or invaded by $CD8^+$ T cells, suggesting that $CD8^+$ immune T cells are the first immune cell population that attacks the cysts. Notably, the penetration of $CD8^+$ T cells does not occur when the T cells lack perforin [12]. In contrast, an attachment of the perforin-deficient T cells on the surface of cyst-containing cells was frequently detected, indicating that perforin mediates the penetration of the T cells into *T. gondii* cysts after their attachment on the surface of cyst-harboring cells.

Morphological deterioration and destruction of cysts occur in the T cell-penetrated cysts, and granular structures intensely positive for granzyme B are detected within these cysts [9]. Granzyme B is one of the cytotoxic proteins that $CD8^+$ cytotoxic T cells secrete into the targets during their attack on the targets. Notably, many of these granzyme B staining co-localize with bradyzoites located within those destroyed cysts [12]. Since Granzyme B is a serine protease, this cytotoxic protein most likely contributes to killing of bradyzoites once

this enzyme is released from $CD8^+$ T cells that have invaded into the cysts. In addition, granzyme B has a pro-inflammatory activity, and in agreement, an accumulation of large numbers of $Iba1^+$ microglia and $Ly6C^+$ inflammatory macrophages are observed within and around these destroyed cysts. Most, if not all, of bradyzoites located within these destroyed cysts are detected within those accumulated microglia and macrophages. Portions of the *T. gondii*-positive materials located within these microglia and macrophages do not maintain a clear morphology of the parasite, suggesting that they have been destroyed within these phagocytes. Treatment of the T cell recipient mice with chloroquine, an inhibitor of endolysosomal acidification, inhibits the T cell-mediated removal of *T. gondii* cysts, indicating that phagosome-lysomose fusion is involved in the *T. gondii* cyst elimination [21]. Thus, the microglia and macrophages are most likely the scavenger cells that eliminate the bradyzoites once $CD8^+$ immune T cells invaded into the cysts and initiate anti-cyst immune process.

A large-scale comparison of mRNA levels for 734 immunity-related genes in the brains between infected SCID mice that received perforin-sufficient or -deficient $CD8^+$ immune T cells revealed that mRNA levels for only six molecules among the 734 gene tested are greater in the recipients of the perforin-sufficient T cells than the recipients of the perforin-deficient T cells [22]. Those six molecules include two T cell co-stimulatory molecules, inducible T cell costimulator receptor (ICOS) and its ligand (ICOSL), two chemokine receptors, C-X-C motif chemokine receptor 3 (CXCR3) and 6 (CXCR6), and two molecules related to an activation of microglia and macrophages, interleukin 18 receptor 1 (IL-18R1) and chitinase-like 3 (Chil3) [19]. Thus, it appears that ICOS-ICOSL interactions are crucial for activating $CD8^+$ cytotoxic immune T cells to initiate their perforin-mediated penetration into *T. gondii* cysts, and that CXCR3, CXCR6, and IL-18R are involved in recruitment and activation of microglia and macrophages to the T cell-attacked cysts for their elimination.

5. $CD8^+$ cytotoxic T cells is able to detect *T. gondii* cysts regardless of sizes and maturity of the cysts

The most of studies described earlier are performed by transferring $CD8^+$ immune T cells into infected and sulfadiazine-treated nude or SCID mice at 3 weeks after infection. It may be argued that the T cells can attack only early stage of cysts present in the hosts infected for only 3 weeks. However, our recent study demonstrated that this is not the case. In addition to the $CD8^+$ immune T cell transfer to SCID mice that have been infected for 3 weeks, the T cell transfer to SCID mice that have been infected for 6 weeks efficiently eliminated the cysts from the brains of the recipients (Fig, 1B) [22]. This evidence strongly suggests that $CD8^+$ immune T cells are able to recognize and eradicate not only early stage of *T. gondii* cysts but also mature stage of the cysts.

The thickness of the cyst wall is an indicator of the maturity of *T. gondii* cysts. The cyst wall thickness of the cysts detected in the brains of the SCID mice at each of 3 and 6 weeks after infection was both around 3 μm (Fig. 1D, left panes) [22]. Importantly, the cyst wall thickness of the cysts in the brains of SCID mice at each of these two times points after infection were equivalent to that of the cysts detected in the brains of CBA/J mice at 6 weeks

after infection [22]. Thus, the cysts present in the brains of infected SCID mice at each of 3 and 6 weeks after infection are mature cysts equivalent to those present in immunocompetent CBA/J mice infected for 6 weeks. This point is further supported by the evidence that diameters of the cysts detected in the brains of SCID mice at each of 3 and 6 weeks after infection does not differ from those of the cysts detected in the brains of the infected CBA/J mice (Fig. 1D right panel) [22]. Furthermore, the distributions of the sizes of cysts attached or invaded by T cells are equivalent to those of general cyst populations without the T cell association [13]. These evidences all together indicate that $CD8^+$ immune T cells are able to eliminate mature cysts of *T. gondii* from the brains of infected mice regard less of ages and sizes of the cysts.

Another possible argument could be that $CD8^+$ immune T cells can eliminate *T. gondii* cysts formed only in the brains of infected immunodeficient mice treated with sulfadiazine. However, in addition to the equivalent thickness of cyst wall and cyst sizes between the cysts formed in the brains of infected SCID and immunocompetent CBA/J mice as described above, an attachment and invasion of T cells into the cysts as well as destructions of cysts invaded by T cells are detectable in the brains of not only infected, sulfadiazine-treated SCID mice that received a transfer of $CD8^+$ immune T cells but also in the brains of infected CBA/J mice that did not receive any drug treatment including sulfadiazine (Fig. 1A) [13]. Thus, an attack and an elimination of *T. gondii* cysts by the immune T cells are the phenomena that naturally occur during the chronic stage of infection in immunocompetent hosts. Table 1 summarizes the evidence of the capability of the immune system to recognize and eradicate *T. gondii* cysts.

6. Evidence available in literatures that support the possibility of the capability of immunocompetent hosts to cure chronic infection with *T. gondii*

As mentioned earlier in the Introduction section, multiple previous studies showed that *T. gondii* cysts are detected in the brains of immunocompetent mice infected for up to 22 months [9–11]. However, a recent study reported that at least two strains of *T. gondii* became undetectable in the brains of mice at 20 months after infection [23], although we would need to be careful about the interpretation of this result because 1) the parasite strains used in this study had been maintained *in vitro* cultures, 2) the presence of *T. gondii* cysts at a earlier time point(s) of infection was not confirmed, and 3) the parasite used in the infection was genetically engineered to express mCherry and Cre recombinase [23]. In humans, it has been recognized that individuals serologically positive to Toxoplasma IgG antibodies remain seropositive for many years [6, 15]. Therefore, it is often considered that the infected individuals remain positive for the antibodies for lifelong period. However, a recent study reported two cases of congenital infection with the parasite who became negative for the IgG antibodies at later times of their lives [24]. These conversions from seropositive to seronegative for Toxoplasma IgG antibodies are not often reported. However, an occurrence of disappearance of the IgG antibodies in some limited individuals may suggest a possibility that *T. gondii* cysts can be targeted and eradicated once the immune system is appropriately activated. In these serological observations, it may be argued that these conversion from

seropositive to seronegative for Toxoplasma IgG antibodies could be, at least in part, due to insufficient sensitivities of serological tests to detect the antibodies [24]. On the other hand, it could also be argued that the persistence of Toxoplasma IgG antibodies is at least in part due to an occurrence of repeated infections with this parasite, not simply due to the persistence of *T. gondii* cysts. [24]. The conversions of seropositive to seronegative for the IgG antibodies may be more frequently detected in immunocompetent individuals when the individuals positive for the antibodies are followed up serologically in a consistent manner, for example every 6 months, for a long period of time.

7. Conclusion

It is well appreciated that chronic infection with *T. gondii* can reactivate and cause serious toxoplasmic encephalitis in immunocompromised individuals such as those with AIDS, neoplastic disease, and organ transplants [6]. Even in immunocompetent individuals, recent epidemiological studies reported increased incidences of brain cancers in Toxoplasma seropositive individuals [25–27]. Thus, it is critical to develop a method for eradicating *T. gondii* cysts from chronically infected individuals. However, there are currently no drugs available to target the cyst stage of this parasite. The recent advances in understanding the capability of the perforin-dependent, $CD8^+$ T cell-mediated protective immunity to eliminate *T. gondii* cysts along with the evidence of serological conversion from positive to negative for IgG antibodies against this parasite in a limited portion of infected immunocompetent individuals support a possibility of developing an immunological method to potently activate anti-cyst $CD8^+$ cytotoxic T cells for eradicating *T. gondii* cysts from chronically infected individuals and curing this widespread infection. Notably, this immunological approach will most likely be able to eliminate the mature cysts beyond multiple genotypes of the parasite.

Chronic infection with *T. gondii* was generally considered as a “latent” or “quiescent”. However, the capability of the protective immunity to recognize and eliminate the cysts illustrated that this chronic infection is not latent or quiescent but under active interplays between the host immune system and the parasite. Elucidating the mechanisms of these dynamic interactions between the $CD8^+$ cytotoxic T cell-mediated protective immunity and *T. gondii* cysts will be able to open a door for better understanding of the immunopathogenesis of chronic infection with *T. gondii* and developing an immunological intervention to efficiently activate the anti-cyst protective immunity and eradicate this widespread infection in the world.

Acknowledgements

The studies described in this review article were supported, in part, by NIH grants (AI095032, AI152597, AI134323, and AI136821). The author appreciates contributions from individuals in my laboratory who participated in the studies and numbers of collaborators who provided valuable assistance in our studies. Two postdoctoral scholars, Ashish Tiwari, Ph.D. and Jenny Lutshuma, Ph.D. contributed to many of our studies published in recent two publications [12, 22]. The author also appreciates the service provided by Markey Shared Resource Facility at University of Kentucky supported by P30CA177558. The images in Figure 1A are rearranged from Figures 2 and 3 of our previous publication, Tiwari, A, et al, Am. J. Pathol., volume 189: 1594–1607, 2019 [12] with permission from Elsevier. Figure 1B–1D are adapted from Figure 6 in in our recent publication, Lutshumba et al, mSystems, volume 5, 2020, e00189–20, DOI: 10.1128/mSystems00189-20 [22], Copyright © American Society for Microbiology.

References

1. Suzuki Y, Orellana MA, Schreiber RD, and Remington JS. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. Science 240 (1988) 516–518. [PubMed: 3128869]
2. Suzuki Y, Sa Q, Gehman M, and Ochiai E. Interferon-gamma- and perforin-mediated immune responses for resistance against *Toxoplasma gondii* in the brain. Expert. Rev. Mol. Med 13 (2011) e31. [PubMed: 22005272]
3. Munoz M, Liesenfeld O, and Heimesaat MM. Immunology of *Toxoplasma gondii*. Immunol. Rev 240 (2011) 269–285. [PubMed: 21349099]
4. Gazzinelli RT, Denkers EY, Sher A. Host resistance to *Toxoplasma gondii*: model for studying the selective induction of cell-mediated immunity by intracellular parasites. Infect. Agents Dis 2 (1993) 139–149. [PubMed: 7909708]
5. Dubey JP. The History and Life Cycle of *Toxoplasma gondii*, (2007) p 1–18. In Weiss LMaK K (ed), Toxoplasma gondii: The Model Apicomplexan Parasite: Perspectives and Methods. Elsevier, London, United Kingdom.
6. Montoya JG, and Liesenfeld O. Toxoplasmosis. Lancet 363 (2004)1965–1976. [PubMed: 15194258]
7. Remington JS, and Cavanaugh EN.1965. Isolation of the encysted form of *Toxoplasma gondii* from human skeletal muscle and brain. N. Engl. J. Med 273 (1965) 1308–1310. [PubMed: 5852454]
8. Alvarado-Esquivel C, Sanchez-Anguiano LF, Mendoza-Larios A, Hernandez-Tinoco J, Perez-Ochoa JF, Antuna-Salcido EI, Rabago-Sanchez E, and Liesenfeld O. Prevalence of Toxoplasma gondii infection in brain and heart by Immunohistochemistry in a hospital-based autopsy series in Durango, Mexico. Eur. J. Microbiol. Immunol 5 (2015) 143–149.
9. Ferguson DJ, Hutchison WM. An ultrastructural study of the early development and tissue cyst formation of *Toxoplasma gondii* in the brains of mice. Parasitol. Res 73 (1987) 483–491. [PubMed: 3422976]
10. Ferguson DJ, Graham DI, Hutchison WM. Pathological changes in the brains of mice infected with *Toxoplasma gondii*: a histological, immunocytochemical and ultrastructural study. Int. J. Exp. Pathol 72 (1991) 463–474. [PubMed: 1883744]
11. Pavesio CE, Chiappino ML, Setzer PY, Nichols BA. *Toxoplasma gondii*: differentiation and death of bradyzoites. Parasitol. Res 78 (1992) 1–9. [PubMed: 1584739]
12. Tiwari A, Hannah R, Lutshumba J, Ochiai E, Weiss LM, and Suzuki Y. Penetration of $CD8^+$ cytotoxic T cells into large target, tissue cysts of *Toxoplasma gondii*, leads to its elimination. Am. J. Pathol 189 (2019) 1594–1607. [PubMed: 31301754]
13. Suzuki Y, Wang X, Jortner BS, Payne L, Ni Y, Michie SA, Xu B, Kudo T, and Perkins S. Removal of *Toxoplasma gondii* cysts from the brain by perforin-mediated activity of $CD8^+$ T cells. Am. J. Pathol 176 (2010) 1607–1613. [PubMed: 20167872]
14. Ghatak NR, and Zimmerman HM. Fine structure of *Toxoplasma* in the human brain. Arch. Pathol 95 (1973) 276–83. [PubMed: 4348725]
15. Merritt EF, Johnson HJ, Wong ZS, Buntzman AS, Conklin AC, Cabral CM, Romanoski CE, Boyle JP, and Koshy AA. 2020. Transcriptional profiling suggests T cells cluster around neurons injected with *Toxoplasma gondii* proteins. mSphere 5 (2020).
16. Howe DK, and Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J. Infect. Dis 172 (1995) 1561–1566. [PubMed: 7594717]
17. Howe DK, Honore S, Derouin F, and Sibley LD. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J. Clin. Microbiol 35 (1997) 1411–1414. [PubMed: 9163454]
18. Ajzenberg D, Yera H, Marty P, Paris L, Dalle F, Menotti J, Aubert D, Franck J, Bessieres MH, Quinio D, Pelloux H, Delhaes L, Desbois N, Thulliez P, Robert-Gangneux F, Kauffmann-Lacroix C, Pujol S, Rabodonirina M, Bougnoux ME, Cuisenier B, Duhamel C, Duong TH, Filisetti D, Flori P, Gay-Andrieu F, Pratlong F, Nevez G, Totet A, Carme B, Bonnabau H, Darde ML, and Villena I.Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. J. Infect. Dis 199 (2009) 1155–1167. [PubMed: 19265484]

19. Ochiai E, Sa Q, Perkins S, Grigg ME, and Suzuki Y. CD8$^+$ T cells remove cysts of *Toxoplasma gondii* from the brain mostly by recognizing epitopes commonly expressed by or cross-reactive between type II and type III strains of the parasite. Microbes Infect (2016) 18: 517–522. [PubMed: 27083473]

20. Sa Q, Ochiai E, Tiwari A, Mullins J, Shastri N, Mercier C, Cesbron-Delauw MF, and Suzuki Y. Determination of a key antigen for immunological intervention to target the latent stage of T*oxoplasma gondii*. J. Immunol 198 (2017) 4425–4434. [PubMed: 28446567]

21. Sa Q, Tiwari A, Ochiai E, Mullins J, and Suzuki Y. Inducible nitric oxide synthase in innate immune cells is important for restricting cyst formation of *Toxoplasma gondii* in the brain but not required for the protective immune process to remove the cysts. Microbes Infect 20 (2018) 261–266. [PubMed: 29287983]

22. Lutshumba J, Ochiai E, Sa Q, Anand N, and Suzuki Y. 2020. Selective upregulation of transcripts for six molecules related to T cell costimulation and phagocyte recruitment and activation among 734 immunity-related genes in the brain during perforin-dependent, CD8+ T cell-mediated elimination of *Toxoplasma gondii* cysts. mSystems 5 (2020) e00189–20. [PubMed: 32291349]

23. McGovern KE, Cabral CM, Morrison HW, Koshy AA. 2020. Aging with *Toxoplasma gondii* results in pathogen clearance, resolution of inflammation, and minimal consequences to learning and memory. Sci. Rep 10 (2020) 7979. [PubMed: 32409672]

24. Rougier S, Montoya JG, Peyron F. 2017. Lifelong persistence of *Toxoplasma* cysts: A questionable dogma? Trends Parasitol 33 (2017) 93–101. [PubMed: 27939103]

25. Thomas F, Lafferty KD, Brodeur J, Elguero E, Gauthier-Clerc M, and Misse D. Incidence of adult brain cancers is higher in countries where the protozoan parasite *Toxoplasma gondii* is common. Biol. Lett 8 (2012) 101–103. [PubMed: 21795265]

26. Vittecoq M, Elguero E, Lafferty KD, Roche B, Brodeur J, Gauthier-Clerc M, Misse D, and Thomas F. Brain cancer mortality rates increase with *Toxoplasma gondii* seroprevalence in France. Infect. Genet. Evol 12 (2012) 496–498. [PubMed: 22285308]

27. Cong W, Liu GH, Meng QF, Dong W, Qin SY, Zhang FK, Zhang XY, Wang XY, Qian AD, and Zhu XQ. *Toxoplasma gondii* infection in cancer patients: prevalence, risk factors, genotypes and association with clinical diagnosis. Cancer Lett 359 (2015) 307–313. [PubMed: 25641340]

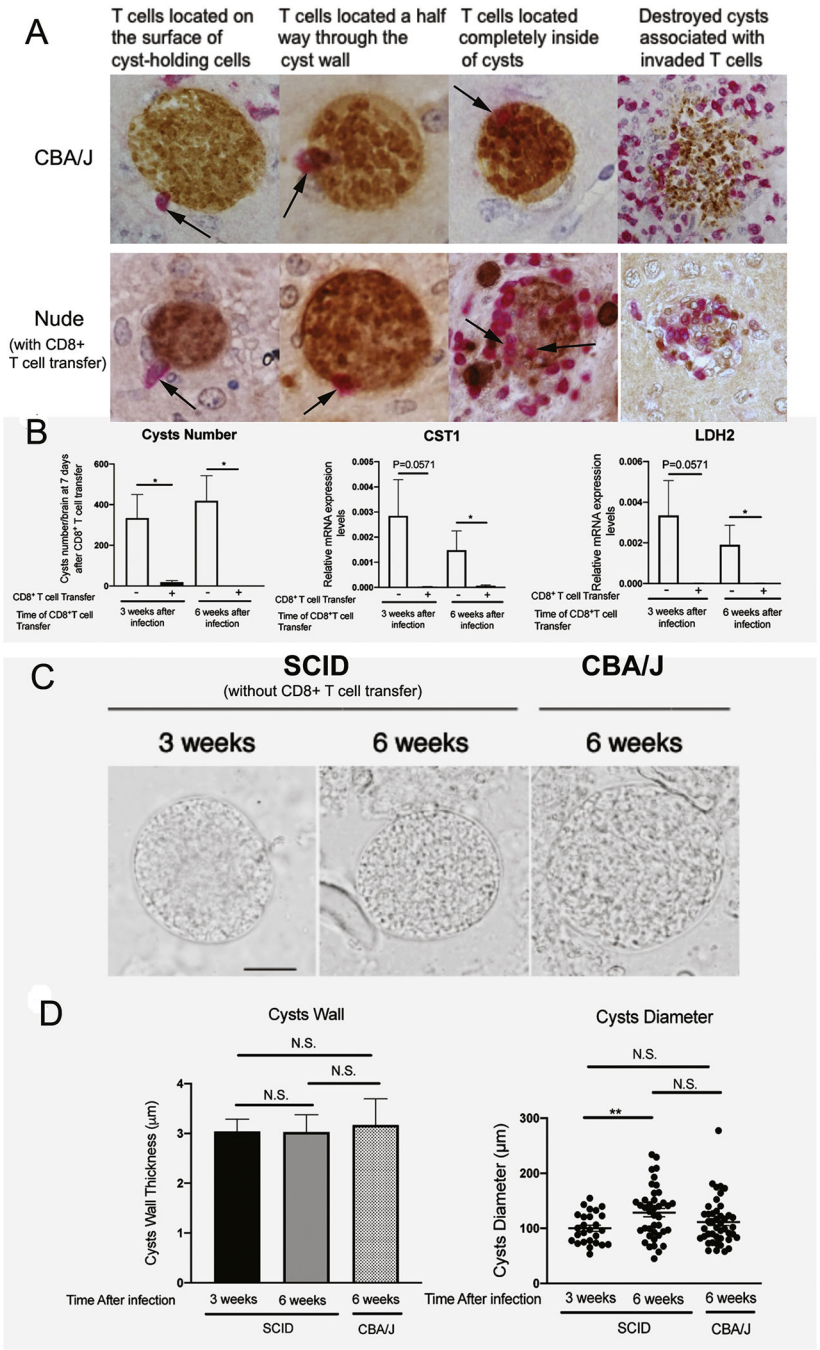

Figure 1.

The capability of $CD8^+$ immune T cells to recognize host cells harboring *T. gondii* cysts and eliminate the cysts. (A) Attachment of T or $CD8^+$ T cells on the surface of cyst-harboring cells, and the presence of these T cells located a half way through the cyst wall or located completely within the cysts in the brains of CBA//J mice infected for 6–8 weeks and athymic nude mice that received a systemic transfer of $CD8^+$ immune T cells (3.5×10^6 cells) at 3 weeks after infection (and treated with sulfadiazine to maintain the chronic infection beginning at 7 days after infection). T cells ($CD3^+$) are stained in red and *T. gondii* cysts are

stained in brown. Arrows in the figures indicates the T cells. (These images are rearranged from Figures 2 and 3 of our previous publication, Tiwari, A, et al, Am. J. Pathol., volume 189: 1594–1607, 2019 [12], with approval from Elsevier). (B-D) Infected and sulfadiazine-treated SCID mice received a transfer of $CD8^+$ immune T cells (1.8×10^6 cells) at either 3 or 6 weeks after infection. CBA/J mice were infected for 6 weeks. (B) Cyst numbers and mRNA levels for bradyzoite (cyst)-specific CST1 and LDH2 in the brains of the SCID mice at 8 days after the T cell transfers at either 3 or 6 weeks after infection. (C) Images of a representative of *T. gondii* cysts detected in the brain suspensions of SCID mice that had been infected for 3 or 6 weeks, and the brain suspension of CBA/J mice that had been infected for 6 weeks. A bar in the image at the left panel indicates 50 μm distance. All images are at the same magnification. (D) The thickness of cyst wall in the cysts (the left panel) and diameters of the cysts (the right panel) detected in brain suspensions from SCID mice infected for 3 or 6 weeks and CBA/J mice infected for 6 weeks. In the right panel, each dot indicates the size of each single cyst in the group. (B and D) Data shown are the mean ± SEM. In the figure of cyst diameter, each bar indicates the mean value in the group. $*P<0.05$, and $**P<0.01$. Figures in B-D are adapted from Figure 6 in in our recent publication, Lutshumba et al, mSystems, volume 5, 2020, e00189–20, DOI: 10.1128/mSystems00189-20 [22], Copyright © American Society for Microbiology

Table 1.

Evidence on the perforin-dependent, $CD8^+$ T cell-mediated protective immunity to eliminate the cyst stage of *Toxoplasma gondii*

Evidence	References
$CD8^+$ immune T cells have the capability to remove the cysts from the brains of infected mice	[12, 13, 19, 22]
Perforin is required for the anti-cyst activity of $CD8^+$ T cells	[12, 13]
$CD8^+$ T cells attach the surface of cyst-harboring cells and penetrate into the cysts in a perforin-dependent manner	[12]
Cysts penetrated by $CD8^+$ T cells show morphological deterioration and destruction	[12]
Granzyme B, a cytotoxic protein that cytotixic T cell secret, is detected in most of the destroyed cysts	[12]
Microglia and macrophages accumulate into and around the destroyed cysts, and most of bradyzoites located within the cysts are detected within these microglia and macrophages	[12]
Treatment of the T cell recipient mice with chloroquine, an inhibitor of endolysosomal acidification, inhibited the T cell-mediated removal of *T. gondii* cysts	[21]
$CD8^+$ T cells primed with a type II *T. gondii* are able to eliminate the cysts of both types II and III, and the T cells primed with a type III *T. gondii* remove the cysts of type II parasite	[19]
$CD8^+$ T cells are able to remove cysts present in mice infected not only for 3 weeks but also for 6 weeks, indicating the capability of the T cells to eliminate manure cysts	[22]
$CD8^+$ T cells are able to attack and eliminate mature tissue cysts regardless of their sizes	[12]
The penetration of T cells into the cysts and their destruction are detectable in the brains of not only mice following an adoptive transfer of $CD8^+$ immune T cells but also in the brains of chronically infected immunocompetent mice	[12]