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A study of Toxoplasma gondii cysts in mouse brains

Varunee Vongvichian*
Medhi Kulkumthorn* Toshihide Fukuma**

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A study of Toxoplasma cysts in mouse brains was carried out by inoculating the proliferative form of T. gondii intraperitoneally in female albino mice which were sacrificed at 14, 21 and 28 days after inoculation. Only two of the mice sacrificed at day 28 had infections, one with 42 cysts and the other 254 cysts. All 296 cysts collected during the experiment were examined and classified in to four stages of development: early, mature, old and ruptured. The formation and degeneration of the cyst wall, the morphology of the cystozoites inside and outside the cysts were found to be related to the stages of development.

Key word : *Toxoplasma gondii cyst.*

Reprint request : Vongvichian V, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
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* Department of Parasitology, Faculty of Medicine, Chulalongkorn University.
** Department of Parasitology, Kurume University, School of Medicine, Kyushu 830, Japan.

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การศึกษาซีสของท๊อกโซพลาสมา กอนดิไอ ในสมองหนู ทำโดยฉีดระยะ proliferative form ของ Toxoplasma gondii เข้าไปในช่องท้องหนูขาว แล้วตรวจซาก ในวันที่ 14, 21 และ 28 หลังจากนั้นนำเนื้อสมองมาทำ impression smear ย้อมสี Giemsa แล้วตรวจด้วยกล้องจุลทรรศน์แสงกำลังขยาย 1000 เท่า พบว่าหนูที่ถูกตรวจซาก ในวันที่ 28 มีเพียง 2 ตัวเท่านั้นที่พบซีส ตัวแรกพบเพียง 42 ซีส เป็น mild infection ตัวที่ 2 พบ 254 ซีส เป็น heavy infection ได้ศึกษาซีสทั้งหมดจำนวน 296 ซีส และจัดแบ่งออก เป็นระยะ early, mature, old และ ruptured cyst ศึกษา formation และ degeneration ของผนังซีส และรูปร่างลักษณะของ cystozoites ภายในและนอกซีส ซึ่งสัมพันธ์กับระยะต่าง ๆ ของซีส

Toxoplasma gondii is a coccidian parasite which has world-wide distribution. The cat is the definitive host of this parasite, but many warm blooded animals, including man,can serve as intermediate hosts. The parasite exists in two forms: i.e. as a proliferating trophozoite and in an encysted form.[1] During the proliferative phase, T. gondii can multiply rapidly extra- or intra-cellularly by endodyogeny. Following the immune response of the host, the parasite enters a chronic phase, forming tissue cysts which prefer the central nervous system, skeletal tissue and cardiac muscle. Virulent strains of T. gondii can cause rupture of the host cells within 3-4 days due to unrestricted multiplication, and can kill the host.[2-4] In contrast, avirulent strains can remain dormant as tissue cysts for years or even throughout the life of the host producing no evident symptom.Until now, many cases of cerebral toxoplasmosis were reported in man specially in patients with AIDS.[5-7] Datry et al.[6] 1984, demonstrated both trophozoites and cystic form of Toxoplasma gondii from the cerebral biopsy of two patients with AIDS by a simple laboratory technique. Navia et al.[7] in 1986 reported that chronic infection of T. gondii in the brain causes morbidity and mortality in patients suffering from the acquired immune deficiency syndrome (AIDS). Though, up to now, the colonization and development of T. gondii as cyst form in the brain of immunocompetent hosts is little known,[3, 8, 9] our purpose is to clearify various stages of cyst development, the morphology and relationship of the cysts and cystozoites by light microscope.

Materials and Methods

Tachyzoites of the RH strain of Toxoplasma gondii preserved in liquid nitrogen at the Department of Protozoology, Institute of Tropical Medicine, Nagasaki University were used in our study. They were thawed in a waterbath by gradually increasing the temperature to 45 °C. Under a Normanski's interference microscope, the parasites were counted with a hemocytometer. About 5 X 10^5 tachyzoites in phosphate buffered saline (PBS) were intraperitoneally inoculated in 12 female albino mice (aged five weeks and 0.5 ml of PBS were injected intraperitoneally in each of three control mice. The mice were divided into three groups. Each group of four infected mice and one control mouse were killed and their brains examined at day 14, 21 and 28 respectively, following incoulation.

The brain parts containing the olfactory lobe,cerebrum and cerebellum were removed. Small pieces of brain were dissected out and gently pressed onto glass slides, air dried, fixed in absolute methanol and stained with Giemsa. All slides of stained brain smear were studied and photographed under a light microscope.

The cysts were counted in number and differentiate to be the early, mature, old and ruptured cyst by considering the diameter of the cysts, the arrangement and the activeness of the cystozoites together with the formation of the cyst wall.

A small cyst with average 18 mu in diameter, about 80 active cystozoites surrounded with thin cyst wall is considered to be an early cyst. A cyst of moderate size (average 36 mu in diameter), about 420 active cystozoites surrounded with well-formed cyst wall is considered to be a mature cyst, while a cyst of moderate size with more or less in active, poor arranged, smaller cystozoites is considered to be an old cyst.

Result

Of the five mice comprising each of the three study groups, four mice. infected with T. gondii and one control mouse were killed at day 14, 21 and 28, respectively, following the date of inoculation. The brain smears of the infected mice sacrificed at day 14 and 21 post-inoculation showed no evidence of the presence of T. gondii in either the proliferative form or the cyst form. However, in the last group of four mice infected with T. gondii the one control mouse, two of them were negative for parasites in the brain (no pathological changes were noted), but the other two infected mice which seemed to be quite healthy, had cysts : one mouse showed a mild infection with 42 cysts and the other a heavy infection with 254 cysts. Intracellular parasites were observed within both inflammatory cells (macrophages and neutrophils) and neural cells. The parasites were present within a parasitophorous vacuole in the host cytoplasm and they had started to construct the cyst wall while the cystozoites inside the cyst wall propagated by endodyogeny to form the cysts in various stages of development: early, mature, old and ruptured cysts. The cystozoites released from ruptured cysts has morphologically changed to the proliferative form, penetrating into new host cells; they were starting to form the very early cysts. A total of 296 cysts were studied in detail under the oil-immersion objective of the light microscope as shown in Table 1.

Table 1. Stages and the number of cysts in mouse brains infected with Toxoplasma gondii.

Infected mouse	Stage of cyst	Number of cysts	Total number of cysts
No.1	Early	6	
	Mature	22	
	Old	12	42
	Ruptured	2	
No.2	Early	41	
	Mature	143	
	Old	52	254
	Ruptured	18	

Note : Infected mice No.1 and No.2 were sacrificed at day 28 following inoculation with T. gondii.

The early cysts (Figs 1, 2, 3, 4) were newly formed cysts, rather round or oval, about 10-24 mu in diameter, with 20-180 cystozoites inside the parasitophorous vacuole in the host cell cytoplasm. Very few infected host cells had apparent nuclei. The size of the cysts and the cystozoites inside the early and mature cysts seemed to be related, as shown in Table 2. The bigger the cysts were, the greater was the number of cystozoites inside them. Six early cysts of mouse No.1 and 41 early cysts of mouse No.2 were 10-24 mu in diameter (average 18 mu), with 20-180 cystozoites (average 80 cystozoites) inside them. Twenty-two mature cysts of mouse No.1 and 143 mature cysts of mouse No.2 were 20-58 mu in diameter(average 36 mu) with 200-1,100 cystozoites (average 420 cystozoites) inside them. Some cysts were quite difficult to identify as early cysts or mature cysts because of their overlapping sizes. However, the morphology of the cysts and the number of cystozoites inside them together with the arrangement of the cyst wall were considered in the identification process. For example, a cyst, 25 mu in diameter with 240 cystozoites inside it and having a well- surrounded cyst wall was identified as a mature cyst.

Table 2. The relationship between the size of the cyst and the number of cystozoites inside early and mature cysts in the brain of two infected mice.

Stage of cyst	Number of cysts	Size of cysts (diameter in mu)	Number of cystozoites inside the cysts
Early	47	10 - 24 (average 18)	20 - 180 (average 80)
Mature	165	20 - 58 (average 36)	200 - 1100 (average 420)

* Because the relationship between the size of the cysts and the number of cystozoites inside the old cyst and the ruptured cysts is not a reliable redality, no attempt was made to measure the diameter of such cysts or count the cystozoites in the ruptured cyst.

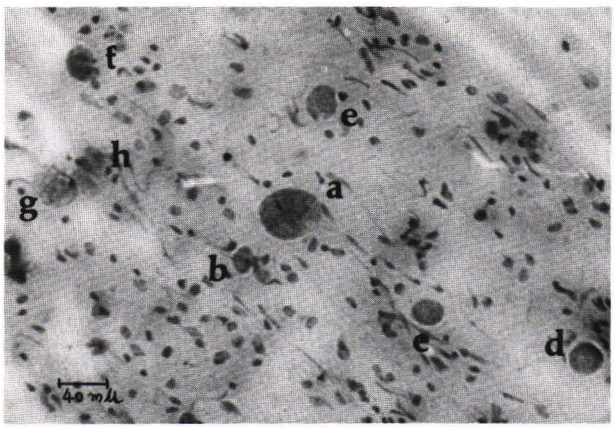

Figure 1. Eight cysts in one field (by 10 x objective) of brain smear.

The biggest one (a) is a rupture cyst with degeneration of the cyst wall at one area while near by is the smallest early cyst (b) as magnified in Fig 2. Four other mature cysts of medium size (c, d, e, f) are with well-formed cyst wall. The other 2 cysts in which the bigger one (g) is another ruptured cyst with loosly packed cystozoites inside while besides is an smaller early cyst (h) as magnified in Fig 3.

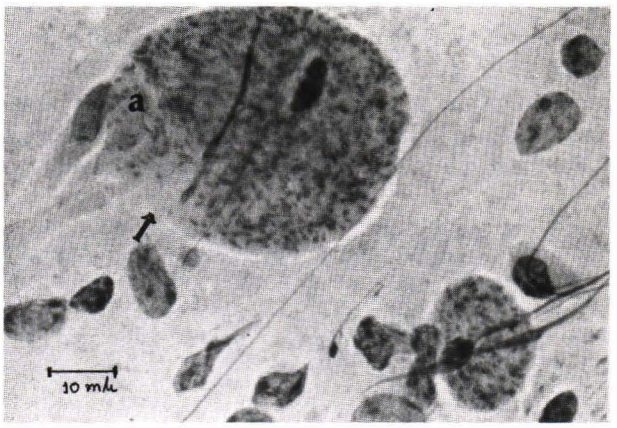

Figure 2. Two cysts of different sizes:-

The bigger one is the ruptured cyst showing degeneration of the cyst wall at one side (arrow). The cystozoites are loosly packed at that area (a) than the other areas in the cyst indicating the certain amount of cystozoites has already flown out. Dividing zoites are quite very rare among 720 cystozoites in this cyst of about 42 x 49 mu in diameter. The smaller one is the early cyst, 18 x 22 mu in diameter, surrounded with distinct cyst wall and with about 140 cystozoites inside.

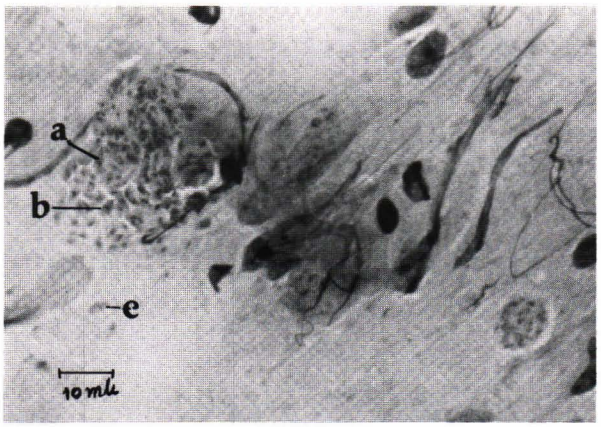

Figure 3. Two cysts in comparison.

The bigger one is the late ruptured cyst on its burst, note that the surrounding cyst wall has disappeared completely. All cystozoites disperse out freely into the brain tissue, among them are many ovoid and stumpy dividing cystozoites (a, b, c) with nuclei at about the centre. The smaller one is the very early cyst, 10 x 11 mu in diameter, 24 cystozoites inside and the cyst wall is only half evident, seems to be under construction.

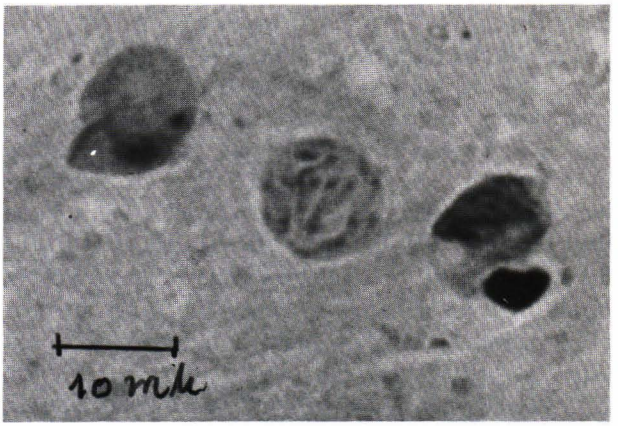

Figure 4. A very early cyst:-

The smallest cyst among all 296 cysts, 10 x 10 mu in diameter and 20 cystozoites inside. Most cystozoites are cresent-shaped with nuclei at the subterminal end. Cyst wall is evidently, partly surrounded, no nucleus of the host cell is seen.

The size of the cyst and the number of cystozoites inside was significantly related only in the early and mature cysts but not in the case of old and ruptured cysts. In the old cysts, most of the cystozoites showed degeneration of the cells, no definite shape, and the the cytoplasmic membrane having no clear limitation; only nuclei were evident but they were not compact. Dividing cystozoites were rarely identified (Fig 6).

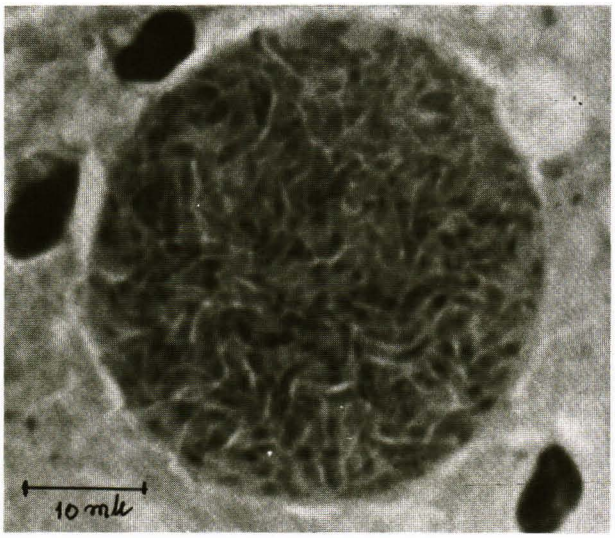

Figure 5. A full grown mature cyst, 35 x 37 mu in diameter containing about 440 cystozoites tightly packed and surrounded with well-formed cyst wall. Most cystozoites are cresent shaped with nuclei at the subterminal end, still some dividing zoites can be seen.

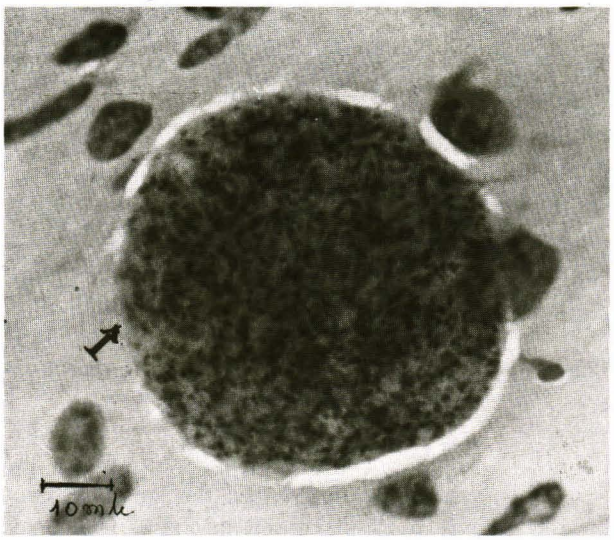

Figure 6. An old cyst with 53 x 58 mu in diameter and about 1100 cystozoites inside. The cyst shows very distinct cyst wall more than half of the cyst but very faint cyst wall on the remaining (arrow). It seems that the cyst is about to be burst out at the area the degeneration of cyst wall appear. Cystozoites are highly compacted, poor arranged in which dividing zoites are difficult to be identified.

In the cases of ruptured cysts (Figs. 3, 7, 8 ,9) there was no relation ship between the size of the cystsand the cystozoites inside them; this was pasticulerly in old cysts (Fig9). It was cause difficulties in measuring the diameter of the cyst, because the gradual degeneration of the cyst wall. And because the cystozoites had already started dispersing out of the ruptured cyst, the number of the zoites remaining inside was not an accurate reflection of the number of cystozoites that had been in the cyst; this was especially so in very old cysts. In very old cysts such as in Fig.9 almost all cystozoites were cresent in shape, with subterminal nuclei becoming the active proliforative form.

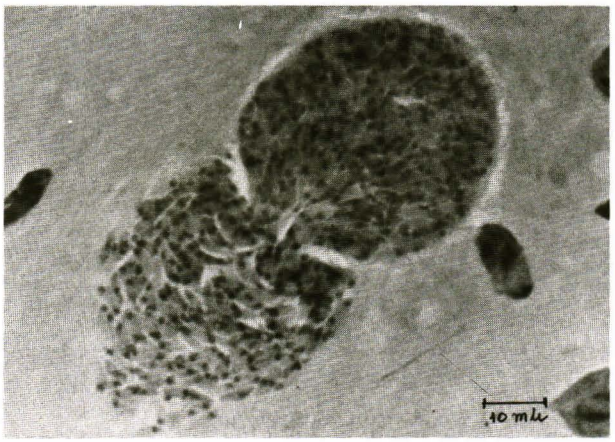

Figure 7. A ruptured cyst at its burst

The cyst of 41 x 43 mu in diameter containing about 480 cystozoites inside is just bursting out, releasing the zoites through the narrow area of the torn cyst wall. About one-third of all zoites released from the cyst are still packed together indicating its newly burst. Most of them are cresent shaped with subtermina lnuclei. Many dividing zoites can be seen.

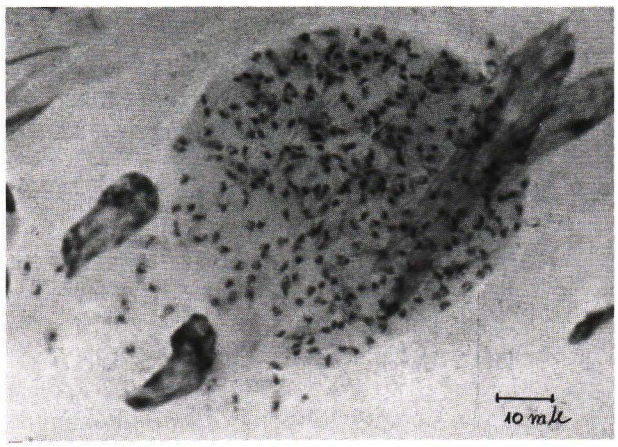

Figure 8. An old ruptured cyst

A ruptured cyst of 56 mu in diameter with about 300 cystozoites inside and outside the cyst. The surrounding cyst wall is too fainted and has disappeared completely specially at the area the cystozoites released out. The zoites inside the cyst are loosly packed because most of them had already released out long before. Some dividing zoites still can be seen.

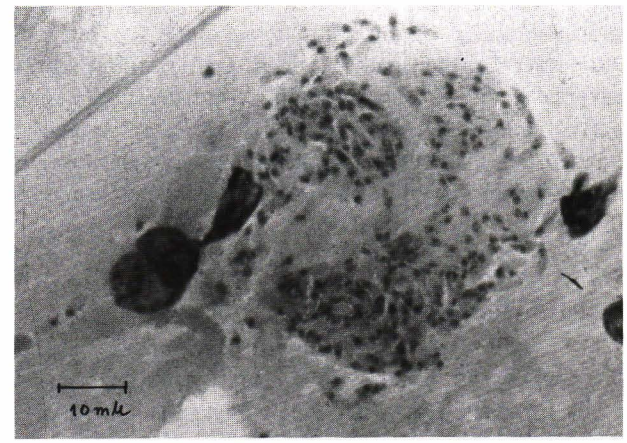

Figure 9. A very old ruptured cyst

A ruptured cyst with more than half of cystozoites released out, surrounded with fainted, torn cyst wall. Cystozoites dispersed at all direction around the cyst into the mouse brain tissue, morphologically changed to become active proliferative trophozoites, long and slender cresent shaped with subterminal nuclei.

Discussion

Usually RH strains of Toxoplasma gondii are virulent enough to cause death in infected mice three or four days following their inoculation.[2-4, 10] In our experiment, out of 12 mice intraperitoneally inoculated, only two were infected: one having a mild infection with 42 cysts in the brain while the other was heavily infected with 254 cysts in the brain. This might have been because the parasites had been preserved in liquid nitrogen for about two years prior to inoculation, which was long enough to decrease their virulence. Nonetheless, two mice developed an infection. The total number of 296 cysts in these mice was sufficient to explain the stages of development of the cysts, the formation and degeneration of the cyst wall, the relationship between the size of the cysts and the number of cystozoites inside early and mature cysts, and the features of ruptured cysts.

Dividing cystozoites inside cysts could also indicate the stages of development of the cyst, as they were prevalent in the early and mature cysts but very rare in old cysts. The capacity of the cystozoites inside the cysts to propagate resulted in an increase in the size of the cysts and their number. When the increase in size of the cysts and number of cystozoites inside them ceased, the zoites gradually degenerated, becoming old cysts. When the cystozoites propagated too much, then the mature cyst burst becoming a ruptured cyst; numerous cystozoites were released, their morphology changed into a cresent shape. Thereafter, they started to penetrate into new hosts.

The fine structure of cystic form of T. gondii were studied by electron microscopy[8, 9, 11, 12] that early tissue cyst, developing cyst and ruptured cyst were shown. Ferguson and Hutchison, 1987,[9] showed an early cyst with about 14 mu in diameter containing at least 20 cystozoites by TEM which might be as the same stage of a very early cyst of about 10 mu in diameter and 20 cystozoites inside in our study by light microscope, 100 x as shown in Fig. 4 Also a tissue cyst of 31.5 mu in diameter containing loosely packed cystozoites as shown by TEM[9] might be as the same stage as an old ruptured cyst in our study by light microscope, 100 x as shown in Fig.8. The detail of cyst wall was also described by Ferguson and Hutchison, 1987.[9] Which was in different point of view as in our study that could demonstrate the rupture of the cyst wall while studying by TEM might be not able to show. According to Datry et al, 1984,[6] the isolate and extracellular trophozoites were 5 to 8 mu long, cresent shaped with a big, slightly eccentric nuclei as similar to the zoites released from a ruptured cyst shown in Fig.7 in our study. That might be the active proliferative form of T. gondii which were ready to penetrate into the new host cell, forming the cyst form again. In the case of cyst form Datry et al[6] also found as the same as our study that the size of the cyst varied in relation to the number of parasites they contained. But that might be only in the early and mature cyst not in the old cyst.

However, our study of T. gondii cyst in mouse brain using the simple technique as Datry et al, 1984[6] might lead to the diagnosis of cerebral toxoplasmosis in man in detail. That the finding the early, mature and ruptured cysts indicate the active phase of the parasites and the progress of the disease while finding the old cyst indicated the innert stage of the disease.

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