ELSEVIER

International Journal for Parasitology 28 (1998) 1019–1024

The Australian Society for Parasitology
INTERNATIONAL Journal for PARASITOLOGY

Advances in the life cycle of *Toxoplasma gondii*

J.P. Dubey

U.S. Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Parasite Biology and Epidemiology Laboratory, BARC-East, Bldg. 1040, Beltsville, Maryland 20705-2350, U.S.A.

Received 3 Novembr 1997; in revised form 22 January 1998; accepted 22 January 1998

Abstract

This paper reviews recent studies on the life cycle of *Toxoplasma gondii*. Tachyzoites, bradyzoites, and sporozoites are the three infectious stages of *T. gondii*. Humans and animals become infected mainly by ingesting bradyzoites or oocysts. After ingestion, both bradyzoites and sporozoites convert to tachyzoites inside tissues. The conversion of tachyzoites to bradyzoites and bradyzoites to tachyzoites is of biological and clinical significance because bradyzoites are less susceptible to chemotherapy and reactivation of bradyzoites to tachyzoites is considered the cause of fatal toxoplasmosis in AIDS patients. Of all the methods currently available to assess stage conversion of *T. gondii*, feeding infective stages to cats is the most reliable method. Felidae, the definitive hosts of *T. gondii* excrete oocysts 3–10 days after ingesting tissue cysts/bradyzoites, $\geq$18 days after ingesting oocysts, and $\geq$13 days after ingesting tachyzoites. Published by Elsevier Science Ltd.

Keywords: *Toxoplasma gondii*; Toxoplasmosis; Life cycle; Tachyzoites; Bradyzoites; Sporozoites; Tissue cysts

1. General biology of *T. gondii*

Infections by *Toxoplasma gondii* are widely prevalent in humans and animals. The ingestion of food or water contaminated with oocysts from cat faeces or the ingestion of tissue cysts in undercooked meat are the two major ways of postnatal transmission of *T. gondii* [1]. After infection with any infective stage, tachyzoites multiply in a variety of cells and eventually encyst in several tissues, particularly in the brain [1, 2]. Tissue cysts persist for a long time, perhaps for the life of the host. It has been hypothesised that tissue cysts rupture occasionally and the released bradyzoites are killed in immunocompetent hosts. However, in immunosuppressed hosts, such as AIDS patients, bradyzoites released from tissue cysts may multiply locally and spread to other organs. Encephalitis is the predominant clinical manifestation of toxoplasmosis in AIDS patients and is believed to be due to reactivation of latent infections [3, 4]. The mechanism of reactivation of toxoplasmosis is unknown. It is not known whether bradyzoites from older tissue cysts can directly give rise to new tissue cysts or have to go through the tachyzoite stage first. Bradyzoites are less susceptible to chemotherapy that is effective against tachyzoites. Therefore, the fate of bradyzoites in host tissues is of clinical significance.

Recently, there has been great interest in studying conditions needed for stage conversion of *T. gondii*, principally between tachyzoites and bradyzoites and vice versa [5–7].

Several criteria are used to distinguish tachyzoites from bradyzoites. Structurally, tachyzoites have a centrally located nucleus, few or no PAS-positive granules and are found during acute infection, whereas bradyzoites have a terminally located

S0020-7519/98 $19.00 + 0.00. Published by Elsevier Science Ltd. Printed in Great Britain
PII: S0020-7519(98)00023-X

nucleus, many PAS-positive granules, are enclosed in a resistant cyst wall and are more prevalent during the chronic phase [2]. However, the transitional stages between tachyzoites and bradyzoites and vice versa are not well defined structurally or antigenically.

2. Life cycle in cats

Bioassay in mice and cats is one of the methods used to trace the development of *T. gondii*. The life cycle of *T. gondii* in the definitive host (Felidae) differs from that in the intermediate hosts (human, mouse). Felidae excrete *T. gondii* oocysts in faeces 3 to 10 days after ingesting bradyzoites, ≥ 18 days after ingesting of sporulated oocysts, and ≥ 13 days after ingesting tachyzoites (Fig. 1). Of these, the bradyzoite-induced cycle in cats is the most efficient because nearly all cats fed tissue cysts shed oocysts whereas $<30\%$ of cats fed tachyzoites or oocysts shed oocysts [2]. Unlike many other coccidia, oocysts of *T. gondii* are less infective and less pathogenic in the definitive host (cat) as compared with intermediate hosts (mice, pigs, humans). Cats fed 10 oocysts did not become infected whereas one oocyst of the same lot was infective for mice and pigs [8, 9]. Millions of oocysts are produced because of profuse multiplication of *T. gondii* in the feline intestine, usually without clinical signs [10].

Only the bradyzoite-induced cycle in cats has been studied in detail [10]. After ingestion of bradyzoites, five morphologically distinct asexual types are formed in feline enterocytes eventually, leading to a sexual cycle resulting in the production of oocysts. The oocyst- and tachyzoite-induced cycles are unknown except that the prepatent period to oocyst shedding is longer and unpredictable compared with the bradyzoite induced infections. It is hypothesised that after oocyst ingestion, *T. gondii* invades many cat tissues and bradyzoites produced in extra intestinal tissues return to the intestine to initiate the bradyzoite-induced coccidian cycle [11]. Because tissue cyst rupture is considered infrequent, the complete coccidian cycle occurs in only a few cats after ingestion of oocysts.

3. Oocyst-induced cycle in the intermediate host after oral ingestion

After the ingestion of oocysts, mice can die of severe enteritis before other organs are severely parasitised [12]. Recently, we studied the early migration and development of sporozoites in mice fed oocysts [13, 14]. Sporozoites were found in

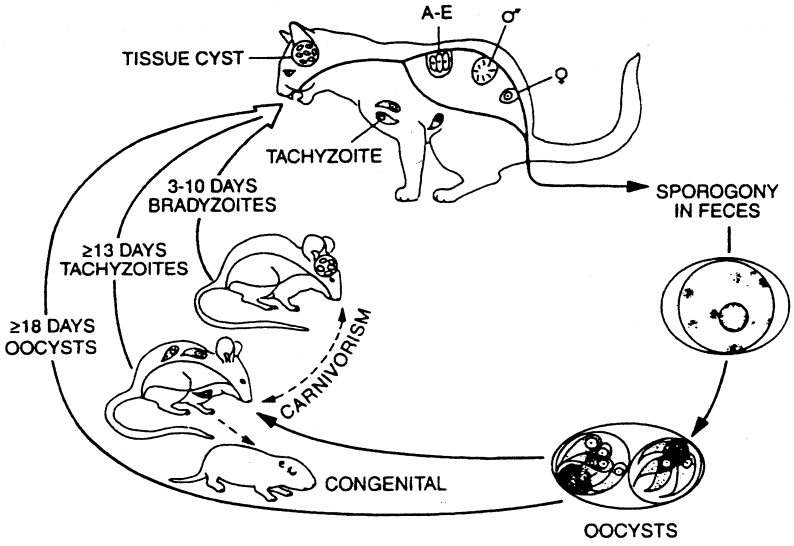

Fig. 1. Life cycle of *T. gondii* in cats.

sections of the small intestinal enterocytes within 30 min after oocyst feeding. Within 2 h of ingesting oocysts, most sporozoites had formed a parasitophorous vacuole (PV) in enterocytes and a few had entered the lamina propria cells. At 6 h p.i. sporozoites were mainly in the lamina propria and infected capillary endothelial cells, macrophages, plasma cells, lymphocytes, neutrophils, eosinophils, smooth muscle cells, and fibroblasts [13, 14]. Sporozoites developed in the lamina propria cells but not in the enterocytes they initially entered. Most sporozoites had converted to tachyzoites in the lamina propria by 12 to 18 h p.i. The enterocytes became infected 48 to 72 h p.i. from tachyzoites originating from the lamina propria. In some mice, parasitaemia was detected as early as 4 h p.i., but more consistently at 48 h p.i. By 3 days after inoculation, several extra-intestinal organs were infected. Results of bioassays of murine organs in cats indicated that bradyzoites were formed between 6 and 7 days after infecting with oocysts (Fig. 2). Depending on the dose and strain of *T. gondii*, mice fed oocysts died of enteritis during the first week, of pneumonia during the second week, and of pneumonia and encephalitis during the third week. Encephalitis was the main lesion in mice that died 4 weeks after ingesting oocysts [13].

TIME POST FEEDING	DEVELOPMENTAL STAGES
0.5 - 4 Hours	Sporozoites excyst, penetrate enterocytes, parasitaemia
	↓
6 - 12 Hours	Sporozoite convert to tachyzoites, divide into two in intestinal lamina propria cells
	↓
≥ 2 Days	Enterocytes and other cells parasitised by tachyzoites
	↓
6 Days	Parasites in brain
	↓
7 Days	Bradyzoites formed

Fig. 2. Proposed developmental cycle of *T. gondii* in tissues of mice fed oocysts.

4. Bradyzoite-induced cycle in intermediate hosts

The fate of bradyzoites after feeding tissue cysts or bradyzoites was traced in mice. Compared with oocyst-induced infections, *T. gondii* bradyzoites were less infective and less pathogenic to mice infected orally, irrespective of the dose or strain of *T. gondii* given [15]. Consistent infections were induced only by feeding 1000 infective bradyzoites to mice. These results indicate that a proportion of bradyzoites are destroyed in the lumen of the gut. Bradyzoites converted to tachyzoites in the small intestinal lamina propria within 18 h after ingestion of bradyzoites. Tachyzoites, not bradyzoites, migrated to extraintestinal organs. Parasitaemia was not detected until 24 h p.i. Results of bioassays in cats indicated that bradyzoites formed in tissues of mice between 5 and 6 days after bradyzoite ingestion (Fig. 3).

Little is known of site specificity of *T. gondii*. Although *T. gondii* multiplies in a variety of cells in the body, there appears to be some site specificity. In mice fed bradyzoites and oocysts, most organisms

TIME POST FEEDING	DEVELOPMENTAL STAGES
	Tissue cysts/bradyzoites fed
	↓
2 Hours	Penetrate enterocytes
	↓
	Enter intestinal lamina propria cells
	↓
18 Hours	Divide
	↓
	Tachyzoites
	↓
24 Hours	Parasitaemia
	↓
≥ 4 days	Invasion of lungs, brain, other organs
	↓
≥ 6 days	Tissue cysts/bradyzoites

Fig. 3. Proposed developmental cycle of *T. gondii* in tissues of mice fed tissue cysts/bradyzoites.

entered the tissues via enterocytes in the distal half of the small intestine [13, 15].

5. Resistance of *T. gondii* tachyzoites and bradyzoites to pepsin and trypsin digestion

Biologically, bradyzoites differ from tachyzoites. Bradyzoites are resistant to gastric digestion and thus are infectious orally whereas tachyzoites are destroyed by gastric juice [16]. However, there are conflicting reports with respect to the susceptibility of tachyzoites to acid pepsin digestion [2, 17–22].

The effect of trypsin and pepsin on *T. gondii* tachyzoites in vitro and in vivo was re-examined recently because sensitivity to these enzymes is used to distinguish tachyzoites from bradyzoites. Tachyzoites of the RH strain were incubated in vitro at 37°C in an acid pepsin solution for various times and then their infectivity was then bioassayed in mice. Although tachyzoites were often killed by exposure to acid pepsin solution for 30 min, occasionally even some extracellular tachyzoites of *T. gondii* survived in acid pepsin for 2 h in vitro [23].

For in vivo studies, mice were inoculated orally with tachyzoites of the RH strain of *T. gondii* obtained from the peritoneal exudates of i.p. inoculated mice and doses of 1000 extracellular tachyzoites of *T. gondii* were found to be infective [23]. Additionally, cats fed tachyzoites of an oocyst producing strain (VEG) became infected and shed *T. gondii* oocysts. Therefore, infectivity of *T. gondii* by the oral route should not be used as a criterion to distinguish tachyzoites from bradyzoites [23]. The oral infectivity of tachyzoites observed might explain the recent case of toxoplasmosis in a breast milk fed child whose mother had recently acquired toxoplasmosis [24]. Riemann et al. [25] had reported toxoplasmosis in a child fed unpasteurised goat milk.

6. Formation and persistence of tissue cysts in animals

Tissue cysts/bradyzoites are an integral part of the life cycle of *T. gondii*. Tissue cysts predominate during chronic infection but they are produced early in infection. In mice, tissue cysts were formed between 2 and 3 days after parenteral inoculation with tachyzoites [2]. The formation of bradyzoites was slightly delayed after oral ingestion of oocysts or bradyzoites. Tissue cysts were formed between 5 and 6 days after ingesting bradyzoites [15] and between 6 and 7 days after ingesting oocysts [13]. Functionally and structurally, early tissue cysts-/bradyzoites were more susceptible to digestion by gastric juice [13, 15, 26] and the bradyzoites in young tissue cysts were not tightly packed compared with those in older tissue cysts.

The location and number of tissue cysts in animals differed with hosts and the strain of *T. gondii*. In mice and rats, more tissue cysts were found in the brain than in visceral tissues, irrespective of the strain given. However in higher mammals (cattle, cats, sheep, goats) more tissue cysts were present in muscular tissues than in the brain [26, 27].

7. Comparison of in vivo and in vitro studies

The recent availability of stage stage-specific antibodies, and molecular markers and techniques has made it possible to study the mechanism of stage transformation of *T. gondii* [28–36]. One of the bradyzoite-specific antibodies against the BAG-5 antigen has been used to study the development of bradyzoites in vitro and in vivo. Weiss et al. [35], using the BAG-5 antibody, found that tissue cysts had formed by 3 days after p.i. of human foreskin fibroblasts with bradyzoites of the ME-49 strain; it appears that some bradyzoites directly formed tissue cysts without conversion to tachyzoites. Using a mAb specific for the tissue cyst wall, Halonen et al. [31] found tissue cysts in human foetal neuronal culture beginning at 2 days p.i. with the ME-49 strain. However, when bradyzoites were inoculated into mice by any route, the minimum period to form biologically functional tissue cysts was 6 days [2]. In contrast to the results from in vitro studies, all bradyzoites had converted to biologically defined tachyzoites by 18 h p.i. and organisms had become BAG-5 negative by 48 h p.i. [15]. BAG-5 positivity was seen again 5 days after ingestion of bradyzoites [15].

Another example of different results from in vitro

and in vivo studies was revealed by the development of *T. gondii* sporozoites in cell culture and in mice.

All stages of *T. gondii* develop within a PV in the host cell. *T. gondii* sporozoites formed two types of PV in cell culture; the first vacuole (PV1) was 10–15 times the size of the sporozoite and did not have a membranous network [37, 38]. The second vacuole (PV2) was formed 18 h p.i. of cell cultures with sporozoites and contained the typical membranous network. However, only one type of PV was found after feeding oocysts to mice; PV1 was not seen in vivo [13, 14]. Therefore, biological measurements should be examined in the context of parasitological and host factors [5] and caution should be used in transferring results obtained from in vitro studies to in vivo systems.

References

[1] Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, FL: CRC Press 1988;1–220.

[2] Dubey JP, Frenkel JK. Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. J Protozool 1976;23:537–546.

[3] Bertoli F, Espino M, Arosemena Jr, Fishback JL, Frenkel JK. A spectrum in the pathology of toxoplasmosis in patients with acquired immunodeficiency syndrome. Arch Pathol Lab Med 1995;119:214–224.

[4] Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clin Infect Dis 1992;15:211–222.

[5] Frenkel JK. The stage-conversion time of *Toxoplasma gondii*: interpretation of chemical–biologic data out of parasitologic or host context. Parasitol Res 1996;82:656–658.

[6] Gross U, Bohne W, Soête M, Dubremetz JF. Developmental differentiation between tachyzoites and bradyzoites of *Toxoplasma gondii*. Parasitol Today 1996;12:30–33.

[7] Bohne W, Wirsing A, Gross U. Bradyzoite-specific gene expression in *Toxoplasma gondii* requires minimal genomic elements. Mol Biochem Parasitol 1997;85:89–98.

[8] Dubey JP. Infectivity and pathogenicity of *Toxoplasma gondii* oocysts for cats. J Parasitol 1996;82:957–960.

[9] Dubey JP, Lunney JK, Shen SK, Kwok OCH, Ashford DA, Thulliez P. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. J Parasitol 1996;82:438–443.

[10] Dubey JP, Frenkel JK. Cyst-induced toxoplasmosis in cats. J Protozool 1972;19:155–177.

[11] Freyre A, Dubey JP, Smith DD, Frenkel JK. Oocyst-induced *Toxoplasma gondii* infections in cats. J Parasitol 1989;75:750–755.

[12] Dubey JP, Frenkel JK. Experimental *Toxoplasma* infection in mice with strains producing oocysts. J Parasitol 1973;59:505–512.

[13] Dubey JP, Speer CA, Shen SK, Kwok OCH, Blixt JA. Oocyst-induced murine toxoplasmosis: life cycle, pathogenicity, and stage conversion in mice fed *Toxoplasma gondii* oocysts. J Parasitol 1997;83:870–882.

[14] Speer CA, Dubey JP. Ultrastructure of early stages of infections in mice fed *Toxoplasma gondii* oocysts. Parasitology 1998;116:35–42.

[15] Dubey JP. Bradyzoite-induced murine toxoplasmosis: stage conversion, pathogenesis, and tissue cyst formation in mice fed bradyzoites of different strains of *Toxoplasma gondii*. J Euk Microbiol 1997;44:592–602.

[16] Jacobs L, Remington JS, Melton ML. The resistance of the encysted form of *Toxoplasma gondii*. J Parasitol 1960;46:11–21.

[17] Hoff RL, Dubey JP, Behbehani AM, Frenkel JK. *Toxoplasma gondii* cysts in cell culture: new biologic evidence. J Parasitol 1977;63:1121–1124.

[18] Pettersen E. Destruction of *Toxoplasma gondii* by HCI solution. Acta Pathol Microbiol Scand B 1979;87:217–220.

[19] Sharma SP, Dubey JP. Quantitative survival of *Toxoplasma gondii* tachyzoites and bradyzoites in pepsin and in trypsin solutions. Am J Vet Res 1981;42:128–130.

[20] Lindsay DS, Dubey JP, Blagburn BL, Toivio-Kinnucan M. Examination of tissue cyst formation by *Toxoplasma gondii* in cell-cultures using bradyzoites, tachyzoites, and sporozoites. J Parasitol 1991;77:126–132.

[21] Lindsay DS, Toivio-Kinnucan MA, Blagburn BL. Ultrastructural determination of cystogenesis by various *Toxoplasma gondii* isolates in cell culture. J Parasitol 1993;79:289–292.

[22] Popiel I, Gold MC, Booth KS. Quantification of *Toxoplasma gondii* bradyzoites. J Parasitol 1996;82:330–332.

[23] Dubey JP. Re-examination of resistance of *Toxoplasma gondii* tachyzoites and bradyzoites to pepsin and trypsin digestion. Parasitology 1998;43–50.

[24] Bonametti AM, Passos JN, Koga de Silva EM, Macedo ZS. Probable transmission of acute toxoplasmosis through breast feeding. J Trop Pediatr 1997;43:116.

[25] Riemann HP, Meyer ME, Theis JH, Kelso G, Behymer DE. Toxoplasmosis in an infant fed unpasteurized goat milk. J Pediatr 1975;87:573–576.

[26] Dubey JP. Tissue cyst tropism in *Toxoplasma gondii*: a comparison of tissue cyst formation in organs of cats, and rodents fed oocysts. Parasitology 1997;115:15–20.

[27] Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites and sporozoites, and biology and development of tissue cysts. Clin Microbiol Rev 1998;in press.

[28] Bohne W, Gross U, Ferguson DJP, Heesemann J. Cloning and characterization of a bradyzoite-specifically expressed gene (hsp30/bag1) of *Toxoplasma gondii*, related to genes encoding small heat-shock proteins of plants. Molecular Microbiology 1995;16:1221–1230.

[29] Gross U, Bohne W, Lüder CGK, Lugert R, Seeber F, Dittrich C, Pohl F, Ferguson DJP. Regulation of developmental differentiation in the protozoan parasite *Toxoplasma gondii*. J Euk Microbiol 1996;43:114S–116S.

[30] Gross U, Bormuth H, Gaissmaier C, Dittrich C, Krenn V, Bohne W, Ferguson DJP. Monoclonal rat antibodies directed against *Toxoplasma gondii* suitable for studying tachyzoite–bradyzoite interconversion in vivo. Clin Diagn Lab Immunol 1995;2:542–548.

[31] Halonen SK, Lyman WD, Chiu FC. Growth and development of *Toxoplasma gondii* in human neurons and astrocytes. J Neuropathol Exp Neurol 1996;55:1150–1156.

[32] Lane A, Soete M, Dubremetz JF, Smith JE. *Toxoplasma gondii*: appearance of specific markers during the development of tissue cysts in vitro. Parasitol Res 1996;82:340–346.

[33] Soete M, Camus D, Dubremetz JF. Experimental induction of bradyzoite-specific antigen expression and cyst formation by the RH strain of *Toxoplasma gondii* in vitro. Exp Parasitol 1994;78:361–370.

[34] Soete M, Fortier B, Camus D, Dubremetz, JF. *Toxoplasma gondii*: kinetics of bradyzoite–tachyzoite interconversion in vitro. Exp Parasitol 1993;259–264.

[35] Weiss LM, Laplace D, Takvorian PM, Tanowitz HB, Cali A, Wittner MA. Cell culture system for study of the development of *Toxoplasma gondii* bradyzoites. J Euk Microbiol 1995;42:150–157.

[36] Sahm M, Fischer HG, Gross U, Reiter-Owona I, Seitz HM. Cyst formation by *Toxoplasma gondii* in vivo and in brain-cell culture: a comparative morphology and immunocytochemistry study. Parasitol Res 1997;83:659–665.

[37] Speer CA, Tilley M, Temple ME, Blixt JA, Dubey JP, White MW. Sporozoites of *Toxoplasma gondii* lack dense-granule protein GRA3 and form a unique parasitophorous vacuole. Mol Biochem Parasitol 1995;75:75–86.

[38] Speer CA, Dubey JP, Blixt JA, Prokop K. Time lapse video microscopy and ultrastructure of penetrating sporozoites, types 1 and 2 parasitophorous vacuoles, and the VEG strain of *Toxoplasma gondii*. J Parasitol 1997;83:565–574.