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The host-parasite relationship of *Toxoplasma gondii* in the brains of chronically infected mice

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Summary. The host parasite relationship in the brains of asymptomatic mice chronically infected with *Toxoplasma gondii* was examined at 3, 6 and 12 months post-infection (PI) using electron microscopy. The parasites were located in large numbers within tissue cysts which ranged in size from 10–50 μm in diameter. The cysts were predominantly found in the grey matter. The toxoplasms were enclosed by a cyst wall consisting of a membrane, with irregular invaginations, and an underlying layer of homogeneous osmiophilic material. A detailed examination of 50 cysts revealed that all the cysts were present within intact host cells irrespective of their size or the period PI. The majority of host cells could be positively identified as neurons by the presence of synapses. No extracellular cysts were observed. It is probable that the intracellular location of the cysts protects them from recognition and attack by the host immune system.

Key words: *Toxoplasma gondii* – Mouse brain – Tissue cyst – Host parasite relationship – Ultrastructure

Introduction

Toxoplasma gondii is an ubiquitous protozoan parasite which can infect any warm blooded animal with a high incidence within the human population (Jacobs 1967). The majority of infections are acquired after birth from the ingestion of the parasite within either tissue cysts present in meat or resistant oocysts from contamination with cat faeces (Jacobs 1973). The resultant infection has an initial acute phase in which the parasite rapidly multiplies within nucleated cells. However, with the onset of the host immune response (both cellular and humoral) the infection enters a chronic phase in which the parasite is present within tissue cysts predominantly located within the central nervous system, striated and heart muscles (Jacobs 1967). Within the cysts, viable organisms persist for long periods possibly throughout the life of the host. The majority of chronic infections are asymptomatic but if the host immune system is compromised then recrudescence of the activity proliferating form of the parasite results in serious or fatal toxoplasmosis. In the human, this has been reported where the immune system has been affected by treatment of neoplasms, immune suppression associated with transplants and acquired immune deficiency syndrome (AIDS) (Frenkel et al. 1975; Luft et al. 1983; Ruskins and Remington 1976; Navia et al. 1986).

The organism within the tissue cysts can therefore form an important source for human disease. Although the structure of the cysts is well known, a number of aspects of the host/parasite relationship in the CNS are unclear. In the present study, we have used electron microscopy to examine the host/parasite relationship in the brains of asymptomatic mice with chronic *T. gondii* infections. This has been carried out in an attempt to characterise the cell type within which cysts develop and whether the cysts retain an intracellular location.

Materials and methods

In the present study, an outbred albino (STR) strain of mice and the avirulent SRRA strain of *Toxoplasma gondii* were employed (for details see Hutchison 1986). The mice were infected by the subcutaneous inoculation of 12 tissue cysts of *T. gondii* obtained from the brains of mice infected three months previously. The mice were examined at 3 months (4 mice), 6 months (4), and 12 months (4) post-infection. In addition,

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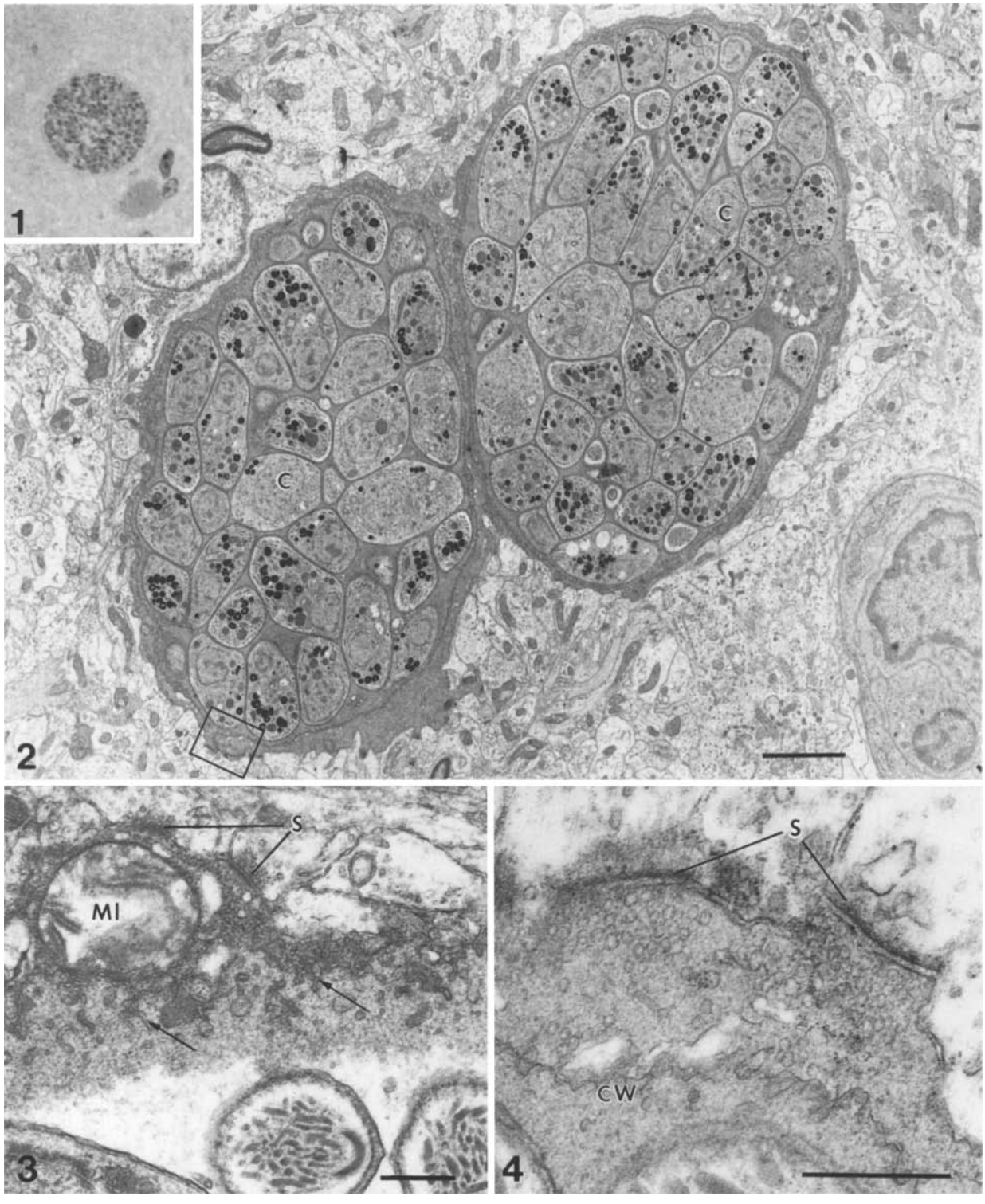

Fig. 1. Light micrograph of a tissue cyst filled with cystozoites in mouse brain. Plastic embedded, Azure A stained. × 500

Fig. 2. Electron micrograph of two tissue cysts containing cystozoites (*C*) within the same neuron. 3 months PI. × 7000. Bar is 2 μm

Fig. 3. Section through the cyst wall illustrating the invagination in the limiting membrane (*arrows*) and the underlying zone of homogeneous material. *MI*, mitochondrion; *S*, synapses. × 25000. Bar is 0.5 μm

Fig. 4. Detail from the enclosed area in Fig. 2 illustrating the cyst wall (*CW*) and the synapses (*S*) between the axon of the host cell and adjacent dendrites. × 50000. Bar is 0.5 μm

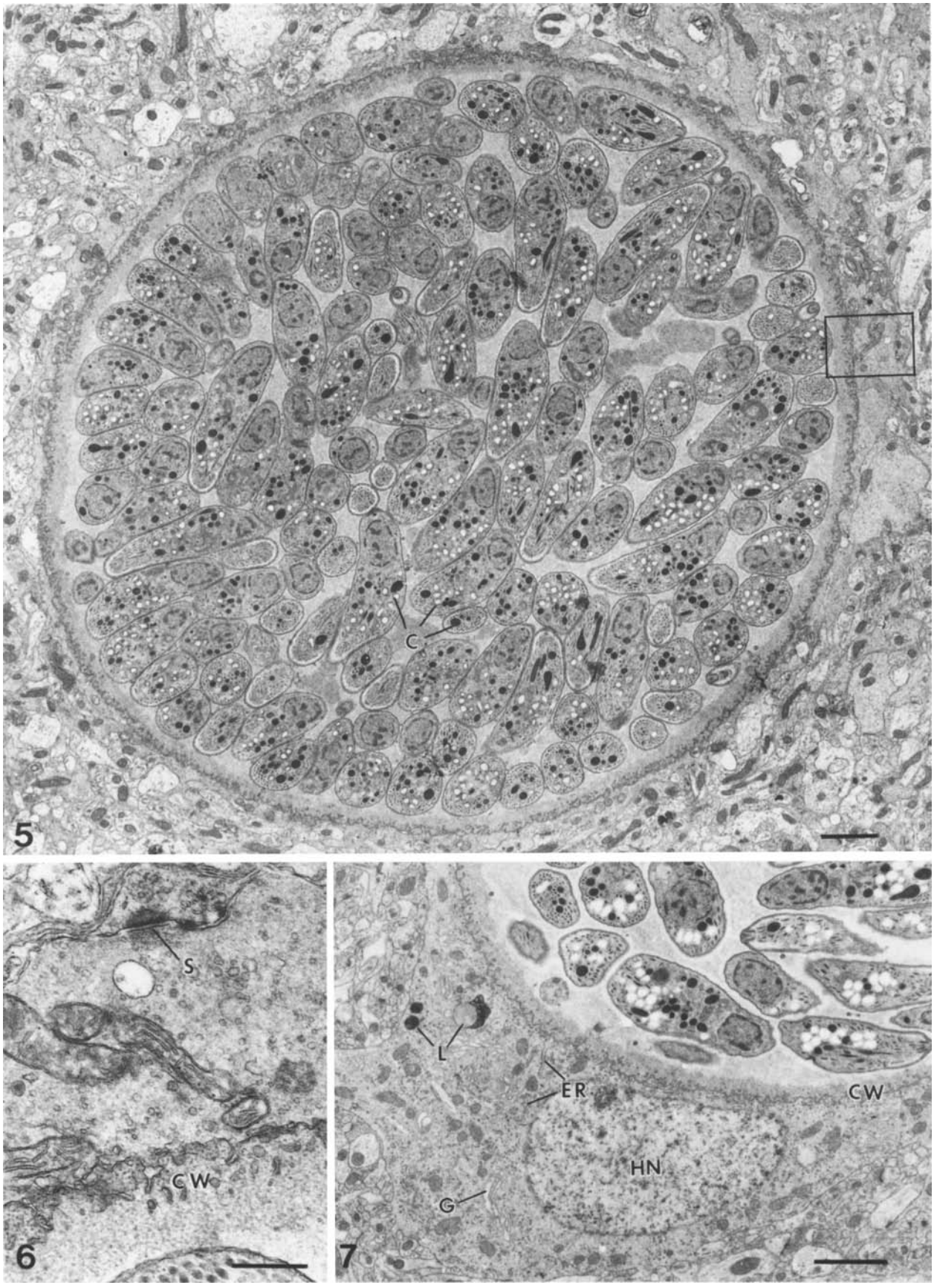

Fig. 5. Large cyst containing numerous cystozoites (*C*) surrounded by the attenuated cytoplasm of a neuron. 12 months PI. × 5000. Bar is 2 μm

Fig. 6. Detail from the enclosed area in Fig. 5 illustrating the host cell cytoplasm and synapse (*S*) formation. *CW*, cyst wall. × 30000. Bar is 0.5 μm

Fig. 7. Part of a tissue cyst present in the cell body of a neuron is illustrated. *CW*, cyst wall; *G*, Golgi body; *L*, lysosome; *ER*, rough endoplasmic reticulum; *HN*, host cell nucleus. × 7000. Bar is 2 μm

six age-matched uninfected mice were included as controls. When the mice were killed, the brain was immediately removed and placed directly in 3% glutaraldehyde in phosphate buffer pH 7.3 where it was chopped into 1 mm cubes to allow adequate fixation. The tissue was fixed overnight then post-fixed in 1% osmium tetroxide in phosphate buffer, dehydrated in ethanol, treated with propylene oxide and embedded in epoxy resin (E-mix). The tissue cysts were initially identified in Azure A stained 1 μm sections. Thin sections of the cysts were stained with uranyl acetate and lead citrate prior to examination with a Jeol 100 CX electron microscope.

The results in this report are based on the ultrastructural examination of 50 tissue cysts of which 45 were located in grey matter and 5 in white matter.

Results

The gross appearance of the brains from infected mice was normal and similar to that of the brains from the uninfected control animals.

Histologically, tissue cysts containing large numbers of toxoplasms were observed within the brains of all the infected mice but not in the control animals. The majority (approximately 90%) of cysts were present in the grey matter with only a few (10%) in the white matter. The cysts were characterised by a total lack of a host immune reaction in their immediate vicinity and ranged in size from 10 to 50 μm in diameter (Fig. 1).

Ultrastructurally, the tissue cysts were seen to contain large numbers of parasites termed cystozoites enclosed within a specialised wall (Figs. 2 and 5). The wall consists of an osmiophilic membrane with numerous short branching invaginations into a zone, approximately 700 nm wide, of homogeneous granular material (Fig. 3). Within the cysts, the cystozoites were embedded in electron-lucent granular material. The parasites were crescentic shaped, each with a basally positioned nucleus. They contain the characteristic organelles of a conoid, rhoptries, micronemes and polysaccharide granules enclosed within a two layered pellicle with a single micropore (Fig. 5). The structure of the cyst wall and the cystozoites was similar, irrespective of the size of the cysts, the period post-infection or whether they were located in white or grey matter.

The ultrastructural features of the host/parasite relationship were examined for 50 tissue cysts (45 in grey and 5 in white matter). In all cases the cysts, irrespective of their size or location, were present within the cytoplasm of intact host cells showing no evidence of degenerative changes (Figs. 2–6). Extracellular cysts were never observed. From the ultrastructural examination of the host cells, the majority could be positively identified as neurons by the presence of synapses with neurosecretory vesicles (Figs. 4, 6 and 7). The electron density and organelle content of the host cell cytoplasm varied with the location of the cyst within the neuron. The neurons contained mitochondria, Golgi bodies, microtubules, neurofilaments, rough endoplasmic reticulum, ribosomes, lysosomes and a nucleus. From differences in the organelle content and the location of the neurosecretory vesicles associated with the synapses, the tissue cysts could be identified within axons (Figs. 2, 4–6), dendrites (Fig. 3) or the cell body (Fig. 7) of neurons. The growth of the cysts resulted in stretching and distortion of the host cell. Certain large cysts were only enclosed by a narrow attenuated 30 nm wide band of host cell cytoplasm which always contained a few host cell mitochondria (Fig. 3). There did not appear to be any specific redistribution of the host cell organelles associated with cyst formation (Figs. 3, 4, 6 and 7).

Discussion

The light and electron microscopic appearances of the tissue cysts and cystozoites of *Toxoplasma gondii* observed in the present study are similar to those described previously in chronic infections in animals and man (Reviewed by Jacobs 1967).

However, the present study clarifies a number of aspects of the host/parasite relationship of the tissue cysts of *Toxoplasma* within the central nervous system. *Toxoplasma gondii* is an obligate intracellular parasite and as such the tissue cysts develop within host cells. Within the CNS we have observed that the tissue cysts appear to develop predominantly within neurons. The unambiguous identification of the host cells as neurons was based on the ultrastructural appearance of the cytoplasm and the finding of synapses in the majority of cases (Peters et al. 1976). This preferential location of the cysts within neurons has not been reported previously. It contrasts with earlier reports of cysts within neuroglial cells but these observations were based on a few poorly fixed samples which made definite identification of the host cell difficult (Wanko et al. 1962; Ghatak and Zimmerman 1973).

Our observation that the cysts are retained within viable host cells irrespective of size or age is at variance with the generally accepted view that in chronic infections many extracellular cysts, resulting from host cell death, are present in the

brain (van der Zypen 1966; Adams et al. 1984; Frenkel 1974). Although our results cannot disprove the existence of a few extracellular cysts, the rationale for this belief requires reconsideration. Histologically, the apparent extracellular location could result either as a preparative artifact (shrinkage during processing resulting in a space around the cyst) or from the inability of the light microscope to resolve the narrow rim of attenuated host cell cytoplasm which we observed around the large cysts located in axons and dendrites. The ultrastructural reports (Wanko et al. 1962; van der Zypen 1966; Ghatak and Zimmerman 1973; Scholtyseck et al. 1974) could be equally well explained as resulting from post mortem changes or poor fixation adversely affecting the host cell. In addition, the finding of intracellular cysts in the brain is consistent with the observation that the cysts in muscle are retained within host cells (Mehlhorn and Frenkel 1980). The advantage of this intracellular location for an obligate intracellular parasite is that the minimal metabolic requirements of the resting stage could be provided by the host cells. If this relationship is required for the maintenance of the integrity of the tissue cyst, then the predelection for neurons, which are long living cells, would ensure the long term survival of the latent infection. It would appear that the cysts contain viable organisms, since cysts isolated from the brains of similarly infected mice produced *Toxoplasm* infections when inoculated into uninfected mice. In addition, the location of the cysts within intact host cells will mask the parasite antigens and this would explain the absence of an inflammatory response to the presence of the large cysts containing numerous parasites.

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