

PATHOLOGIC STUDY OF ACUTE TOXOPLASMOSIS IN EXPERIMENTAL ANIMALS

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Abstract. We studied the pathology of acute toxoplasmosis in experimental mice inoculated with RH strain tachyzoites of *Toxoplasma gondii*. All died from severe disseminated toxoplasmosis involving the liver, spleen and pancreas. Pathological features of acute toxoplasmosis in susceptible mice could be regarded as an excellent model for acute reactivation of *Toxoplasma* in the immunosuppressed host.

INTRODUCTION

Toxoplasma gondii, an intracellular protozoan, was first discovered by Nicholle and Manceaux in 1908 from the liver of the African rodent, *Ctenodactylus gondii*. About 50% of the world's population is infected by that obligate intracellular parasite that multiplies in all nucleated cells of the vertebrate host (Cesbron-DeDelauw and Capron, 1994). It is a crescent-shaped organism, size 2-3 µm in width and 4-8 µm in length, with one end slightly broader than another. There are three infectious stages of this parasite in humans that are relevant to the understanding and diagnosis of the disease. Firstly, the trophozoite includes a tachyzoite, the rapidly proliferative intracellular form and a bradyzoite, the low-diving form inside the tissue cyst. Secondly, the pseudocyst in the host cell contains tachyzoites. Lastly, the cyst, the chronic stage of the parasite, is filled with bradyzoites in a well-defined cyst wall.

T. gondii, as well as other members of the Apicomplexans are intracellular parasites. Invasion of the host cell is a major step in its biology and pathogenesis. All three stages possess characteristic apical organelles; rhoptries, micronemes and dense granules that are in-

volved in parasite attachment, penetration and parasitophorus vacuole maturation (Carruthers and Sibley, 1997; Conseil *et al*, 1999).

Populations at risk of toxoplasmosis are immunocompromized persons, pregnant women and their fetuses in whom the clinical manifestations such as encephalitis, brain abscess, hydrocephalus, hepatosplenomegaly and retinitis are apparent. Conversely, in the immunocompetent host, clinical signs and symptoms are mostly mild, non-specific and acute toxoplasmosis thus usually passes unnoticed and is followed by a chronic quiescent stage. Presently, available evidence suggests that the host tissue pathology associated with *T. gondii* infection may play an important role in latent infection and the reactivation process (Herion and Saavedra, 1993). However, the pathological features of acute toxoplasmosis in humans are rarely documented since it is not often feasible.

We, therefore, studied acute toxoplasmosis in experimental mice infected with RH strain tachyzoites. The findings from light and electron microscopic studies could be applied to humans for further understanding of the pathogenesis of *Toxoplasma* in the immunocompromized host.

MATERIALS AND METHODS

Toxoplasma gondii strain

Tachyzoites of the RH strain of *Toxo-*

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plasma gondii, maintained in inoculated mice passage, were used in this experiment.

Animals

Outbreed and disease-free, 3-4 week old mice of both sexes were used. They were supplied by the National Laboratory Animal Center, Mahidol University, Thailand. Diets and bedding materials were maintained free from pathogenic microorganisms by skilled technical staff.

Infection and autopsy

Mice were intraperitoneally inoculated with 5×10^5 RH strain tachyzoites of *T. gondii*. At days 3-, 4-, 6- and 9-post infection (PI), peritoneal fluids were aspirated for electron microscopic study. Autopsies were performed for light microscopic examination on the 4th, 6th and 9th days after infection.

Light and electron microscopy

The liver, spleen, pancreas and brain of each studied mouse were fixed in 10% formalin, dehydrated and embedded in wax for light histo-pathologic examination. Hematoxylin and eosin (H&E) and Giemsa staining were used.

Tachyzoites in the peritoneal fluid of all acutely infected mice were processed for electron microscopic study as previously described (Ferguson *et al*, 1999). In brief, they were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, post-fixed in osmium tetroxide and treated with uranyl acetate prior to dehydration and embedded in Spurr's epoxy resin, before sectioning and staining for electron microscopy.

RESULTS

All studied mice were acutely ill with toxoplasmosis. They were less active and preferred to stay at the corners of their cages. Their entire body hair stood on end and they seldom took food or water.

Light microscopy

Different degrees of congestion were seen

in all organ studies: the brain, liver, spleen and pancreas. In the brain, mild to moderate congestion of the meningeal and interstitial brain parenchyma were observed, but neither *T. gondii* tachyzoites nor pseudocysts were seen.

In the liver, marked congestion was found in the central, portal and sinusoidal vessels. Generalized necrosis of the liver cells was present, including the portal areas, which were filled with numerous, intracellular and extracellular *Toxoplasma gondii* tachyzoites (Fig 1A-B). Numerous tachyzoites were located along the thickened liver capsule (Fig 1C). Pseudocysts with rosette formation were seen in the liver (Fig 1D).

In the spleen, marked congestion and increased megakaryocytes were also found. Eosinophilic and histiocytic infiltrations containing intracellular and extracellular *Toxoplasma* organisms were seen in the spleen (Fig 2A-B). Trophozoites were found at the thickened capsule, and proteinaceous fluid mixed with fibrinous material was found adjacent to hepatic and splenic capsules (Fig 2C). As in the liver, pseudocysts were also present. In a piece of pancreas, fatty tissue septa and peri-pancreatic fatty tissue as well as cytoplasm of histiocytes were also filled with tachyzoites (Fig 3A-B).

Electron microscopy

Numerous *T. gondii* tachyzoites were seen in the peritoneal fluid of the studied mice. They were crescent-shaped, with one end broader than the other. Organelles belonging to Apicomplexans such as conoid, rhoptries, dense body, micronemes as well as the nucleus were present (Fig 4A). There were numerous macrophages containing several tachyzoites. The parasites were in parasitophorous vacuoles and some appeared divided (Fig 4B). Eosinophils with intracellular *Toxoplasma* organisms were also found (Fig 4C).

DISCUSSION

Most animals can be classified as either

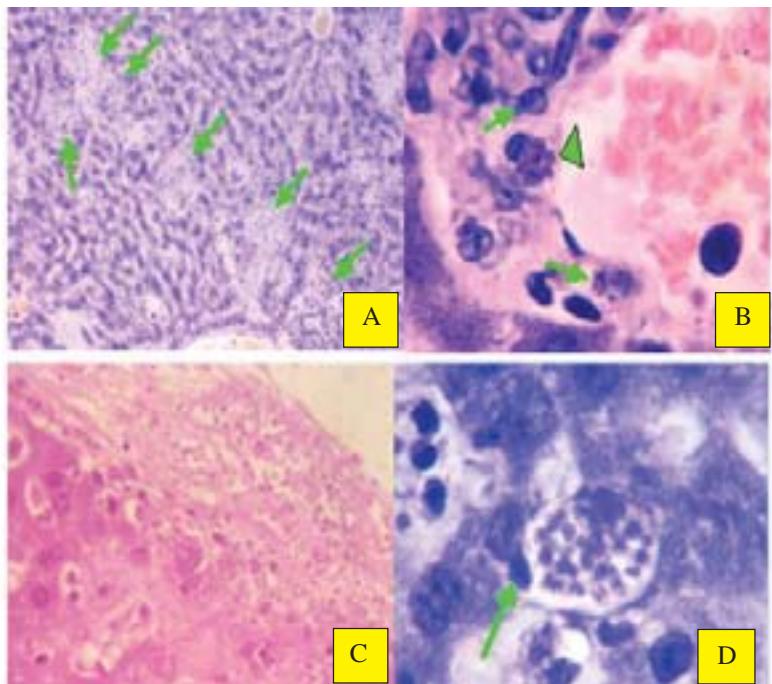


Fig 1—Light microscopic features of acute toxoplasmosis in the liver of the studied mice. A, section stained with Giemsa showed generalized necrosis of the liver cells (arrows) x 400, the portal areas were filled with *Toxoplasma* tachyzoites (B), both intracellular (arrowhead) and extracellular (arrows) x 1,000. C, thickened liver capsule stained with hematoxylin and eosin showed numerous tachyzoites along the capsule x 400. D, pseudocysts with rosette formation were observed in the liver. Note host cell nucleus (arrowhead) x 1,000.

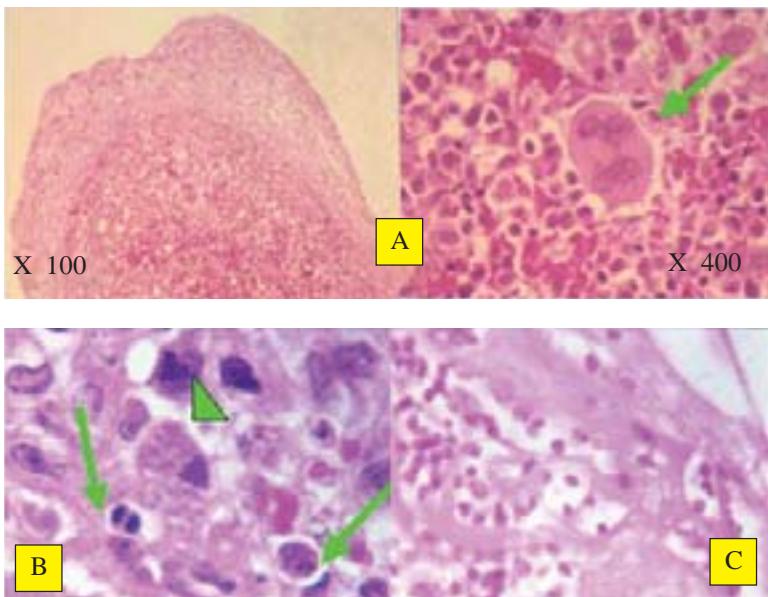


Fig 2 A-C—Section of the spleen showed marked congestion and increase of megakaryocytes (A) (arrow) x100 and x 400. Note eosinophilic (arrows) and histiocytic (arrowhead) infiltration containing intracellular tachyzoites (B) x 1,000. C, proteinaceous fluid mixed with fibrinous material containing numerous *Toxoplasma* tachyzoites (arrows) x 1,000.

resistant or susceptible to *T. gondii* with respect to clinical disease. In general, man, cattle, horses and rats belong to the resistant species, which may produce mild or non-specific clinical symptoms, whilst mice, guinea pigs and hamsters are susceptible hosts (Darcy and Zenner, 1993). The stage of the infected parasite (tachyzoite, bradyzoite and sporozoite), the route of inoculation (oral, intraperitoneal and subcutaneous) and the susceptibility of the host, influence the virulence of toxoplasmosis (Kaufman *et al.*, 1959; Ferguson *et al.*, 1981; Suzuki *et al.*, 1989).

With regard to the pathogenesis and virulence of toxoplasmosis, the presently available literature has only considered natural infection by the oral route acquired by oocyst and tissue cyst (De Rover-Bonnet, 1966; Reikvam and Lorentzen-Styr, 1976; Ferguson *et al.*, 1981; Suzuki *et al.*, 1989).

Different strains of *T. gondii* have been observed to have very different outcomes on infection in intermediate hosts, with some strains being virulent and cause fatal, acute toxoplasmosis, while others causing chronic infection by forming cysts in the brain and muscle tissue of the infected animal (Miller *et al.*, 1999). In the present study, we used the RH strain of *T. gondii*, which is well known to be the most virulent (Remington *et al.*, 1958; Dubey and Beattie, 1988). Moreover, the infected trophozoites derived from repeated passages in the mouse and had become more virulent. Thus the pathology found in the experimental mice was widespread into many organs.

There are 3 stages of *T. gondii* in intermediate hosts, including humans that are relevant to the understanding and diagnosis of the disease; the trophozoite, pseudocyst and tissue cyst, all of which are intracellular. Oocysts contaminate an environment by cat excreta and are transmitted to the host via ingestion. The tachyzoite, which is one of the trophozoite stages, is the most aggressive when infecting the host. The bradyzoite is less aggressive, the organism dividing slowly within a tissue cyst (Dubey and Frenkel, 1973). The mice in the present study were infected with tachyzoites.

The clinical manifestations were therefore present within a short time after infection and all died from severe disseminated toxoplasmosis. However, we could not demonstrate tachyzoites or pseudocysts in the brain. This may be due to the artificial route of infection, the intraperitoneal inoculation, which caused severe necrosis of the liver, spleen and pancreas. Many organs were severely damaged before toxoplasmic encephalitis developed. Only mild to moderate congestion was observed in the brain parenchyma and meninges.

Among the susceptible hosts; mice, guinea pigs and hamsters, the mouse is the most susceptible (Darcy and Zenner, 1993). According to Dubey and Beattie (1988) 100,000 oocyst of all strains of *T. gondii* were lethal to mice by the oral route. We used the most virulent strain given to the most susceptible host by the artificial infection route. The pathological features seen in our study were therefore widespread, involving many organs within a short period of time. A very severe disseminated toxoplasmosis caused death in all experimental mice, whether they were treated with pyrimethamine or not.

In resistant hosts, including man, there are usually no major effects of infection with chronic cyst formation unless the immune system is weakened, at which time those cysts may be reactivated, resulting in acute toxoplasmosis and the death of the host. This is a common situation in human suffering from AIDS. Acute toxoplasmosis in susceptible mice in the present study was severe and life-threatening. In our opinion, this could be regarded as a model for acute reactivation of toxoplasmosis in the immunosuppressed host.

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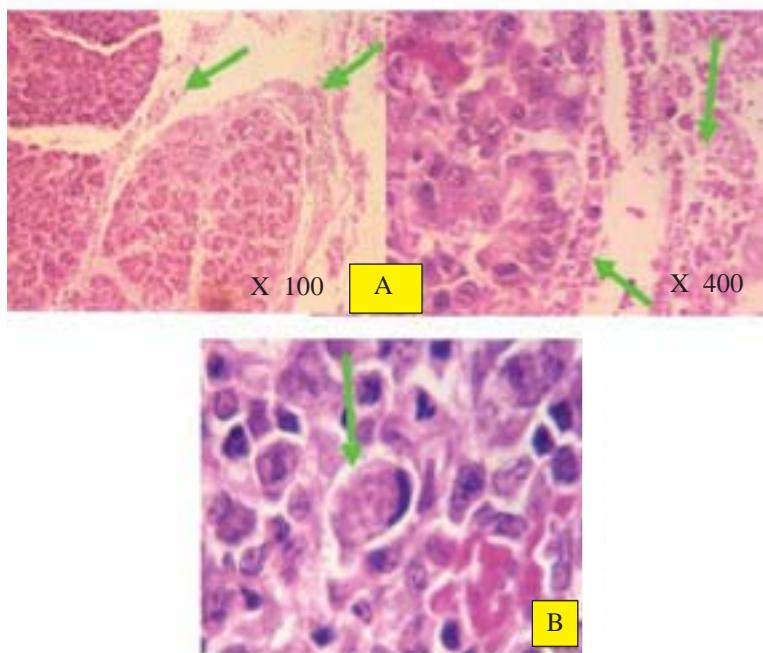


Fig 3 A-B—A piece of the pancreas, many *Toxoplasma* tachyzoites (arrows) were seen at the fatty tissue septa and tissues surrounding it (A) x 100 and x 400. B, note intracellular *Toxoplasma* organism in the histiocyte x1,000.

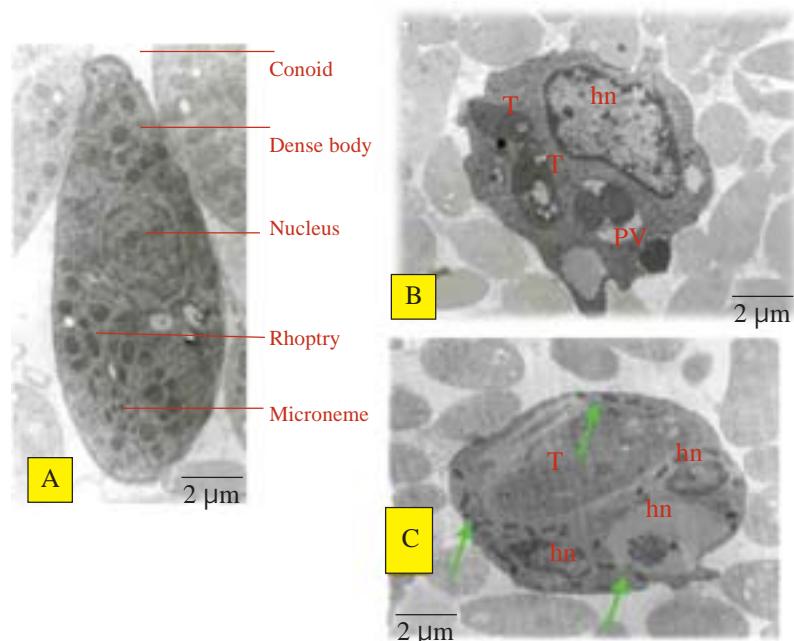


Fig 4 A-C—Electron micrograph of *Toxoplasma gondii* tachyzoite from peritoneal fluid of studied mice (A). Note the conoid, dense bodies, nucleus, rhoptry and micronemes, which are important for invading the cell, x 12,000 Bar = 2 μm. B, macrophage with several intracellular *Toxoplasma* tachyzoites (T) and parasitophorous vacuole (PV) containing multiplication of tachyzoite. x 3,500 Bar = 2 μm. Eosinophils with intracellular organisms were seen (C), x 9,000. Note host cell nucleus (hn) and eosinophilic granule (arrows). Bar = 2 μm.

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