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Pathological changes in acute experimental toxoplasmosis with *Toxoplasma gondii* strains obtained from human cases of congenital disease

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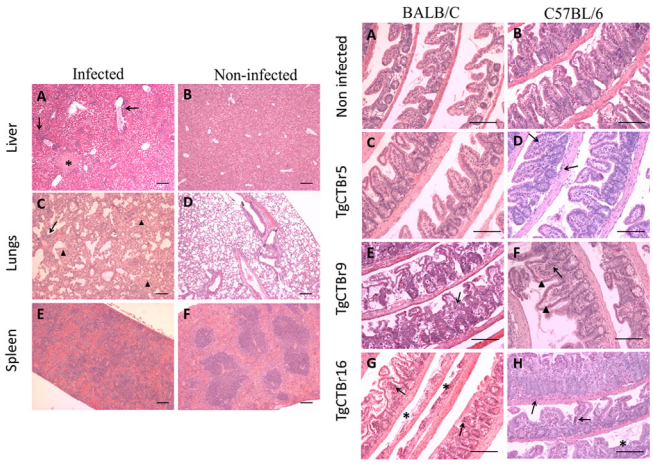
HIGHLIGHTS

- We conducted a pathological study using mice as a model of *T. gondii* infection.
- The strains used were obtained from children congenitally infected in Brazil.
- The strains induced similar tissue damage, although genetically diverse.
- Histological changes in the small intestine seem to be dependent on the strain.

GRAPHICAL ABSTRACT



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ABSTRACT

There is a lack of studies using *Toxoplasma gondii* strains isolated from human patients. Here, we present a pathological study of three strains obtained from human cases of congenital toxoplasmosis in Brazil using inbred mice after oral infection with 10 tissue cysts. Multiplex-nested PCR-RFLP of eleven loci revealed atypical genotypes commonly found in Brazil: toxodb #8 for TgCTBr5 and TgCTBr16 strains and toxodb #11 for the TgCTBr9 strain. BALB/c and C57BL/6 mice were evaluated for survival and histological changes during the acute phase of the disease. All mice inoculated with the non-virulent TgCTBR5 strain survived after 30 days, although irreversible tissue damage was found. In contrast, no mice were resistant to infection with the highly virulent TgCTBR9 strain. The TgCTBr16 strain resulted in 80% survival in mice. However, this strain presented low infectivity, especially by the oral route of infection. Despite being identified with the same genotype, TgCTBr5 and TgCTBr16 strains showed biological differences. Histopathologic analysis revealed liver and lungs to be the most affected organs, and the pattern of tissue injury was similar to that found in mice inoculated perorally with strains belonging to clonal genotypes.

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However, there was a variation in the intensity of ileum lesions according to *T. gondii* strain and mouse lineage. C57BL/6 mice showed higher susceptibility than BALB/c for histological lesions. Taken together, these results revealed that the pathogenesis of *T. gondii* strains belonging to atypical genotypes can induce similar tissue damage to those from clonal genotypes, although intrinsic aspects of the strains seem critical to the induction of ileitis in the infected host.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite that infects most warm-blooded animals including one-third of the human population (Tenter et al., 2000). Although asymptomatic in most cases, toxoplasmosis can cause severe symptoms in congenitally infected fetuses such as retinochoroiditis, hydrocephalus, intracranial calcifications and fetal growth retardation (Montoya and Liesenfeld, 2004).

Molecular studies using multilocus markers revealed a higher genetic diversity of *T. gondii* isolates in Brazil than in isolates from clonal genotypes commonly found in Europe and North America (Carneiro et al., 2013; Dubey et al., 2008; Ferreira et al., 2006; Pena et al., 2008). Ocular disease in children with congenital toxoplasmosis is more frequent in Brazil than in countries where the parasite has clonal population structure. Therefore, the effectiveness of treatment in North America and Europe may not be the same where more virulent strains of atypical genotypes predominate (Gilbert, 2009).

During the acute phase of acquired infections, lymphoid organs, liver and lungs are the most recurrently affected organs. Lymphadenopathy is the main clinical finding in human toxoplasmosis (Hill et al., 2005). Macroscopic hyperplasia of lymphoid organs is associated with an immune response, and humans or animals that die of toxoplasmosis can present lymphocyte depletion (Frenkel, 1988; Gavrilescu and Denkers, 2001). In livers, tachyzoite replication can lead to the death of infected cells causing necrotic foci accompanied by inflammatory mononuclear infiltrates (Frenkel, 1988). Furthermore, lungs can present a diffuse pneumonia in immunocompromised human patients (Evans and Schwartzman, 1991) as well as lung injuries which can be fatal to mice during the second week of infection (Dubey et al., 1997).

In experimental infection, the severity of toxoplasmosis depends on host and parasite factors including both parasite and mouse strains as well as the dose and route of inoculation. Oral infection with tissue cysts in mice has been widely used for pathological studies, and the small intestine, liver, lungs, spleen and brain are often found to be affected (Djurković-Djaković et al., 2006; Dunay et al., 2008; Egan et al., 2011; Liesenfeld et al., 1996; McLeod et al., 1984; Schreiner and Liesenfeld, 2009; Smiley et al., 2005). However, most of the strains used in these studies were obtained from animals as the isolation of *T. gondii* from human patients is extremely challenging. Furthermore, the aforementioned studies were carried out using strains belonging to clonal genotypes isolated from North America and Europe.

Recently, our group was able to isolate *T. gondii* strains from the peripheral blood of newborn infants with congenital toxoplasmosis in Brazil (Carneiro et al., 2013). Thus, the aim of this study was to conduct a pathological study using inbred mice as a model of acute infection after oral inoculation with tissue cysts of strains isolated from children with congenital toxoplasmosis in Brazil, as well as to access whether the strain genotypes induce different tissue lesions in mice.

2. Materials and methods

2.1. T. gondii *strains*

The *T. gondii* strains used in this study were previously isolated from newborn infants and had their virulence determined in BALB/c mice (Carneiro et al., 2013). The TgCTBr5 strain was obtained from a newborn asymptomatic at birth and presented no virulence in mice. The TgCTBr9 strain was isolated from a child presenting severe congenital toxoplasmosis with death after 9 months and is highly virulent to mice. Finally, the TgCTBr16 strain was obtained from a newborn with ocular disease and showed intermediate virulence in mice. The strains were maintained cryopreserved or in Swiss mouse passages by successive oral inoculation of brain cysts of animals infected 6 months previously. For the TgCTBr9 virulent strain, mice were treated with sulfadiazine for 10 days beginning at day 2 post infection, for survival of mice and thus development of tissue cysts.

2.2. Mice

Female Swiss, BALB/c and C57BL/6 mice, between 8 and 10 weeks old, were obtained from the animal breeding facility (Centro de Bioterismo – CEBIO) of the Universidade Federal de Minas Gerais (UFMG). BALB/c and C57BL/6 mice were used for experimental infections, whereas Swiss mice were used for parasite maintenance and the obtainment of tachyzoites for DNA extraction. All experiments using animals were approved by the Ethics Committee on Animal Use (CEUA-UFMG) certified by the Protocol no 266/2012.

2.3. *Multiplex-nested PCR-RFLP genotyping of* T. gondii

Genotyping of strains TgCTBr5 and TgCTBr9 were previously conducted by Carneiro et al. (2013). The TgCTBr16 strain was genotyped in the present study. DNA for molecular analysis was extracted from tachyzoites obtained from the peritoneal cavities of outbred Swiss mice after intraperitoneal inoculation with tissue cysts of the TgCTBr16 strain. Extraction was performed using the Promega *Wizard* genomic DNA purification kit following the manufacturer's instructions. Two steps of amplification were taken in multiplex-nested PCR-RFLP using eleven molecular markers as previously described (Su et al., 2010).

The genotype of the TgCTBr16 strain was determined by DNA banding patterns after treatment with restriction enzymes (Su et al., 2010) and resolved in a polyacrylamide gel (5%) stained with silver nitrate. Strains RH and GT1 (type I), ME49 and PTG (type II), VEG and CTG (type III), as wells as strains with unusual alleles (TgCgCa1, MAS and TgCatBr5) were used as banding pattern references. For identification, the genotypes were compared to those listed in ToxoDB virtual database at http://toxodb.org.

2.4. *Experimental infection and survival analysis*

The mice were divided into 6 groups according to mouse lineage and *T. gondii* strain. Sixteen mice were inoculated per group, 10 for survival analysis, and 6 for histopathological assay at two time points: three mice 7 days post infection (dpi) and three mice 12 dpi. Inoculation was carried out by gavage of 10 cysts harvested from Swiss mouse brains between 2 and 3 months after previous infection with each of the three strains. Mouse was sacrificed by cervical dislocation; the brain was removed and homogenized in 1 ml of phosphate buffered saline, pH 7.2. The tissue cysts were counted in duplicate in 10 μl of brain homogenate under light microscope. For experimental infections, BALB/c and C57BL/6 mice received 10 cysts