MAJOR ARTICLE

Congenital Toxoplasmosis: Continued Parasite Proliferation in the Fetal Brain Despite Maternal Immunological Control in Other Tissues

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Background. Congenital toxoplasmosis is a serious condition but little is known of the natural history of parasite development and associated fetal tissue destruction.

Methods. Two cases identified by ultrasound underwent induced abortion at 21 and 30 weeks' gestation. At autopsy, the placenta and fetal organs were examined by histology and immunocytochemistry employing anti-*Toxoplasma* stage-specific antibodies to confirm diagnosis and also provide information on the stage of parasite development.

Results. In both cases, maternal serology prior to termination showed both specific immunoglobulin M (IgM) and immunoglobulin G (IgG), whereas retrospective analysis of an earlier sample (12–14 weeks' gestation) showed only IgM reactivity consistent with infection occurring in the first trimester. The finding of a number of tissue cysts but few or no tachyzoites within the placenta and fetal adrenal and heart is characteristic of a chronic infection. However, in contrast, there were still areas of the fetal brain with large numbers of actively dividing, tissue-destructive tachyzoites.

Conclusions. These observations show that continued parasite proliferation and tissue destruction can occur within the fetal brain even when there is a marked maternal immune response including maternal IgG. This finding strongly suggests that there may be benefits from treating cases of recently acquired congenital infection to destroy any remaining proliferating parasites located in immunologically protected sites such as the fetal brain.

Keywords. congenital toxoplasmosis; *Toxoplasma gondii*; pathology; immunocytochemistry; parasite proliferation.

Toxoplasma gondii is a protozoan parasite with a worldwide distribution. It is a coccidian parasite with the cat as its definitive host; however, any warm-blooded animal, including humans, may act as intermediate hosts [1]. In intermediate hosts, after an initial acute systemic phase with proliferating parasites (tachyzoites), it reverts to a chronic infection with parasites (bradyzoites) limited to tissue cysts in the brain or musculature resulting in lifelong persistent infection. Although infection can cause devastating disease in immunocompromised individuals, it causes no, or mild and transient, symptoms in immunocompetent individuals. However, if a woman (or other female mammal) becomes infected for the first time during pregnancy, proliferating parasites, circulating in the blood, may cross the placenta during the acute phase and infect the developing fetus [2, 3]. The results of this prenatal infection vary widely, ranging from intrauterine fetal death to birth of children who may present with severe symptoms or remain asymptomatic [2, 4]. The chances of congenital transmission and severity of the symptoms can vary depending on when in gestation infection occurs [5]. Because *Toxoplasma* has a tropism for the central nervous system, severe symptoms are caused by the parasite replicating in the brain or eye.

Different countries have divergent policies on serological screening for evidence of primary maternal

infection during pregnancy and also on the benefits of treating mothers in whom infection has been identified. If seroconversion is detected during pregnancy, then it is possible that congenital infection may occur. In countries where there is no screening, the first evidence of congenital infection can come from abnormalities observed during prenatal ultrasound examinations or only at the time of birth, when the newborn is already severely affected. In addition, and especially when infection occurs at late gestational age, infection of the fetus might be asymptomatic. The 2 cases reported in this study were identified by prenatal ultrasound examination and subsequently the pregnancy was terminated. In each case, the placenta and fetus were examined by histology and immunocytochemistry to identify not only the presence of T. gondii, but also the developmental stages present in relation to continuing host tissue destruction. The developmental stage was identified by the expression of stage-specific proteins, in this case antibody to a surface protein (SAG1) only expressed by the actively proliferating tachyzoites [6] and a cytoplasmic protein (BAG1) only expressed by the chronic phase bradyzoites located within tissue cysts [7]. These observations further our understanding of the natural history of T. gondii infection in pregnancy.

CASE REPORTS

Case 1

The mother was a 28-year-old in her second pregnancy. Her first pregnancy resulted in a healthy live-born baby at term. A routine anomaly scan at 20 weeks had been reported as normal. Repeat ultrasonography at 30 weeks' gestation revealed severe bilateral ventriculomegaly with almost total destruction of the cerebral cortical tissue posteriorly. A fine thread-like echo pattern in the posterior horns of the lateral ventricles was thought to represent intracranial bleeding. The pregnancy was terminated.

Mother's serology at termination was positive for both Toxoplasma immunoglobulin M (IgM; Siemens ADVIA Centaur Toxoplasma IgM assay, index 25.9) and immunoglobulin G (IgG; Siemens ADVIA Centaur Toxoplasma IgG assay). When a serum sample taken at 12 weeks was retrospectively analyzed, it was positive for Toxoplasma IgM (index 19.7) but negative for IgG.

At autopsy, the placenta and fetal organs, including the brain, were processed for histopathological examination.

Case 2

The mother was a 28-year-old in her first pregnancy. Routine anomaly scanning at 21 weeks' gestation revealed mild bilateral ventriculomegaly (anterior and posterior horns of the lateral ventricles: 13 mm), echogenic bowel with ascites, and a head circumference of less than the 3rd centile for gestation. The pregnancy was terminated.

Mother's serology at termination was positive for both Toxoplasma IgM (Siemens ADVIA Centaur Toxoplasma IgM assay, index >40) and IgG (Siemens ADVIA Centaur Toxoplasma IgG assay). When a serum sample taken at 14 weeks' gestation was retrospectively analyzed, it was positive for IgM (index >40) but negative for IgG.

At autopsy, the placenta and fetal organs, including the brain, were processed for histopathological examination.

MATERIALS AND METHODS

Histology

Tissues were fixed in formaldehyde and processed for wax histology. Sections were stained with hematoxylin and eosin prior to examination.

Immunohistochemistry

Sections were de-waxed and treated by pressure cooking before immunostaining. The sections were double labeled with Toxoplasma stage-specific antibodies (tachyzoite-specific rabbit anti-SAG1 raised to the recombinant protein [6] and bradyzoite-specific mouse anti-BAG1 raised to the recombinant protein [7]) and visualized using goat antirabbit immunoglobulin conjugated to fluorescein isothiocyanate and goat antimouse immunoglobulin conjugated to Texas red and counterstained with 4′,6-diamidino-2-phenylindole.

RESULTS

Case 1

Histological examination confirmed the diagnosis of T. gondii by the identification of a number of tissue cysts within the placenta and umbilical cord (Figure 1A) plus a few cysts in the adrenal gland and heart. Double-labeled immunocytochemistry confirmed these structures as Toxoplasma tissue cysts due to the presence of SAG1⁻/BAG1⁺ bradyzoites (Figure 1A and 1B). In addition, a few SAG1⁺/Bag1⁻ tachyzoites, intermediate SAG1⁺/BAG1⁺, and also SAG1⁻/BAG1⁻ parasites were observed within the umbilical cord (not shown). It is probable that these represent dead (lysed) parasites.

When the brain was examined, there appeared to be areas with apparently normal architecture and areas with marked necrosis. When sections from the "normal" area were examined the architecture appeared intact but with some inflammatory cell infiltration. In addition, a few tissue cysts were identified confirmed by immunocytochemistry as containing SAG1⁻/BAG1⁺ bradyzoites (Figure 1D). However, no SAG1⁺/BAG1⁻ proliferating tachyzoites were observed. In contrast, in other areas of the brain, particularly in those regions adjacent
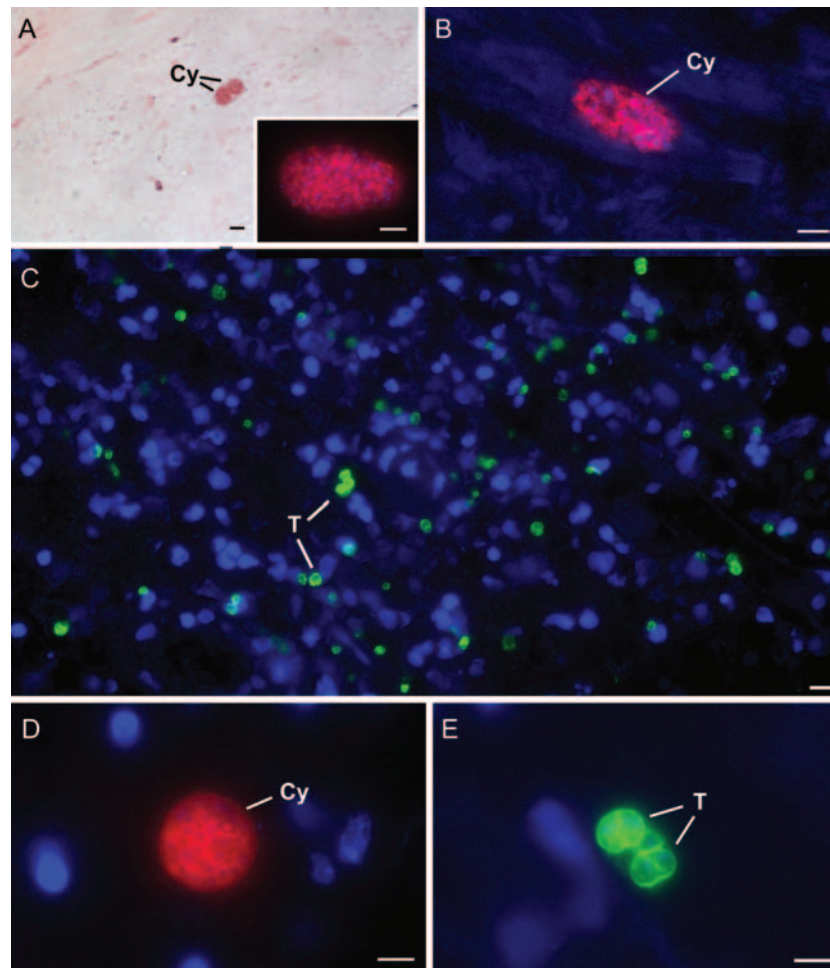
Figure 1. Images from case 1 of the umbilical cord (*A*), heart (*B*), and brain (*C–E*) stained with hematoxylin and eosin (*A*) or double immunolabeled with antibodies to SAG1 and BAG1 and visualized with fluorescein isothiocyanate (green) and Texas red (red), respectively (*B–E*). Bars represent 10 μm (*A–D*) and 1 μm (*E*). *A*, Section through the umbilical cord showing the presence of 2 tissue cysts (Cy). Magnification, ×200; inset shows immunostained section of a tissue cyst containing SAG1$^-$/BAG1$^+$ bradyzoites. Magnification, ×800. *B*, Section of a SAG1$^-$/BAG1$^+$ tissue cyst (Cy) in the heart. Magnification, ×800. *C*, Low-power image through a damaged area showing the very large numbers of proliferating SAG1$^+$/BAG1$^-$ tachyzoites (T). Magnification, ×600. *D*, Section of a SAG1$^-$/BAG1$^+$ tissue cyst (Cy) surrounded by normal neural tissue. Magnification, ×800. *E*, Detail showing dividing tachyzoites (T). Magnification, ×3000.

to necrotic foci, very large numbers of proliferating tachyzoites were observed (Figure 1*C* and 1*E*). No SAG1$^-$/BAG1$^+$ bradyzoites containing tissue cysts were observed in these lesions (Figure 1*C*).

Case 2

By histology, low numbers of tissue cysts were identified within the placenta (Figure 2*A*) but parasites were not identified in fetal organs except the brain. Immunocytochemistry confirmed the identity of the tissue cysts as containing SAG1$^-$/BAG1$^+$ (Figure 2*B*). In addition, a few small tissue cysts were identified within myocytes of the heart (Figure 2*C*). However, no SAG1$^+$/BAG1$^-$ proliferating tachyzoites were observed in the placenta or fetal tissues.

In contrast, in sections from various regions of the brain, areas with numerous individual and small groups of proliferating SAG1$^+$/BAG1$^-$ tachyzoites were observed (Figure 2*D*). In addition, a few parasites were SAG1$^+$/BAG1$^+$ representing intermediate stages (Figure 2*F*) and a few small SAG1$^-$/BAG1$^+$ tissue cysts were also present (Figure 2*E*).

DISCUSSION

The autopsy examination of the placentas and fetal tissue confirmed the diagnosis of toxoplasmosis. Review of the serological investigations showed positive results for both IgM and IgG at time of termination of pregnancy (21 and 30 weeks, respectively). The presence of specific IgG antibodies together
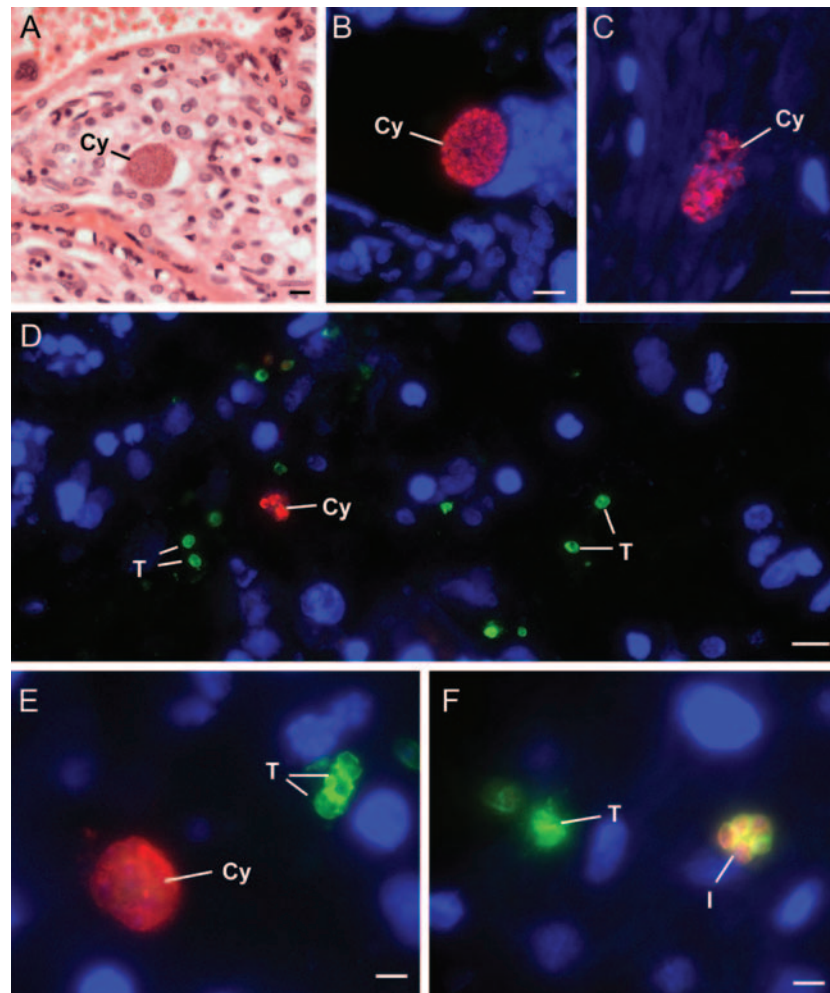
Figure 2. Images from case 2 of sections of the placenta (*A–B*), heart (*C*), and brain (*D–F*) stained with hematoxylin and eosin (*A*) or double immunolabeled with antibodies to SAG1 (green) and BAG1 (red). Bars represent 10 μm (*A–D*) and 1 μm (*E* and *F*). *A*, Section through the placenta showing the presence of a tissue cyst (Cy). Magnification, ×400. *B*, A similar section to (*A*) showing a tissue cyst (Cy)-containing SAG1⁻/BAG1⁺ bradyzoites. Magnification, ×600. *C*, Section of heart showing a SAG1⁻/BAG1⁺ tissue cyst (Cy) within a myocyte. Magnification, ×600. *D*, Low-power image showing a large number of SAG1⁺/BAG1⁻ tachyzoites (T) and a group of SAG1⁻/BAG1⁺ bradyzoites forming a small cyst (Cy). Magnification, ×800. *E*, Detail showing a small SAG1⁻/BAG1⁺ tissue cyst (Cy) and a group of SAG1⁺/BAG1⁻ tachyzoites (T). Magnification, ×2000. *F*, Section showing a group of SAG1⁺/BAG1⁺ intermediate-stage parasites (I) and adjacent SAG1⁺/BAG1⁻ tachyzoites (T). Magnification, ×2000.

with IgM antibodies was consistent with a longer period of infection. This was confirmed by the retrospective finding of only IgM at initial referral (12–14 weeks), pointing to infection occurring in the first trimester and therefore is in excess of 8 weeks' duration (10–16 weeks). In mice, the acute phase of infection with proliferating tachyzoites lasts for approximately 14–21 days, but with the onset of the immune response the tachyzoites are eradicated except for a small subpopulation that convert to bradyzoites and form tissue cysts in the musculature and central nervous system, resulting in lifelong chronic infection (David J. P. Ferguson, unpublished data). The finding of tissue cysts in the placenta and fetal organs would be consistent with the conversion to the chronic stage

of infection. However, within the brain the situation appeared to be completely different with the retention of actively proliferating tachyzoites. Therefore, while the immune response can control parasite proliferation within the placenta and fetal organs and also part of the brain, in case 1, the fetal brain is not completely protected, and uncontrolled tachyzoite proliferation results in continued host tissue destruction.

The cases in this report were identified by abnormal ultrasound findings at 21 and 30 weeks of pregnancy but could have been identified much earlier (at 12 weeks) if the routine blood sample had been tested for *Toxoplasma* antibodies. This would have allowed the patients to undergo more detailed investigations to confirm fetal infection prior to considering

whether to undergo termination or anti-*Toxoplasma* treatment. An ongoing controversy exists over whether to treat women with anti-*Toxoplasma* drugs if seroconversion occurs during pregnancy [8]. There is circumstantial evidence for the efficacy of treatment if initiated early [8–11], consistent with worse outcomes reported in those countries without screening and therefore no prenatal treatment [12]. The present report shows that there can be continuing parasite proliferation within the brain of the fetus for extended periods. Both fetal brains described showed immune privilege, and it could be argued that treatment would have a beneficial affect by destroying any remaining proliferating parasites within the brain.

In conclusion, in both cases the placenta and fetal organs showed features consistent with conversion to the non-tissue-destructive chronic phase with few or no proliferating parasites. However the brains of both fetuses showed evidence of numerous proliferating acute-stage tachyzoites resulting in continued tissue destruction.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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