Histopathological Features of Ocular Toxoplasmosis in the Fetus and Infant

Fiona Roberts, MD; Marilyn B. Mets, MD; David J. P. Ferguson, PhD; Richard O’Grady, MD; Carol O’Grady; Philippe Thulliez, PhD; Antoine P. Brézin, MD; Rima McLeod, MD

Background: Ocular disease is a frequent manifestation of congenital Toxoplasma gondii infection. There are only limited data available in the literature concerning early stages of this disease in fetuses and infants. The purpose of our study was to characterize histopathological features in the eyes of 10 fetuses and 2 infants with congenital toxoplasmosis.

Methods: Fifteen eyes from 10 fetuses, 3 eyes from 2 premature infants, and both eyes from a 2-year-old child with congenital toxoplasmosis were examined by light microscopy. Immunohistochemical analysis to identify inflammatory cells and T gondii antigens was performed. The findings in infected eyes were compared with those of age-matched control eyes.

Results: Retinitis (10/18 eyes), retinal necrosis (4/18 eyes), disruption of the retinal pigment epithelium (12/18 eyes), and choroidal inflammation and congestion (15/18 eyes) were characteristic findings. Optic neuritis was present in 5 of 8 fetal eyes with associated optic nerve available for evaluation. An eye obtained from a 32-week-old fetus showed retinal rosettes at the edge of a scar. T cells predominated in retinal lesions and choroid. Parasites were identified by immunohistochemical analysis in 10 of 18 eyes.

Conclusions: Ocular toxoplasmosis causes irreversible damage to the retina in utero. The fetus and infant mount inflammatory responses that may contribute to ocular damage. These findings have important implications for serological screening programs and in utero therapy.

MATERIALS AND METHODS

EYES

Fifteen eyes were collected post mortem from 10 fetuses with congenital toxoplasmosis. These eyes came from France, where routine serological screening for T. gondii infection has been carried out since 1978. Maternal infections were confirmed by seroconversion in both the Sabin-Feldman dye test and the IgM-immunosorbent agglutination assay. The fetuses were aborted between 19 and 32 weeks of gestation following a positive diagnosis of toxoplasmosis by polymerase chain reaction that demonstrated the presence of the T. gondii B1 gene in amniotic fluid. Prior to termination of pregnancy, additional evidence of severe T. gondii infection, as demonstrated by intracerebral ventricular dilatation on ultrasound, was obtained in 5 cases. In 6 cases the mother received spiramycin therapy in an attempt to prevent transplacental transmission of the parasite. Spiramycin cannot cross the placenta and does not treat the infection in the fetus. The mother of the 26-week-old fetus had received pyrimethamine and sulfadiazine therapy for 3 weeks prior to termination of pregnancy. In most cases, necropsy and/or subincision of placental tissue, amniotic fluid, or brain into mice was carried out to confirm the diagnosis. One second-trimester fetus was also infected with cytomegalovirus.

The infants and the child in this study had died of complications of congenital toxoplasmosis. Three infant eyes were available from 2 cases. Both infants were born prematurely, one at 34 weeks’ gestation, the other unreceived. The infants survived 7 days and 5 days, respectively. For each of these cases there was either serological confirmation of the infection during life, autopsy documentation, or positive subincision of tissues into mice. The histopathological findings of one infant were previously described in a case report by Frenkel and Friedlander. The 2-year-old child had well-documented changes of severe ocular and cerebral toxoplasmosis, which had been partially treated. This case was included in the study for comparison with the earlier stages of the disease in the fetus and infant.

Eyes from normal, uninfected, age-matched fetuses, infants, and a 2-year-old child were used as controls. Institutional review board approval was obtained for this study.

HISTOPATHOLOGICAL ANALYSIS

Specimens retrieved from archives were already embedded in paraffin. The remainder of the eyes were received in 10% buffered formalin. These were opened horizontally, processed, and embedded in paraffin. Sections (6 µm) were cut and stained with hematoxylin-eosin and periodic acid–Schiff for light microscopy.

IMMUNOHISTOCHEMICAL ANALYSIS

Inflammatory Cells

Following antigen retrieval, immunohistochemical analyses for pan–B cell (anti-CD20; 1:200), pan–T cell (anti-CD3; 1:10), CD4+ T cells (anti-CD4; 1:23), CD8+ T cells (anti-CD8; 1:50), and macrophages (anti-CD68; 1:100) (all supplied by DAKO, Santa Barbara, Calif) were performed using a standard ABC technique. The slides were visualized with 3-3′-diaminobenzidine and counterstained with hematoxylin.

T. gondii Antigens

Similarly, for T. gondii immunohistochemical analysis, slides were incubated with a monoclonal antibody (L43; donated by E. Petersen, PhD, Copenhagen, Denmark), which recognizes both bradyzoite and tachyzoite life cycle stages. Immunohistochemical staining was performed using an indirect alkaline phosphatase, anti–alkaline phosphatase (APAAP) technique. The slides were visualized using APAAP substrate solution containing naphthol-AS-BI-phosphate (substrate) and new fuchsin (chromagen) (Sigma-Aldrich Chemical Corp, St Louis, Mo). This gives a red reaction product that allows distinction of extracellular organisms from the brown melanin granules of the disrupted retinal pigment epithelium (RPE).

RESULTS

CONTROL EYES

The uninfected, age-matched, control eyes showed no lesions or inflammation. The histopathological findings are summarized in Table 1 and illustrated in Figure 1.

FETAL EYES

Two eyes, from a 21- and 26-week-old fetus, respectively, were normal. In all other cases the anterior segment showed mild chronic inflammation within the iris and ciliary body. In the posterior segment, focal retinal lesions were identified in 8 of the 15 eyes. Some lesions consisted of frank retinal necrosis with disruption of the underlying RPE (Figure 1A). Free pigment granules were scattered throughout the necrotic areas. Other lesions were devoid of retina and consisted of disruption and proliferation of the RPE. The choroid underlying the lesion was congested with chronic inflammation. There was diffuse choroiditis. Lesions varied in size, from less than 1 mm to more than 7 mm in a 22-week-old fetus. Of 4 cases in which both eyes were available, lesions were bilateral in only 1 case. The most frequent location of lesions was the posterior pole, which was involved in 5 eyes. In 3 of these cases the lesions were confined to the peripapillary area. The remaining eyes had lesions in the peripheral retina. In 2 cases, both peripapillary and peripheral retina were involved. The lesion in the peripheral retina of the 32-week-old fetus was unusual compared with other retinal lesions. Unlike other cases that showed evidence of continuing inflammation, this lesion was almost devoid of inflammatory cells. At the edge of this lesion the

eyes were studied by routine light microscopy as well as with immunohistochemical techniques to identify infiltrating inflammatory cells and T. gondii antigens. The findings were compared with those already described and correlated with the recognized clinical signs and symptoms.
The retina showed abnormal maturation with loss of distinction of nuclear layers and formation of Flexner-Wintersteiner rosettes (Figure 1B, bottom). Distant from this lesion the retina had appropriate maturation for gestational age. These appearances were interpreted as a form of focal retinal dysplasia. In addition to focal retinal lesions, there was retinal gliosis and neovascularization in 5 eyes (Figure 1C, right). In 3 eyes in which no focal retinal lesions were identified, the retina was normal in 2 eyes but showed widespread gliosis and neovascularization in 1 eye.

Inflammation extended into the vitreous in 7 eyes with condensation bands, scattered inflammatory cells, and necrotic debris. Remnants of the tunica vasculosa lentis were identified in 9 eyes. The hyaloid artery and primary vitreous were present in 2 eyes, and were surrounded by inflammatory cells (Figure 1D).

The optic nerve was present in sections from 8 eyes and was normal in only 3 eyes. In 5 eyes there was a leptomeningitis associated with optic neuritis (Figure 1E and 1F). In 3 of these eyes, the nerve architecture was disrupted. In 2 of the eyes with optic neuritis there was a peripapillary lesion.

**INFANT EYES**

Both eyes from the 7-day-old infant had a large retinal detachment with atrophy of the photoreceptors and a subretinal serous exudate (Figure 1C, left). There was bilateral extensive retinal necrosis with lesions extending from the pars plana to the posterior pole. Within the areas of retinal necrosis there were scattered pigment granules and focal calcification. The adjacent RPE was disrupted, showing tubuloacinar proliferation. There was diffuse choroiditis. Elsewhere the RPE was intact; however, the retina showed edema, gliosis, and mononuclear cell infiltration. There was an organizing vitritis with continuing inflammation. The optic nerve was not present in sections examined.

In the eye from the 5-day-old infant there was also a large retinal detachment with subretinal exudate and atrophy of photoreceptors (Figure 1C, left). However, there were only small foci of retinal necrosis and the RPE was intact. The retina showed changes similar to those described above, but in addition there were collections of intracellular organisms. The optic nerve showed leptomeningitis. Condensation bands were present in the vitreous with necrotic debris and mononuclear cells. In both cases the anterior segment was normal.

**CHILD EYES**

Both eyes showed features of end-stage disease. The right eye contained 2 retinochoroidal scars situated in the posterior pole and superior region of the peripheral retina (Figure 1A, bottom). Overlying these lesions there was organization of the vitreous. The left eye was firm, partially collapsed, and smaller than the right eye. On opening, the anterior chamber was shallow with a cataractous lens displaced into the posterior chamber. Within the posterior chamber there was massive retinal gliosis.

**INFLAMMATORY CELLS**

Findings were similar for both fetal and infant eyes and are summarized in Table 2 and illustrated in Figure 2. The inflammatory cells present in the lesions consisted of lymphocytes, plasma cells, and macrophages. Immunohistochemical staining showed both B and T lymphocytes. Overall, T lymphocytes predominated although scattered B lymphocytes were present, confined mainly to the choroid. There were focal localizations of T lymphocytes in the choroid underlying lesions and in the retina adjacent to lesions (Figure 2B). These were of both CD4+ and CD8+ subtypes although CD4+ cells predominated (Figure 2C). In addition, in the cases with optic neuritis, the nerve contained B cells as well as T cells of both CD4+ and CD8+ subtypes. Similar to the inflammatory infiltrate in the retina and choroid, CD4+ T cells predominated in the optic nerves. The eye from the 32-week-old fetus and both eyes from the 2-year-old child contained only rare lymphocytes. In 2 fetal eyes numerous macrophages were identified in the choroid underlying retinal lesions (Figure 2D). In all other eyes only occasional macrophages were identified within the choroid.
Figure 1. Ocular histopathology in congenital toxoplasmosis. A, top, A well-demarcated area of retinal necrosis (n) at the posterior pole in the eye of a 22-week-gestation fetus (hematoxylin-eosin, original magnification ×250). A, bottom, The edge of a large retinochoroidal scar from the eye of the 2-year-old child. The scar is well demarcated with tubuloacininar proliferation of the retinal pigment epithelium (rpe) at the edge of the scar. The center of the scar is devoid of retina (hematoxylin-eosin, original magnification ×250). B, top, Eye from the 32-week-gestation fetus showing a large hyperpigmented scar, with a white rim, in the superotemporal region of the eye (arrow). B, bottom, The retina from the edge of the scar shows disorganization with formation of Reimer-Wintersteiner rosettes (arrows) (hematoxylin-eosin, original magnification ×400). C, left, Retina from the 5-day-old infant eye showing retinal detachment with an exudate (e) between the retina and choroid. The inner retinal layer is edematous and inflamed (hematoxylin-eosin, original magnification ×100). C, right, Retina from the eye of a 22-week-gestation fetus showing gliosis (g) of the inner retinal layers (hematoxylin-eosin, original magnification ×250). D, Eye from a 23-week-gestation fetus showing a moderate inflammatory infiltrate (i) within the primary vitreous and surrounding the hyaloid artery (ha) (hematoxylin-eosin, original magnification ×20). E, Optic nerve from the eye of a 24-week-gestation uninfected fetus showing normal nerve architecture (hematoxylin-eosin, original magnification ×100). F, Optic nerve from the eye of a 23-week-gestation fetus with congenital toxoplasmosis. The nerve architecture is disrupted with an inflammatory cell infiltrate (hematoxylin-eosin, original magnification ×100).
Tissue cysts were not identified in the eye from the 5-day-old infant. Immunohistochemical analysis for organs were identified in the perivascular space around the central retinal vessels. In the 7-day-old infant's eye, very few parasites were identified despite the presence of extensive necrosis.

Parasites were not identified within the choroid despite the presence of numerous inflammatory cells. In 1 fetal eye with optic neuritis, a few extracellular parasites were identified in the perivascular space around the central retinal vessels. However, parasites were not identified within the substance of the optic nerve.

Retinitis, retinal necrosis, and disruption of the RPE with marked choroidal inflammation and congestion are the characteristic findings in the eyes of fetuses with congenital toxoplasmosis. These lesions represent irreversible in utero damage to the retina. They frequently affect extensive areas including the peripapillary area, posterior pole, and peripheral retina. The 2 infants in this study had severe ocular disease with retinal necrosis, retinal detachment, and large subretinal exudates. Within the surviving retina there was neovascularization and gliosis. Much of this damage must have occurred in utero since postnatal survival was only a few days.

It is not possible to document the kinetics of T gondii infection in human fetuses in utero. In this study, the time from maternal seroconversion to fetal diagnosis and subsequent termination of pregnancy estimated the maximum length of fetal infection to be on average 11.5 weeks. However, in reality, it is probably much shorter than this because there is a delay between maternal and fetal infection. The length of this delay varies but can be longer than 16 weeks. In addition, delay between fetal infection and ocular involvement may occur. Furthermore, unlike an animal model, in human infections, it is rarely feasible to document the clonal derivation of the parasite (ie, clonal type I, II, or III). Parasite life cycle stage, or the size of the inoculum with which the mother has been infected. Despite these differing parameters, the findings in the fetal eyes are remarkably similar and seem to represent a continuum of the same process. Specifically, retinal necrosis occurs in the acute lesions of ocular toxoplasmosis. As these lesions heal they leave an early scar devoid of normal retina but with proliferation of the RPE. The end stage of this process is formation of a retinochoroidal scar. End-stage scars such as these were seen in the eyes of the 2-year-old child.

Acute retinitis with necrosis may also be seen in children and adults following reactivation of ocular toxoplasmosis. This is rarely as extensive as that seen in the fetus except in the immunocompromised host.

Clinically established retinochoroidal scars have been identified at birth in some infants, implying that ocular damage, healing, and repair can all occur in utero. Indeed a retinochoroidal scar, almost devoid of inflammatory cells, was the main abnormality detected in the eye of the 32-week-old fetus. Adjacent to this scar, the retinal disorganization and rosettes, which we consider to represent a form of retinal dysplasia, was an unusual finding. Retinal dysplasia occurs most frequently with multiple congenital anomalies associated with chromosomal aberrations. At necropsy, this fetus (case 10) was not dysmorphic and showed no evidence of any malformations other than those associated with congenital toxoplasmosis. In addition, the retinal dysplasia was confined to the area immediately adjacent to the scar.

To our knowledge this is the first report of retinal dysplasia associated with T gondii infection. There have been several reports of infectious agents other than T gondii causing retinal dysplasia in animals. For example, retinal dysplasia develops in 2-day-old hamsters but not 25-day-old hamsters following intracerebral injection of measles virus. Similarly, retinal dysplasia was the most common finding following intraocular injection of feline leukemia virus into fetal kittens. Finally, canine herpesvirus infection in puppies produces severe ocular inflammation with subsequent retinal dysplasia. The mechanism(s) whereby these infectious agents cause abnormal retinal development remains to be determined. However, it has been postulated that progressive disor-

### Table 2. Immunohistochemical Staining for Inflammatory Cells and Toxoplasma gondii Antigens in Congenital Toxoplasmosis

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of Eyes</th>
<th>B Cells (CD20)</th>
<th>T Cells (CD3)</th>
<th>CD4+ T Cells</th>
<th>CD8+ T Cells</th>
<th>Macrophages (CD68)</th>
<th>T gondii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second-trimester fetus</td>
<td>12</td>
<td>4/4/4/0</td>
<td>2/2/0/8</td>
<td>2/2/6/2</td>
<td>5/3/4/0</td>
<td>8/2/0/2</td>
<td>5/1/2/4</td>
</tr>
<tr>
<td>Third-trimester fetus</td>
<td>1</td>
<td>1/0/0/0</td>
<td>0/1/0/0</td>
<td>0/1/0/0</td>
<td>1/0/0/0</td>
<td>1/0/0/0</td>
<td>1/0/0/0</td>
</tr>
<tr>
<td>Child</td>
<td>3</td>
<td>3/0/0/0</td>
<td>0/0/0/3</td>
<td>0/0/3/0</td>
<td>0/0/3/0</td>
<td>3/0/0/0</td>
<td>0/3/0/0</td>
</tr>
<tr>
<td>Infant</td>
<td>2</td>
<td>2/0/0/0</td>
<td>0/2/0/0</td>
<td>0/2/0/0</td>
<td>0/2/0/0</td>
<td>2/0/0/0</td>
<td>2/0/0/0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>10/4/4/0</td>
<td>2/5/0/11</td>
<td>2/5/5/6</td>
<td>8/3/7/0</td>
<td>12/2/2/2</td>
<td>8/4/2/4</td>
</tr>
</tbody>
</table>

* Data are presented as no/few/moderate/large numbers of positively stained cells or organisms. EC indicates extracellular; AA, amorphous antigens.
ganization of the retina and necrosis are followed by differential repair of the retinal layers.23,24 Furthermore, the RPE seems to be important in normal retinal development.20,21 Therefore, disruption of the RPE also may contribute to retinal dysplasia and rosette formation, perhaps owing to loss of polarity of retinal cells or lack of specific growth or maturation factors produced by the RPE.20,27 Both retinal necrosis and RPE disruption occur in ocular toxoplasmosis. This form of retinal dysplasia might therefore represent a rare complication of early T. gondii infection where the insult occurs prior to organization of the retinal layers.

The location of lesions is important because patients are at risk for visual loss if tissues such as the macula...
or optic nerve are involved. In clinical studies there seems to be a definite predilection for the macular region in patients with congenital toxoplasmosis. In the Chicago study, peripheral lesions were present in 64% of patients whereas macular scars were present in 58%. Considering the much smaller area of the macula, these results support a predilection for the macular region.

The development of the macular region is markedly retarded relative to that of the rest of the retina and differentiation in this region continues for at least the first 4 years of life. In view of this lack of specialization and small size we were unable to identify the macular region in the fetal or infant eye. However, where lesions were extensive and involved the posterior pole, it seems likely that the cells that form the macula were involved.

In congenital infection, T gondii probably reaches the eye by a hematogenous route. The identification of parasites around an inner retinal vessel in one of our cases supports a hematogenous route. Therefore, this predilection for the posterior pole may reflect the fact that it is vascularized earlier in development than other portions of the retina. In addition, although the macula is avascular, it obtains its blood supply from end arterioles, which form a capillaryplexus around it. Lodging of parasites in these capillaries could facilitate establishment of infection in this delicate region of the eye.

The optic nerve was present in sections from only 8 fetal eyes; however, optic neuritis was present in 5 of these with distortion of the normal nerve architecture in 3 cases. Distortion of the nerve architecture may represent another instance in which inflammation of a structure early in gestation results in its abnormal development. Because of a lack of appropriate neural connections, optic atrophy may be the clinical outcome in surviving patients. In the Chicago study, optic atrophy was present in 20% of patients with congenital toxoplasmosis. However, fetuses in our study were known to have intracranial disease with ventricular dilatation and optic neuritis may simply reflect the severity of encephalitis. Papillitis, papilledema, and optic atrophy are recognized clinical manifestations of toxoplasmic encephalitis.

True optic neuritis is a rare complication of disease reactivation, although T gondii may involve the optic disc or retina immediately adjacent to the optic disc, resulting in a papillitis sometimes referred to as Jensen juxtapapillary retinitis. This may represent a clinical correlate for the 2 cases of optic neuritis associated with a peripapillary lesion. Optic neuritis with necrosis and numerous parasites has been described in a patient with human immunodeficiency virus-associated ocular toxoplasmosis and a patient with fulminant congenital infection. This has led to the suggestion that congenital infections may be transmitted to the eye from the brain via the optic nerve. Alternatively, parasites infecting the eye anlage may accompany it to the developing eye. However, in our cases although there was inflammation and distortion of the normal nerve architecture, there was no necrosis or parasites. It is possible that the inflammatory response had killed parasites within the optic nerve. However, it seems unlikely that the optic nerve is the usual means of T gondii spread to the eye. Indeed the presence of parasites in the optic nerve may simply reflect hematogenous spread via the ocular branches of the ophthalmic artery. This is supported by the finding of a few parasites surrounding the central retinal artery in 1 fetal eye.

Immunity to T gondii requires a cell-mediated immune response that involves macrophages, natural killer cells, and their monokine and cytokine products. In particular interferon γ (IFN-γ) seems to be a critical cytokine for effective immunity. The fetus and neonate are unduly susceptible to infections with intracellular pathogens such as T gondii. Nonetheless, the presence of inflammatory cells in the eyes of infected fetuses suggests that the fetus is capable of mounting, albeit a less-effective, immune response to T gondii infection. Early in gestation the numbers of T cells and their repertoire for recognition of antigens are limited compared with the latter half of gestation. Indeed, infants with congenital toxoplasmosis have absent or diminished lymphocyte blastogenic responses to T gondii antigen and production of interleukin 2 and IFN-γ is reduced. Their lymphocytes are, however, capable of responding normally to other stimuli. This anergy to T gondii may reflect differences in route of acquisition, cytokine environment, or costimulatory molecules present during gestation. Muri- ne models have shown that the cytokines IFN-γ, tumor necrosis factor α, and CD4+ and CD8+ lymphocytes are important in acquired ocular toxoplasmosis. Since T lymphocytes are clearly present in the fetal eye at the time of infection, a decreased production of IFN-γ or other cytokines by these lymphocytes may contribute to tissue destruction through uncontrolled parasite proliferation. In our future studies we will examine local cytokine production and distribution of costimulatory molecules in these fetal eyes using immunohistochemical techniques.

Tissue cysts were not identified in the eyes of any of the fetuses or infants by light microscopy, although they were identified in the brains of 5 of 7 fetuses and in 1 infant. In the immunocompetent adult, tissue cysts may be found at the edge of retinochoroidal scars or within apparently normal retina at some distance from previous lesions. Encystment usually occurs with the onset of effective immunity and studies have shown a role for immune mediators, such as IFN-γ, in tachyzoite to bradyzoite conversion and thus cyst formation. Extracellular organisms were present in lesions, particularly in areas of necrosis, and numerous intracellular parasites were present in 1 infant case. This may reflect the early acute nature of this disease in the eye as well as a lack of effective immunity.

Our work demonstrates that significant irreversible ocular disease can occur in utero. This finding has substantial implications for fetal and infant treatment. In countries such as France that have prenatal screening programs, studies suggest that antimicrobial treatment in utero may reduce ophthalmic disease caused by this parasite. Similarly, early postnatal treatment of toxoplasmosis is associated with prompt resolution of active retinochoroiditis. documentation of the early stages of this disease in unsuccessful pregnancies is therefore important to allow clinical comparisons and assess effectiveness of therapies. Further studies to examine the ocular
immune response may help us understand the complex interactions between the immature host and T gondii in the eye. A nontoxic, nonteratogenic medicine that crosses the placenta and that kills tachyzoites and bradyzoites and that can be used early in gestation as soon as infection is diagnosed is needed.

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Corresponding author and reprints: Rima McLeod, MD, Department of Ophthalmology/Visual Sciences, University of Chicago, 939 E 57th St, Chicago, IL 60637 (e-mail: rmcleod@midway.uchicago.edu).

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