A Feline Model of Ocular Toxoplasmosis

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Purpose. This study was performed to characterize the clinical, serologic, histopathologic, and immunohistochemical features of an experimental model of ocular toxoplasmosis in cats.

Methods. Seven specific pathogen-free cats were inoculated in the right carotid artery with $5 \times 10^3$ tachyzoites of the ME49 strain of Toxoplasma gondii. Control cats received heat-killed tachyzoites.

Results. Progressive, bilateral, multifocal retinal, and choroidal inflammatory foci developed in the principal cats, beginning 5 to 8 days postinoculation (PI). Lesion development peaked 3 weeks PI, and the lesions varied in size from pinpoint to 5 mm, had a predilection for the central tapetal fundus, and were more numerous ipsilateral to the side of inoculation. Resolution of the lesions 21 to 70 days PI was characterized by foci of tapetal destruction and retinal degeneration. Fluorescein angiography showed disruption of the blood–retinal barrier at the level of the retinal pigmented epithelium, and occasional retinal vasculitis and perivasculitis. Mild anterior uveitis developed in four cats 10 to 13 days PI. Aside from a slight febrile response 2 to 3 days PI, no physical abnormalities were observed. T. gondii antigens were detected intermittently in the serum of four of seven cats as early as 8 days PI. T. gondii-specific immunoglobulin M titers were present on day 7 PI and continued to increase until 28 days PI. Immunoglobulin G production was documented on day 13 PI, and titers continued to increase throughout the study. Evidence of anterior uveal antibody production (mean Goldmann-Witmer coefficient [C value], 80.7; range, 13.4 to 236.6) was present in 11 of 14 eyes on day 70 PI. On histopathologic evaluation 70 days PI, multifocal granulomatous chorioretinitis, with retinal degeneration, retinal vasculitis, and lymphocytic–plasmacytic anterior uveitis, was documented. Tissue cysts in the retina and choroid were found with mouse inoculation of tissue suspensions, immunohistochemical studies, and histopathologic examination.

Conclusions. This nonfatal, noninvasive method of inducing ocular toxoplasmosis may prove to be a useful model for investigation of toxoplasmic retinochoroiditis, particularly with the recent characterization of a naturally occurring, immunosuppressive feline lentivirus with properties similar to human immunodeficiency virus. Invest Ophthalmol Vis Sci. 1993;34:3653–3660.

Toxoplasma gondii currently is considered the most common cause of retinochoroiditis in humans in the United States and is recognized as an important opportunistic pathogen in patients with acquired immune deficiency syndrome. Attempts to experimentally create and study the ocular lesions of toxoplasmosis in laboratory animals have often been difficult because oral administration of oocysts or tissue cysts results in inconsistent ocular tissue replication, and parenteral administration of standard inoculum dosages of the organism often results in fulminant systemic disease and premature death of the experimental subject. These problems have been circumvented in rabbit and primate models of ocular toxoplasmosis by intravitreal inoculation of a relatively low number of T. gondii tachyzoites, followed by attempts to cause reactivation after cessation of the acute inflammatory response. The two species used in these experimental designs have the disadvantages of differences in immunologic response to T. gondii and anatomic dissimilarity of the retina compared with humans (rabbit), and there is often a prohibitive expense and increasingly...
low supply for the laboratory setting (primate). In this article, we report a feline model of ocular toxoplasmosis that involves inoculation of a low number of organisms from a mildly virulent strain of *T. gondii* into the common carotid artery, which results in predictable clinical ocular disease.

**MATERIALS AND METHODS**

**Experimental Animals**

Eight female, specific pathogen-free cats between 8 months and 1 year of age were used in the study. Use of the animals in this experiment adhered to the ARVO Resolution on the Use of Animals in Research and was approved by the University Animal Use Committee. All cats had normal findings on physical and ophthalmic examinations and were free of serum antibodies to *T. gondii* before inoculation.

**Experimental Induction of Toxoplasmosis**

The ME49 strain of *T. gondii* was used in the experiment (oocysts were supplied by J. P. Dubey, United States Department of Agriculture, Beltsville, MD). Strain ME49 originally was isolated from sheep muscle, is only mildly virulent in mice, and characteristically produces high numbers of tissue cysts in the brains of chronically infected mice. Tachyzoites were produced initially by intraperitoneal inoculation of retired breeder Swiss Webster mice with oocysts collected from a cat fed mouse brains containing *T. gondii* bradyzoites. Three serial passages of tachyzoites in peritoneal fluid then were performed in mice with described methods. Immunosuppression of the mice with 1 mg trimacinolone subcutaneously was necessary to enhance tachyzoite replication. Before inoculation into cats, peritoneal exudate was cytocentrifuged and examined for bacterial contamination, and tachyzoite numbers in peritoneal fluid were determined on a hemocytometer. Parasitic inocula were prepared by dilution with Hank’s solution containing 100 IU heparin sodium per 10 ml to a final concentration of 5000 organisms per milliliter. Organisms were harvested within 45 minutes of inoculation into cats.

The cats were anesthetized with ketamine hydrochloride (7.5 mg/kg, intramuscularly), and the right common carotid artery was isolated surgically. One milliliter of the inocula (5000 organisms) was injected through a 27-gauge hypodermic needle. The viability of the inocula was confirmed after inoculation by injection of 0.1 ml intraperitoneally into Swiss Webster mice and examination of peritoneal exudate for tachyzoites 4 days later. One cat, who served as a control, received similarly collected inocula that were heated in a water bath to 56°C for 1 hour to kill the organisms.

Ophthalmologic, Serologic, and Fecal Examination Procedures

Physical and ophthalmic examinations with biomicroscopy and indirect ophthalmoscopy were performed daily for 3 weeks, and three times weekly thereafter. Fluorescein angiography was performed before inoculation and at weekly intervals for 1 month with 25 mg/kg sodium fluorescein injected into the cephalic vein. Blood was collected for serologic studies by jugular venipuncture three times weekly for 4 weeks, then weekly thereafter. *T. gondii*–specific immunoglobulin M (IgM), immunoglobulin G (IgG), and antigens were measured in each serum sample. *T. gondii*–specific IgM, IgG, and antigens, as well as total IgM and IgG, were measured in serum and aqueous humor collected on day 70 postinoculation (PI). The Goldmann-Witmer coefficient for local production of antibodies in aqueous humor was calculated for these samples as previously described. Fecal samples from the cats were examined every other day between days 8 and 33 PI with a sucrose flotation method for the presence of *T. gondii* oocysts.

**Tissue Techniques**

The cats were killed with an overdose of intravenous thiopental sodium (Pentothal, Abbott Hospital Products, North Chicago, IL) 70 days PI. Aqueous humor (0.1 ml) was collected through a 27-gauge needle inserted at the limbus, just before the animals were killed. Globes were removed and fixed in Zenker’s or Trump’s fixative and processed for light microscopic examination with paraffin embedding and hematoxylin and eosin staining. Four left globes with clinical evidence of chorioretinitis were bisected at the equator before fixation, and the lateral retina and choroid were dissected aseptically from the adjacent tissues. Swiss Webster mice then were inoculated with a trypsin-digested retina–choroid suspension, according to previously described procedures, to assay for the presence of *T. gondii* tissue cysts.

Ocular tissues were examined for the presence of tachyzoites, tissue cysts, or *T. gondii*–specific antigens with the following adaptation of a *T. gondii*–specific immunohistochemical staining procedure. Polyclonal mouse IgGs containing *T. gondii*–specific antibodies were produced and isolated as previously described and used as the primary antibody at a concentration of 1:100 diluted in Tris-buffered saline solution (pH, 7.6). A commercial kit containing biotinylated antiserum IgG and streptavidin–alkaline phosphatase complex (Histomark, catalog no. 710039; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used as recommended by the manufacturer. A commercial kit containing substrate and contrast stain (Histomark Red, catalog no. 55-69-00, Kirkegaard...
and Perry Laboratories) also was used as directed by the manufacturer.

RESULTS
Clinical Findings
The clinical findings throughout the experiment were limited to the eyes and were not present in the control animal receiving heat-killed tachyzoites. Three of the cats had a mild increase in rectal temperature compared with preinoculation and control values (39.3° to 39.7°C) on days 7 to 12 PI; however, no other physical abnormalities were found on examination.

Focal areas of choroidal and retinal inflammation were evident beginning on day 5 PI and were present in all animals by day 8 PI. Lesions were bilateral in six of the seven cats; however, in three cats the areas of inflammation were more profound in the right eye (ipsilateral to inoculation site). The lesions were characterized initially by multifocal, slightly gray discoloration in the tapetal fundus (Fig. 1). Whitish-gray lesions of a similar size appeared in the nontapetal fundus. Circumferential progression in the size of the foci, 1- to 2-mm lesions, was evident over the next 7 days. Then the lesions typically varied in size and were areas of gray to black discoloration of the tapetum, with overlying retinal inflammation and edema, and slight vitreous haze. In five animals, large (4 to 5 mm) subretinal exudative lesions, with a granulomatous appearance, and overlying shallow retinal detachment developed. The intensity and number of posterior segment lesions peaked on days 14 to 16 PI, with 5 to 30 focal areas per eye. Over the next 2 to 3 weeks, there was regression of these areas to focal chorioretinal scars with tapetal color alteration, retinal degener-

![FIGURE 1. Tapetal fundus photograph of a cat with experimental ocular toxoplasmosis (day 14 PI). Multiple grayish lesions represent areas of chorioretinitis.](image)

ation, and, in some cases, organization and consolidation of subretinal exudative material. Black or bronze discoloration of the affected tapetum was common. Larger nontapetal foci appeared, along with retinal and choroidal degenerative lesions, with resultant visibility of scleral tissues.

Fluorescein angiography showed multifocal disruption of the blood-retinal barrier at the level of the retinal pigmented epithelium, with late-phase fluorescein dye leakage into the sensory retina. Hypofluorescence from blocked fluorescence was present in the central aspect of large funduscopy lesions with cellular infiltrate (Fig. 2). Occasionally, retinal vasculitis and perivasculitis with late hyperfluorescence adjacent to focal segments of affected retinal vessels also were observed. Fluorescein angiographic abnormalities first were observed 2 weeks PI. Regeneration of the blood-retinal barrier was evident with most posterior segment lesions at 4 weeks PI.

In four cats, mild unilateral (right eye) anterior uveitis also developed between 10 and 13 days PI, characterized by peripheral iridal swelling and hyperemia, 2+ of 4+ aqueous flare, and medium-sized keratic precipitates. Fibrin and a small hyphema were seen in one cat. Anterior segment changes resolved in 4 to 7 days. In one cat, a similar, mild anterior uveitis developed in the left eye 24 days PI. The degree of anterior segment inflammation did not preclude adequate visualization of the fundus at any point in the experiment.

Oocyst Shedding
Only one cat had detectable fecal oocysts from days 24 to 27 PI.
Serologic Findings

*T. gondii* antigens were detected intermittently in the serum of four of seven cats (57%). *T. gondii*-specific IgM titers were present on day 7 PI and continued to increase until 28 days PI. Serum *T. gondii*-specific IgG titers greater than 1:64 were documented on day 13 PI; titers continued to increase through day 47 PI and thereafter showed a gradual decline. *T. gondii*-specific IgG was detected in the aqueous humor of 13 of 14 eyes at day 70 PI. The Goldmann-Witmer coefficient was greater than 8 in 11 of 14 eyes (mean, 80.7; range, 13.4 to 236.6).

Mouse Inoculation Studies of Retinal-Choroidal Suspensions

Twelve of 20 mice (4 of 4 eyes) assayed for the presence of tissue cysts from retinal-choroidal suspension had evidence of *T. gondii* infection as established by the presence of brain tissue cysts 8 weeks after inoculation.

Histopathologic Findings

Histopathologic evaluation 70 days PI showed multifocal chorioretinitis characterized by a predominately granulomatous inflammatory reaction with a mixed population of macrophages, lymphocytes, and plasma cells. Retinal degeneration and disorganization of cellular layers and granulomatous inflammation overlying the primary foci of choroiditis were common, as were retinal pigmented epithelium hyperplasia and hypertrophy and pigment migration into the sensory retina (Fig. 3). Lymphocytic-plasmacytic retinal vasculitis and perivasculitis also were evident around many of the larger retinal blood vessels. Focal, predominately perivascular aggregates of lymphocytes and plasma cells were seen in the iris and ciliary body of cats showing clinical evidence of anterior uveitis. *T. gondii* cysts were seen only rarely with standard histopathologic evaluation and were found only in the posterior segment in the choroidal tissues and inner sensory retina.

*T. gondii* Immunohistochemistry

One section from an eye from each cat was evaluated. A cyst morphologically similar to *T. gondii* was detected in the inner retina of one section (Fig. 4). There was no conclusive evidence of free antigens in the tissues examined.

DISCUSSION

In this feline model, intracarotid inoculation of a relatively small number of tachyzoites produced multifocal areas of choroidal and retinal inflammation with many similarities to ocular toxoplasmosis in humans. Among these were the predilection of lesion foci for...
the posterior pole of the eye; inflammation of the choroid, sensory retina, and, in some instances, overlying vitreous; associated mild anterior uveitis; production of chorioretinal scars with the presence of *T. gondii* tissue cysts; and production of lesions with similar histopathologic features, including the type of inflammatory cell infiltrate.\textsuperscript{15} The cat model described in this article differs from typical human ocular toxoplasmosis because it is primary choroidal versus retinal in nature, a primary infection versus a reactivation of a congenitally acquired infection, and multifocal and self limiting. The latter characteristic likely reflected the relative virulence of the organism used in the study and the effective immunologic control of infection by the cats.

The pathogenicity of *T. gondii* in experimental models of ocular infection relates to several factors, including experimental host species, virulence of the strain, inoculum dosage, stage of the organism, and route of inoculum administration.\textsuperscript{2,10} Intracarotid inoculation of tachyzoites in rabbits with the virulent RH strain has produced chorioretinal lesions similar to those described in this article; however, concomitant meningoencephalitis and rapid death usually occurred, preventing detailed and sequential observations of resultant lesions.\textsuperscript{14} Meningoencephalitis also may develop in rabbits after suprachoroidal inoculation of even low numbers of tachyzoites of virulent *T. gondii* strains.\textsuperscript{3,15} Previous experimental infections of cats with the RH strain of *T. gondii* produced a high mortality rate with subcutaneous or intraperitoneal inoculation with a larger number of tachyzoites or tissue cysts. Subcutaneous, intravenous, or intraperitoneal routes of inoculation in these studies also appeared to be associated with inconsistent ocular tissue infection and posterior segment lesion production.\textsuperscript{16–21} Oral administration of oocysts or tissue cysts in cats likewise produces only sporadic ocular lesions.\textsuperscript{16,19,22,23} In the experiment described in this article, in which a low number of tachyzoites of a strain of *T. gondii* that is mildly virulent in mice was used, systemic disease was not produced, and clinically apparent lesions were confined to the ocular tissues.

A congenital murine model of ocular toxoplasmosis involving inoculation of an avirulent strain of *T. gondii* in pregnant mice also has been characterized.\textsuperscript{24} Although this murine model mimics the likely time of exposure and establishment of tissue cysts in humans with ocular toxoplasmosis, it has the disadvantages of producing varying degrees of severity and types of ocular lesions, having retinal lesions that do not resemble the classic retinochoroiditis seen in people, and being difficult to monitor clinically in the small mouse eye. Unlike the well-described animal models of ocular toxoplasmosis that involve inoculation of tachyzoites into the suprachoroidal space or the vitreous adjacent to the retina through a pars plana approach,\textsuperscript{3,4,25} the method used in this experiment does not have the potential for artifactualy disrupting the integrity of the vitreous cavity and causing mechanical damage to the retina. The anterior segment inflammatory response in these cats was mild and did not preclude examination and photography of posterior segment lesions. Although intracarotid inoculation does not mimic the route and time period of natural exposure to *T. gondii* in humans, it probably mimics the route of entry of parasites into the eye (ie, through the vascular system). In this study, intracarotid inoculation was used as previously suggested to concentrate microbes to ocular tissues, resulting in more predictable experimental lesions.\textsuperscript{26}

Posterior segment lesions induced in these cats originated in the choroidal layers (including the tapetum) and involved the sensory retina secondarily. Lesions had a predilection for the central superior fundus, an area that contains the tapetum in the cat, a modified, reflective, cellular layer of inner (vitread) choroid between the larger choroidal vessels and choriocapillaris. Blood-borne pathogens may localize in tapetal tissues preferentially because of rapid slowing and turbulence of blood flow and subsequent embolization created by the perpendicular path of the choroidal capillaries as they traverse the tapetum.\textsuperscript{26} Aside from the presence of a cellular, choroidal tapetum, in most respects, the anatomic features of the feline posterior segment and retina resemble those of human and nonhuman primates, including a duplex retina with a well-defined area centralis (analogue of the human macula) and holangiotic retinal vascular pattern.\textsuperscript{57} The primary choroidal involvement of lesions seen in this experiment contrasts with the disease in humans (and some cases of naturally occurring ocular toxoplasmosis in cats), in which the retina is parasitized preferentially and choroidal involvement is secondary. Lesions produced here more closely resemble the less common outer punctate retinal lesions described in people with ocular toxoplasmosis;\textsuperscript{28} however, overlying retinal inflammation was found clinically and histologically in these cats, and *T. gondii* tissue cysts in the posterior segment were detected histologically with immunohistochemical and mouse inoculation studies. This would suggest that studies of recurrent toxoplasmic lesions of the retina could be conducted in this model system.

Histopathologic and immunohistochemical evaluation found only rare tissue cysts within ocular tissues, and most inflammatory lesions showed no organisms. Tissue cysts within ocular tissues also have been found to be rare in mice and hamsters experimentally inoculated with the same strain of *T. gondii* used in this study;\textsuperscript{19,30} this occurred despite the finding that the ME49 strain characteristically produces high numbers.
of cysts in the brains of chronically infected mice. However, more sensitive means of identification, including polymerase chain reaction and Southern blot hybridization, detected replicating organisms in ocular tissues in the mouse model of ocular toxoplasmosis.\(^8\) Mouse inoculation studies of retinal–choroidal tissue suspensions in the cats in this study did detect \(T. gondii\) tissue cysts in four of four eyes assayed. Additional studies of tissues in the more acute phases of chorioretinitis are needed to determine whether the chorioretinal lesions described in these studies or the cats in the current study resulted solely from tachyzoite replication or other more complex mechanisms related to hypersensitivity.

Serologic evaluation of inoculated cats showed production of serum IgM antibodies as early as 7 days PI, and detectable IgM antibodies were present in all cats by day 11 PI. This time interval correlated closely with the initial development of posterior segment lesions. The discovery of IgM antibody has been useful in the early detection of acute, naturally occurring toxoplasmosis in humans\(^9\) and cats.\(^8,9\) \(T. gondii\)–specific IgM antibodies were not detected in the aqueous humor at day 70 PI; it has not been determined whether IgM was produced locally early in the course of the infection. Naturally infected \(T. gondii\)–seropositive cats with suspected ocular toxoplasmosis commonly have local production of IgM in aqueous humor.\(^9\) In contrast to IgM, the Goldmann-Witmer coefficient for IgG antibodies was greater than 8 in 11 of 14 eyes, confirming the local production of antibodies in most cats. This contrasts with a previous study in rabbits inoculated with \(T. gondii\) in the suprachoroidal space, in which \(T. gondii\)–specific IgG was detected inconsistently in aqueous humor.\(^9\) Differences in the studies may have resulted from the route of inoculation, serologic testing methods, strain of \(T. gondii\) used, time of evaluation PI, and differences in immunologic responses between cats and rabbits. These findings support the use of aqueous humor antibody-detection techniques in cases of naturally occurring feline ocular toxoplasmosis.

Circulating toxoplasma antigens have been detected by the enzyme-linked immunosorbent assay in cases of acquired toxoplasmosis in humans\(^34\) and, along with the development of IgM assays, have increased the specificity of diagnosis of acute toxoplasmosis in some cases. \(T. gondii\)–specific serum antigens were detected only sporadically in the serum of four of seven cats during the 70-day study period. Neither aqueous humor nor serum proved to be reliable sources of detectable antigen in a previous rabbit model of ocular toxoplasmosis,\(^33\) although vitreous \(T. gondii\) antigen was present in active posterior segment lesions. Together, these findings suggest that, although current enzyme-linked immunosorbent assay antigen-detection techniques are highly specific, the sensitivity somewhat limits the diagnostic utility. More sensitive techniques, such as the polymerase chain reaction, probably will be necessary for accurate detection of \(T. gondii\) in body fluids of patients with toxoplasmosis.\(^30,35\)

Although the cat is the definitive host (and therefore may shed oocysts after inoculation) for \(T. gondii\), it also may serve as an intermediate host. As in other intermediate hosts, after initial exposure there is dissemination of the parasite to various extraintestinal tissues, tachyzoite replication, and formation of tissue cysts in the cat.\(^9\) Similar to humans, most cats exposed to \(T. gondii\) have protective immunity develop, remain latently infected with tissue cysts, and are thought to manifest disease only while in an immunocompromised state.\(^16,36\) Although the cat is the definitive host for \(T. gondii\) and may differ from humans in some undefined manner with regard to its immunologic response to the organism, the primary humoral\(^8,17,37\) and cellular\(^8,39\) immune response to \(T. gondii\) in cats has been shown to be similar to that in humans.

For many years, veterinary ophthalmologists have recognized naturally occurring ocular disease due to \(T. gondii\) in the domestic cat. The naturally occurring syndrome in cats shares many common features with human ocular toxoplasmosis, including the tendency of the associated lesions to affect the retina and, specifically, the posterior pole of the eye; the presence of tachyzoites and encysted forms of \(T. gondii\) in the inflammatory retina foci; the common occurrence of toxoplasma retinitis in humans and cats with other systemic disease or a compromised immune status; frequent recurrence; and a refractory nature toward pharmacologic management.\(^32,40-43\) Investigation of naturally occurring feline ocular toxoplasmosis and the experimental model described here could provide pertinent information regarding the pathogenesis of human ocular toxoplasmosis. Additionally, the presence of a feline immunosuppressive lentivirus (feline immunodeficiency virus), with biochemical, molecular, and clinical characteristics similar to human immunodeficiency virus,\(^44\) may provide insight into the interaction of Retroviridae and secondary opportunistic infections, such as toxoplasmosis, that involve the eye. Prior experimental infection with an immunosuppressive virus in cats inoculated with \(T. gondii\) may elucidate mechanisms whereby immunocompromised people are more susceptible to primary ocular infection.\(^45\) Alternatively, suprainfection with feline immunodeficiency virus in cats chronically infected with \(T. gondii\) may provide a useful means to investigate the immunologic alterations responsible for reactivation of latent tissue cysts and recurrent ocular toxoplasmosis.
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**Key Words**
toxoplasmosis, chorioretinitis, feline, animal model, fluorescein angiography

**References**

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