Acquired Retinochoroiditis in Hamsters Inoculated With ME 49 Strain Toxoplasma

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Purpose. These studies were undertaken to establish an animal model for use in studies of ocular toxoplasmosis. An animal model is needed to examine the development, progression, and resolution of ocular Toxoplasma infections and to study the effects on the disease of currently used and experimental therapies.

Methods. Cysts of the ME 49 strain of Toxoplasma gondii were injected intraperitoneally into each of 60 golden hamsters. The hamsters’ eyes were examined before inoculation and at intervals after inoculation, and fundus photographs were taken. Histologic sections were analyzed and photographed to document the ocular effects of the infection.

Results. Retinochoroiditis was found in both eyes of all hamsters within 2 to 3 weeks of inoculation. The disease resolved spontaneously without treatment and was quiescent in most cases at 12 weeks after inoculation. The animals remained in good general health, and those tested had high antibody titers to Toxoplasma (1:256 to 1:32,000) at 6 months after the infection. The discovery of cysts and lesions in the retina confirmed the diagnosis.

Conclusions. Although the lesions were not identical to those of human disease, this animal model of ocular toxoplasmosis offers several advantages: reproducibility, short incubation time, spontaneous resolution without treatment, consistent production of cysts, and ease of inoculation intraperitoneally without intracocular injection. Invest Ophthalmol Vis Sci. 1995;36:2166-2175.

Infection with Toxoplasma gondii is widespread; it has been estimated that approximately 500 million people throughout the world have antibodies to the organism.1 The infection is usually asymptomatic.2 However, congenital toxoplasmic ocular disease has long been recognized in offspring of mothers infected during pregnancy.2,3 The ocular disease recurs frequently, with potentially blinding episodes. The reasons for recurrences are not known, but therapy does not seem to prevent recurrence or alter the course of the disease.

The severity of ocular Toxoplasma infections in normal persons is overshadowed by the devastating disease that occurs in immunocompromised patients.4 Therapy with sulfadiazine and pyrimethamine may hold the disease in check, but relapses occur when treatment is withheld because of its serious side effects.4-6 Despite the potential severity of toxoplasmosis, the efficacy of currently used drugs and of those under development is difficult to evaluate because of the variable course of the disease and the frequency of spontaneous resolution of the lesions as well as the difficulties of using controls in human research.

A small animal model is needed for large-scale, controlled studies of the development, progression, and resolution of ocular Toxoplasma infections in response to various treatments. Efforts to find such a model have been made in the past.7-14 Of particular interest to us was the work of Frenkel,8,15 who found frequent but sporadic ocular disease in golden hamsters several months after inoculation with the RH or CJ strains of Toxoplasma. However, the animals succumbed to encephalitis unless they were treated with sulfonamides. In our search for an appropriate animal model, we chose to reinvestigate the hamster model.
Animal Model of Ocular Toxoplasmosis

Animal Experiments

To maintain stocks of ME 49 strain cysts, the brain tissue of a previously infected mouse was ground in a mortar and pestle and suspended in 1 ml Hanks’ balanced salt solution (HBSS) containing 10 μm streptomycin and 10 U penicillin. The cysts were counted (see next paragraph), and the desired number was inoculated intraperitoneally into other mice.8

Counts of Cysts

To count cysts, each mouse brain was homogenized in 1 ml HBSS with streptomycin and penicillin as noted above. An aliquot of 0.05 ml (containing 1/20 of the total brain tissue) was placed on a microscope slide and covered with a 22 × 40 mm coverslip. The number of cysts was counted by scanning the entire slide by light microscopy, using a ×10 objective. The approximate number of cysts per 1 ml suspension and per brain were calculated.

Inoculation of Hamsters

In preliminary experiments, a few pathogen-free golden Syrian hamsters (Charles River Laboratories, Wilmington, MA) were inoculated intraperitoneally with approximately 10 to 25 cysts of the ME 49 strain. Some were treated with sulfadiazine (4 mg/ml in their drinking water) starting at 3 days after inoculation, in a modification of the method of Frenkel.8 Treated animals did not develop ocular disease, but untreated animals developed retinochoroiditis. Because ME 49 is a relatively avirulent strain, we withheld treatment and gradually increased the dosage of parasites. All hamsters, whether inoculated with 10, 25, 50, or 100 cysts, developed ocular disease without systemic illness. An inoculum of 100 cysts produced more cysts in brain and eye than smaller inocula of 10 to 25 cysts. An appropriate dosage was, therefore, determined to be approximately 100 cysts and was contained in 0.2 ml of brain homogenate from previously infected mice. This amount was inoculated intraperitoneally into each of 60 additional pathogen-free golden hamsters weighing between 140 and 200 g.

Diagnostic Examinations

Fundus observations were made with a 30-D, aspheric, handheld lens in combination with an indirect ophthalmoscope. All animals underwent retinal examinations before inoculation and then at intervals starting at 1 week and ending at 12 weeks after inoculation. A final examination was made at 24 weeks. The pupils were dilated with one drop each of 1% tropicamide and 2.5% phenylephrine. To document the findings, we anesthetized the animals when necessary with ketamine (4 mg/100 g) and xylazine (0.5 mg/100 g) and took fundus photographs with a Zeiss fundus camera (Carl Zeiss, Oberkochen, Germany).

The general health of the animals was monitored three times a week by checking temperature, eating habits, and general activity. Blood drawn from eight hamsters was tested for antibodies to Toxoplasma by the Sabin–Feldman dye test.17

Histologic Studies

At 6 months after inoculation with ME 49 strain of Toxoplasma, 10 hamsters were exsanguinated while under deep ether anesthesia and were then perfused with HBSS followed by 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4. The eyes and brains were removed. Half of each brain was used for counts of cysts. Each eye was bisected into anterior and posterior halves, and each lens was removed. Fixation was continued for 2 days in 0.2% formaldehyde in phosphate-buffered saline, pH 7.4, at 4°C, then the eyes were refixed in 4% formaldehyde as described above before dehydration in acetone and embedment in JB-4 plastic (Polysciences, Warrington, PA). Three-micrometer to 4-μm sections were cut from the posterior eye cups. Alternate sections were mounted serially on two different sets of numbered slides. One set was stained with hematoxylin and eosin; the alternate set of sections was stored at 4°C until stained by immunocytochemical methods (see next paragraph). Cysts were counted in each hematoxylin and eosin section of five retinas, and their locations and other pathologic findings were recorded.

Immunocytochemistry

Three-micrometer sections of JB-4-embedded specimens were cut onto 0.5% ammonia water and mounted on glass coverslips. Immunocytochemical staining was performed by a modification of the method of Beckstead et al.18 Sections were digested in 0.125% trypsin in saline without ethylenediaminetetraacetic acid for 10 minutes at 37°C, washed in several changes of calcium- and magnesium-free phos-
plasma vitritis and rarely severe vitritis that clouded the view were present around arteries and veins and in retinal weeks after inoculation, the whitish lesions enlarged and had diffuse, indistinct edges (Fig. 2). The lesions progressed and became more active between 2 and 4 weeks after inoculation, the whitish lesions enlarged and had diffuse, indistinct edges (Fig. 2). The lesions were present around arteries and veins and in retinal tissue remote from major vessels.

After 4 to 5 weeks, the disease reached its peak in most animals (Fig. 3). There was often vasculitis at the height of the activity. Occasionally, there was minimal vitritis and rarely severe vitritis that clouded the view of the fundus. There were both retinal and vitreous hemorrhages in severely affected animals. The clinical manifestations were not like those of a typical focal necrotizing retinitis in humans; in the hamster, vitritis and vasculitis were less pronounced than in humans. In most cases, regression began by 8 to 9 weeks after inoculation. The vitreous inflammation, if present, became less intense, and the lesions started to resolve. By 12 weeks after inoculation, the lesions were usually quiet, leaving whitish areas in retinas that appeared largely atrophic (Fig. 4), except for small areas where lesions were regressing. In other cases, a few older lesions had dark clusters of pigment granules, and occasionally there was recrudescence of lesions before final quiescence.

One animal developed a unilateral cataract at 12 weeks after inoculation because of severe vitritis. Nevertheless, that hamster and all the others remained in good general health without signs of systemic disease, even when ocular disease was severe. At 6 months, the antibody titer was 1:256 in one hamster, and it ranged from 1:8,000 to 1:32,000 in seven others tested.

**RESULTS**

**Clinical Findings in Hamsters**

The course of the disease varied among hamsters, even in those inoculated on the same day with the same dose of a standard inoculum. Nonetheless, all 60 developed multiple lesions in both eyes. In general, the infection progressed as follows. No retinal disease was observed by indirect ophthalmoscopy at 1 week after inoculation, but retinal lesions were present in both eyes of each animal by 2 to 3 weeks after inoculation. One to 10 small, white, individual lesions were then seen in the inner retina, primarily at the posterior pole (Fig. 1). Sizes varied from pinpoint lesions to lesions as large as the optic disc. As the disease progressed and became more active between 2 and 4 weeks after inoculation, the whitish lesions enlarged and had diffuse, indistinct edges (Fig. 2). The lesions were present around arteries and veins and in retinal tissue remote from major vessels.

At the other extreme was a hamster with 70 cysts in the posterior eyecup in these three animals, totaling 24 cysts. Of these, 15 were in the inner nuclear layer of the retina, two each were in the retinal inner plexiform layer, choroid, and extraocular muscle, and two were at the edge of the vitreous. The remaining cyst was in an extensively damaged area and could not be assigned a definite location. Except in severely affected tissue, there was little or no inflammation immediately adjacent to the cysts (Figs. 6, 7).

**Histology of the Retinal Lesions**

Several retinal lesions were observed (Figs. 8 to 10), including an extensive one measuring more than 1 ml. The lesions usually were limited to the retina and retinal pigment epithelium and were characterized by abrupt thinning of the retina and loss of definable layers (Fig. 8). In two lesions (Figs. 8 and 10), Bruch’s membrane formed a barrier between the disrupted retina and the inflamed but intact choroid below in which there were moderate to extensive infiltrations of mononuclear leukocytes.

In a third instance, a punctate disruption in a
**FIGURE 1.** Focal area of inflammation in the retina, without vitreous involvement, 2 weeks after inoculation.

**FIGURE 2.** Multiple foci of inflammation of the retina, with evidence of inflammation anterior to retinal vessels, 3 to 4 weeks after inoculation.

**FIGURE 3.** Focus of severe retinal inflammation with minimal vitreous involvement, 5 weeks after inoculation.

**FIGURE 4.** Largely atrophic retina with diffuse changes at the level of the retinal pigment epithelium, 12 weeks after inoculation.
chronically inflamed area of the retina penetrated Bruch’s membrane (Fig. 9). There was chronic inflammation in both the retina and the thickened choroid (Figs. 8 to 10). Macrophages engorged with pigment granules were among the photoreceptors in several areas of the retina (Figs. 6, 8).

Severely Affected Animals

In one case, there was moderate vitritis with marked vitreous traction (Fig. 11) and moderate numbers of mononuclear leukocytes at the inner limiting membrane and at the edge of the vitreous (Fig. 12). In severely affected cases, there were both retinal and vitreous hemorrhages. Retinal hemorrhages sometimes separated the photoreceptors from the retinal pigment epithelium (Fig. 13). There were also extensive hemorrhages into the vitreous with displacement of the normal structures of the eye (Fig. 14). In severe cases, there were massive infiltrations of polymorphonuclear leukocytes, mononuclear leukocytes, lymphocytes, and plasma cells that replaced much of the normal tissue so that most landmarks were lost. As a result, it was sometimes difficult to define the localization of cysts. The inflammation in the choroid tended to be less severe; there were few polymorphonuclear leukocytes among the predominant mononuclear leukocytes. Staining with specific antibody verified that the cysts in the retina were cysts of *T. gondii* (Figs. 15, 16).

DISCUSSION

Our hamster model of toxoplasmic disease has distinct advantages over previous models for several reasons. First, because all 60 animals developed disease in both eyes within 2 to 3 weeks of inoculation, we conclude that the ME 49 strain of parasite consistently produces ocular disease in the hamster. Second, the short incubation eliminated time-consuming and costly waiting periods. Third, the disease resolved spontaneously over time, without treatment, as it does in humans when immunity is not impaired. Fourth, the disease consistently induced the production of cysts in the eye. Fifth, the infection was introduced through the peritoneal cavity, without breaching the ocular barrier, so that the ensuing disease was not complicated by trauma to the eye and inflammation. Last, although the hamster is small (between 140 to 200 g), its eyes are large enough to allow fundus photography to document the progression of the infection.
Hamster Disease Compared to Human Disease
In hamsters and humans, systemically acquired toxoplasmic infection has a predilection for the eyes, often without producing other disease. Cysts are found in the retinas of both species, primarily in the inner nuclear layer. In fulminating disease of hamsters and humans, there is frequently vitreous clouding with traction and sometimes vitreous hemorrhage and opacity.

Although there are similarities between hamster and human disease, there are also some differences. In particular, the appearance of the ocular lesions was different between the two species. In humans, the acute lesions are gray or grayish yellow with an indistinct border. Most of the lesions are in the posterior retina and may be accompanied by vascular sheathing, vitreous opacity, and inflammation in the choroid. When the inflammation subsides, the lesions become more distinct and the sclera may become visible through the necrotic retina and choroid. Pigment granules may be prominent, especially at the margins of the scar. In quiescent disease after healing, the central areas may be atrophic.9

In the initial stages of ocular disease in the hamster, the appearance of the lesions is similar to human disease. However, necrosis in lesions of the hamster was usually limited to the retina with chronic inflammation in the choroid so that in quiescent disease the retina appeared atrophic. There were few pigmented lesions. In contrast to human disease, the disease in the hamster was bilateral and multifocal. In some hamsters, there was extensive fibrosis of the retina at the final 6-month examination. Although marked vitritis may be considered a hallmark of human infection, vitritis in the hamster was not usually severe enough to prevent visualization of the fundus.

Other Animal Models
Frenkel8 was the first to report ocular symptoms in hamsters inoculated with RH or CJ strains of Toxoplasma gondii, Strain ME 49

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plasma, but he was unable to produce progressive chronic infection without treatment. His usual protocol was inoculation of the parasites intraperitoneally or subcutaneously, followed by treatment with sulfadiazine on the day of inoculation and for 10 to 30 days thereafter. He found that ocular lesions usually appeared when the virulent RH strain was used as inoculum, although additional sulfadiazine was sometimes needed to control the encephalitis that often resulted. The less virulent CJ strain did not consistently produce ocular lesions.

A number of investigators have used intraocular injection of Toxoplasma parasites to produce infections. This technique has the disadvantage of disrupting the blood–ocular barrier. Furthermore, it is difficult to perform and may cause unintentional damage to intraocular structures, including the retina. In addition, the trauma of injection, although usually minimal, could produce inflammation, with vascular dilation and the influx of inflammatory mediators, cells, and other modifying factors into the arena of the infection. The effects of these events on the subsequent course of the disease are unknown.

Despite these reservations, there have been a number of successful attempts to produce chorioretinitis with intraocular injections. Using a primate model, Culbertson et al. injected living Beverley strain Toxoplasma gondii onto the macula of cynomolgous monkeys and produced toxoplasmic retinitis, along with mild iritis, vitritis, and retinal vasculitis. The retinal lesions enlarged during the first 10 days; after 1 month, they gradually resolved without treatment, providing a good model of the human disease.

In another potentially useful model, Davidson and colleagues reported the production of experimental ocular toxoplasmosis in cats, using the ME 49 strain that we used in our studies. They found bilateral ocular lesions in seven cats inoculated with tachyzoites that we used in our studies. They found bilateral ocular lesions in seven cats inoculated with tachyzoites in the common carotid artery. Progressive, bilateral granulomatous chorioretinitis developed within 5 to 8 days of inoculation and resolved between 21 and 70 days. Immunoglobulin M antibodies appeared at 7 days, and immunoglobulin G antibodies appeared at 13 days. There was no overt evidence of systemic illness except for a mild increase in temperature in three cats.

Despite the apparent success of these experiments with cats, the suitability of the cat as an animal model for the study of toxoplasmic ocular disease is uncertain. Seven cats is a small sample. Furthermore, the cat is the primary host for Toxoplasma, not an intermediate host like humans, the hamster, and other vertebrates. Toxoplasma organisms undergo a complex sexual cycle of development in the cat so that it is unique as a host. What effect, if any, sexual forms of the parasite might have on the development of immunity or the ocular infection in the cat remains to be established.

It may be significant that the ME 49 strain of Toxoplasma used in Davidson’s experiments on cats and in ours on hamsters consistently produced ocular disease in every inoculated animal. This strain may prove to have a predilection for ocular tissues. It is thought to have been isolated originally from sheep (Lunde MN, personal communication, 1991).

Thus far, only two cyst-forming strains of the parasite in vivo have been studied by electron microscopy. Cysts are initially formed within host cells. The membrane of the parasitophorous vacuole becomes the outer limit of the cyst wall, which is gradually thickened by deposits of a dense matrix. Consequently, at early stages of their development, all cysts are intracellular. Reportedly, cysts of the SRA strain are always intracellular in neurons of the brain as long as 22 months after inoculation. However, using the ME 49 strain of Toxoplasma, we observed that cysts in mouse brain were extracellular by 17 days after inoculation, the earliest time an adequate sampling was obtained. These findings indicate that the environments of the two strains of cysts are markedly different. Cysts of the SRA strain are protected by host cells from circulating antibodies, drugs, and a myriad of inflammatory mediators from other cells that might enter their environment. In addition, organisms within intracellular cysts may derive essential growth factors from their host cells. Conversely, cysts of the ME 49 strain have only their own protective thickened wall to shield the enclosed bradyzoites from hazards in their environment. They use previously stored nutrients. These characteristics may be factors in the relative avirulence of the ME 49 strain. It is an advantage that the ME 49 strain is relatively avirulent so that infected animals survive with few or no symptoms other than ocular ones, allowing prolonged examination through the window of the eye of the progression and resolution of the disease and its response to experimental manipulation.

In conclusion, the hamster model of ocular toxoplasmic disease, using the ME 49 strain of Toxoplasma, offers distinct advantages over other animal models. This experimental infection is reproduced easily, has a short incubation time, resolves spontaneously, and produces a readily monitored ocular disease.

Key Words
animal model, ocular toxoplasmosis, opportunistic infection, retinochoroiditis, Toxoplasma gondii

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References