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EXPERIMENTAL MODELS OF OCULAR INFECTION WITH *TOXOPLASMA GONDII*

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Ocular toxoplasmosis is a vision-threatening disease and the major cause of posterior uveitis worldwide. In spite of the continuing global burden of ocular toxoplasmosis, many critical aspects of disease including the therapeutic approach to ocular toxoplasmosis are still under debate. To assist in addressing many aspects of the disease, numerous experimental models of ocular toxoplasmosis have been established. In this article, we present an overview on *in vitro, ex vivo,* and *in vivo* models of ocular toxoplasmosis available to date.

Experimental studies on ocular toxoplasmosis have recently focused on mice. However, the majority of murine models established so far are based on intraperitoneal and intraocular infection with *Toxoplasma gondii*. We therefore also present results obtained in an *in vivo* model using peroral infection of C57BL/6 and NMRI mice that reflects the natural route of infection and mimics the disease course in humans. While advances have been made in *ex vivo* model systems or larger animals to investigate specific aspects of ocular toxoplasmosis, laboratory mice continue to be the experimental model of choice for the investigation of ocular toxoplasmosis.

Keywords: *Toxoplasma gondii,* ocular toxoplasmosis, experimental models of toxoplasmic retinochoroiditis

Introduction

Ocular toxoplasmosis (OT), a vision-threatening ocular disease caused by the protozoan parasite *Toxoplasma gondii* [1], is the most common cause of infectious uveitis. Up to 30% of the human population worldwide is infected with *T. gondii,* and around 2% of infected individuals in Europe and North America develop OT [2]. In spite of extensive research on epidemiology, immunology, and pathophysiology of the disease (comprehensively reviewed recently by Maenz et al.) [1], many aspects of OT still await an answer. One of the major factors limiting progress in understanding the pathophysiology and other aspects of OT is the difficulty to obtain samples from humans with (acute) infection due to the localization and clinical presentation of the disease. Therefore, the establishment of experimental models mimicking the course of OT in humans is essential as a springboard for investigation of the disease.

Parasite biology

Humans, as other animals, are intermediate hosts of *T. gondii*. The sexual cycle of the parasite is complex and explains some of the features of OT [3]. Oocysts shed by felines, i.e., cats, are ingested by an intermediate host and give rise to rapidly replicating tachyzoites that infect any nucleated cell. As infected cells die, they release tachyzoites that infect neighboring cells. Tachyzoites and the surrounding immune response both contribute to the signs and symptoms of OT. Under the pressure of the immune response of the host, intracellular tachyzoites convert into bradyzoites – slowly dividing forms of the parasite – which form intracellular cysts in muscle, brain, and eye. Cysts persist for prolonged times without causing apparent signs and symptoms; however, they form a lifelong reservoir in the host that can lead to reactivation of OT following cyst rupture [4].

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Epidemiology

Infection with *T. gondii* in humans typically occurs postnatally through the ingestion of raw or undercooked meat containing tissue cysts; infection may also be acquired through consumption of food or water contaminated with oocysts [3]. Transmission of the parasite during pregnancy may result in congenital toxoplasmosis often presenting as OT. Reactivation of OT may occur, and it has been hypothesized that most cases of reactivation occur in patients with postnatally acquired infection [5, 6]. However, the route of infection remains undetermined in as many as 70% of OT cases and has no impact on clinical signs or symptoms and patient management [7].

Seroprevalence varies among countries and ranges from 10 to 80% [8]. It reflects hygiene and eating habits of the population, such as consumption of raw meat. *T. gondii* shows a lower prevalence in cooler climates and at high elevations. The number of infected individuals has decreased recently in developed countries [3]. The majority of *T. gondii* strains in Europe and North America belong to one of the three clonal types (type I, II, and III) [9]. In Europe, type II strains are responsible for most of human infections including OT [10]. In South America, however, an abundance of recombinant and "exotic" strains associated with OT has been described [11]; the incidence of OT appears to be higher in South America [12]. The impact of the parasite strain on pathogenesis of toxoplasmosis in humans has not yet been fully understood [13]; a recent report from Germany found an association of clinical disease with atypical genotypes [9].

Clinical presentation

The presentation of OT appears to take a common course independent on the route of infection [6]. OT often remains unnoticed if only peripheral parts of the retina are affected [14]. Focal, unilateral retinal lesions are the most common signs of acute OT: in subjects with reactivation of OT, these lesions can often be found adjacent to old hyperpigmented retinal scars [15]. Choroiditis, vitreitis, retinal vasculitis, and inflammatory infiltrates in the anterior chamber are also commonly observed during the course of disease. OT may result in vision loss if the macula is involved [16]. In congenital OT, a predilection for macular involvement has been observed [17].

Fifty-seven percent of affected individuals experience recurrence within 2 years [18]. Pregnancy [19], the patient's age [20], or lesions to the retina [21] have been reported to trigger recurrence. However, the exact pathomechanism of recurrence still remains unknown.

In immunocompromized patients, OT is most often the result of reactivated infection due to rupture of tissue cysts and may present a broad clinical spectrum [4]. Bilateral lesions, occlusive vasculitis, diffuse necrotizing retinitis, and scleritis have been observed in these individuals [16].

Patient management

The diagnosis of OT is commonly based on clinical, fundoscopic observation. As the absence of *T. gondii* antibodies rules out the diagnosis of OT, serology (IgG, IgM, and other antibodies) is performed. In cases of doubt, detection of *T. gondii* DNA in the vitreous humor by polymerase chain reaction (PCR) may be of help [16, 22, 23].

OT often resolves without treatment. However, as many as 40% of uveitis specialists advise to treat all patients with acute ocular toxoplasmosis regardless of clinical manifestation [24, 25]. Macular disease and severe inflammation are considered strong indications for antiparasitic therapy [24]. The combination of pyrimethamine, sulfadiazine and folinic acid, or clindamycin as a monotherapy is frequently administered. Corticosteroids are added to the regimens to reduce inflammation [25, 26]. In patients with a high frequency of recurrence and in immunocompromised patients, maintenance treatment has been successfully used [1, 27].

Prevention of infection can be achieved by avoidance of primary infection, i.e., consumption of meat containing tissue cysts, or ingestion of contaminated food or water. A vaccine for use in humans does not exist.

A number of excellent reviews on ocular toxoplasmosis have been published [1, 2, 15, 16, 28, 29]. Other reviews covered specific aspects of OT such as diagnosis, pathogenesis, clinical features, recurrence patterns, impact of patient age, and treatment [20, 22, 30–34]. Since a large number of complex experimental models have been described, in the present manuscript we focus on experimental models of OT. We discuss experimental approaches using *in vitro, ex vivo,* and *in vivo* models reported in the literature and also present unpublished data from our own studies in C57BL/6 and NMRI mice orally infected with the ME49 strain of *T. gondii*.

Experimental models of ocular toxoplasmosis

OT has been investigated using both *in vivo, ex vivo,* and *in vitro* models of the disease. *Figure 1* presents an overview of *in vivo* and *in vitro* models.

In vitro models of ocular infection with *T. gondii*

Several *in vitro* models of OT involving human retinal pigmented epithelium [35–38] and human retinal endothelium [39, 40] have been investigated. The retinal pigmented epithelium model serves to study the immunopathogenesis of infection with *T. gondii,* e.g., secretion of pro- and anti-inflammatory cytokines and their influence on parasite replication. The retinal endothelium model system successfully mimics the biological barrier between blood

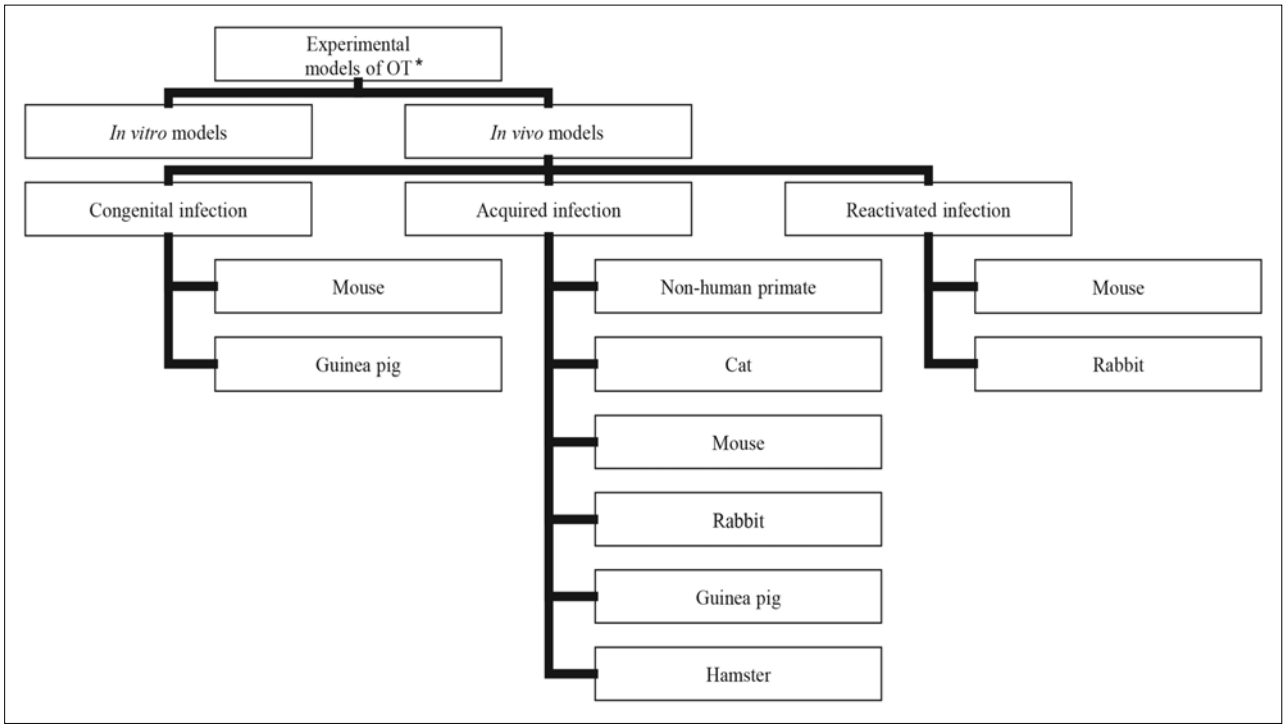

Fig. 1. *In vitro* and *in vivo* models of ocular toxoplasmosis. *****: *ex vivo* models of ocular toxoplasmosis are not depicted in the figure but described in the text

and retina, enabling the investigation of parasite crossing of the blood–retina barrier. The abovementioned models are also applicable to investigate the interaction between the host and different parasite strains, enabling a more in-depth look into parasite biology and the (immuno-)pathogenesis of OT.

Retinal pigmented epithelium (RPE) is a retinal layer situated between the neural retina and choroid. It takes an active part in the ocular pathology after infection with *T. gondii*. First, tachyzoites enter RPE during infection. Second, RPE upregulates the expression of MHC class I and MHC class II molecules on the cell surface to present parasite antigens in the eye. Third, it has been shown in an *in vivo* model that RPE migrates towards the inner layers of retina during the course of OT [41]. RPE cells secrete cytokines that take part in the immunopathogenesis such as interleukin (IL)-6, IL-8, and nitric oxide [38]. The secretion of IL-1β, GM-CSF, and ICAM-1, as well as anti-inflammatory cytokines TGFβ1 and TGFβ2, is also induced [36, 37]. In RPE culture, IFNγ was shown to suppress parasite replication [38].

The eye is a well-protected (immunoprivileged) site due to the blood–retina barrier. The exact mechanism of *T. gondii* migration through the blood–retina barrier remains unknown. Using human retinal endothelial cells, free tachyzoites were found to cross the retinal endothelium, and ICAM-1 facilitated this process [40]. Dendritic cells can taxi tachyzoites to the retina in a Trojan horse manner; ICAM-1, VCAM-1, and ALCAM as well as chemokines CCL21 and CXCL10 take part in the migration of infected dendritic cells to the retina [39].

Ex vivo models of ocular infection with *T. gondii*

Ex vivo models have been described to study different aspects of OT. As the development of OT following congenital infection is more frequent than after acquired infection [2, 15], the proliferation pattern of *T. gondii* using retinal explants from chick embryos was studied [42]. The activity of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), necessary for the synthesis of polyamines that are required for *T. gondii* proliferation and differentiation, was found to decline during retina maturation. Consequently, proliferation of *T. gondii* was lower in retinas acquired from older chick embryos. Recently, Furtado et al. developed a more holistic model to study OT. Posterior eyecups of cadaveric donors were infected with *T. gondii* tachyzoites allowing to observe the dissemination of the parasite within the human retina as a whole; parasites showed a predilection for infection of glial rather than neuronal retinal cells [43].

In vivo models of ocular infection with *T. gondii*

The first animal model of OT was established in 1951 by Hogan [44] and was based on intracarotid infection of rabbits. In 1953, Frenkel [45] managed to evoke OT via intraperitoneal infection of hamsters. To date, many other

animal species have been investigated as models of toxoplasmic retinochoroiditis including non-human primates, cats, rabbits, and guinea pigs. In recent years, the efforts to establish an *in vivo* model of OT have focused mainly on mice. Any animal model of OT has a set of specific features: animal species, parasite strain, stage and dose of the inoculum, and infection route. As the route of parasite inoculation influences the disease outcome, it will be addressed in detail in the next chapter.

Parasite entry

OT has been evoked via intracarotid, intraocular, intraperitoneal, and peroral infection of animals so far. An intracarotid injection of parasites was used in rabbits [44] and cats [46]. This inoculation route inevitably leads to OT in infected animals. On the other hand, intracarotid infection is difficult to perform in small animals and does not represent the natural route of infection in humans.

The intraocular parasite injection evokes OT in infected animals. This inoculation route has been used in non-human primates, guinea pigs, rabbits, and mice. It can be performed intracamerally, periretinally (through the pars plana), or intraretinally. This inoculation route causes mechanical damage to the retina which may interfere with the course of disease. Interestingly, even the administration of parasites onto the surface of the eye of a mouse leads to OT [47]. The latter method maintains the integrity of ocular tissues.

The intraperitoneal infection route is the most common route of infection in models of OT as it is easy to perform and successfully leads to development of toxoplasmic retinochoroiditis in hamsters [45, 48] and mice [41, 49–51]. However, the intraperitoneal parasite inoculation does not reflect the natural peroral route of infection in humans.

The peroral route has rarely been chosen. Hamsters infected orally with 100 cysts consistently developed OT within 4 to 8 weeks postinfection [52] whereas the intraperitoneal parasite inoculation evoked ocular pathology already 2 to 3 weeks postinfection [48]. In mice, histologic changes, characteristic of OT, were observed approximately 1 month post-peroral infection [53, 54]. Thus, development of pathology after peroral infection appears to be delayed compared to the intraperitoneal route; this is likely due to the fact that, during the peroral infection, the parasite is initially exposed to lymphoid barriers in the intestinal mucosa of the host. However, we found that peroral infection of NMRI and C57BL/6 mice with ME49 cysts evoked toxoplasmic retinochoroiditis within 14 to 21 days [this study].

Ocular toxoplasmosis in non-human primates, rabbits, hamsters, guinea pigs, and cats

Among all animals, the visual organ of monkeys most closely resembles the human eye in its anatomy and physiology [32]. Additionally, intraretinal infection is easily achieved in monkeys, and ophthalmoscopy can easily be performed due to the large size of eyes [55]. However, non-human primates are more susceptible to infection with *T. gondii* than humans [56]; furthermore, monkeys are costly and require proper housing conditions that cannot be granted in most laboratories.

Members of the *felidae* family also present with OT. However, unlike in humans, choroiditis is more pronounced than retinitis during the course of the disease [56]. In a cat model, it has been shown that treatment with clindamycin administered 7 days postintraperitoneal infection with *T. gondii* does not alleviate the course of retinochoroiditis. On the contrary, it aggravated signs of systemic disease [57].

The intraocular infection (transvitreal or suprachoroidal) combined with ophthalmoscopy to investigate the clinical course of infection can be easily performed in rabbits. Similar to humans, the retina and not the choroid is primarily affected in OT [58].

Hamsters also proved to be a good animal model of OT. They have been studied to assess the efficacy of atovaquone therapy in comparison with conventional therapies [59]. Hamsters develop retinochoroiditis after both intraperitoneal and peroral infection with *T. gondii* [48, 52]. In addition, retinochoroidal changes can be easily observed using ophthalmoscopy. On the other hand, hamsters often develop multifocal and bilateral ocular lesions, as opposed to humans presenting with OT. Vasculitis and vitreitis are also less pronounced in hamsters than in men [32].

Guinea pigs were one of the first animals used to study OT [60]. However, they have rarely been used for further investigations of the disease since then [61].

The *in vivo* models of ocular toxoplasmosis in animals other than laboratory mice are presented in *Table 1*.

Ocular toxoplasmosis in mice

A wide variety of murine models of ocular toxoplasmosis has been established. Most murine models are using the congenital, intraocular, and intraperitoneal infection. While far fewer studies have been published, the peroral inoculation route also successfully evokes toxoplasmic retinochoroiditis in mice.

Mice have many advantages that make them the current *in vivo* model of choice. They are easily accessible, their genome is completely sequenced, and mice with targeted deletions of genes (knock-outs) and a large number of immunological reagents are available. On the other hand, the anatomy of the murine visual organ varies from the human eye, i.e., mice lack a macula. This can be a major drawback as a predilection for macular disease can be observed during the course of congenital infection in humans. Additionally, due to the small organ size, it is necessary to pool aqueous humor samples from a large number of eyes to perform analyses [62].

Table 1. Animal models of ocular toxoplasmosis other than laboratory mice

Animal species	Parasite dose, strain, and stage	Inoculation route	Observations	References
Cat	5×10^3 ME49 bradyzoites	Intracarotid	Bilateral, multifocal retinitis and choroiditis 5 to 8 days postinfection	[46]
Guinea pigs	5×10^3 RH tachyzoites	Intraocular (posterior chamber)	Retinochoroiditis within 1 to 3 weeks postinfection	[60]
Monkey *(Macaca fascicularis)*	1×10^5 or 2.5×10^5 of living or heat-killed RH tachyzoites	Intraretinal and subcutaneous	Iridocyclitis, vitreitis, and retinal edema up to 2 weeks postinfection; retinal vasculitis 6 days postinfection; no necrotizing retinochoroiditis	[55]
Monkey *(Macaca fascicularis, Cercopithecus aethiops, Macaca mulatta)*	At least 1×10^5 RH-tachyzoites	Intraretinal	Retinitis, vitreitis, iridocyclitis, and keratic precipitates by 24 to 48 h postinfection; rare reactivation 3 weeks postinfection	[101]
Rabbit	5×10^3 BK tachyzoites	Primary subcutaneous infection; transvitreal challenge of infected rabbits vs. primary infection of naïve rabbits	Retinochoroiditis and vitreal infiltration post-transvitreal challenge in 100% of naive and 91% of primed animals; ocular disease more severe in naïve compared to challenged animals	[58]
Rabbit	RH or Beverly strain	Intraocular (suprachoroidal space)	Retinochoroiditis within 3 to 4 days postinfection. Inflammation resolved spontaneously. Cortisone therapy, but not epinephrine, local trauma, or challenge infection evoked disease recurrence	[102, 103]
Syrian golden hamster	100 ME49 cysts	Intraperitoneal	Bilateral, white lesions, varying in number and size 2–3 weeks postinfection; severe retinochoroiditis with vasculitis and, in some animals, vitreitis 4–5 weeks postinfection. No influence of commonly used therapy regimens (atovaquone, pyrimethamine with sulfadiazine, clindamycin, spiramycin) on the course of acute disease	[48, 59]
Syrian golden hamster	100 ME49 cysts	Peroral	Small inflammatory foci, varying in size, predominantly at the posterior pole 4 weeks postinfection	[52]

Murine models of congenital ocular toxoplasmosis as well as models of acquired ocular toxoplasmosis in immunocompetent and immunocompromized mice will be discussed in detail in the following chapters.

Models of congenital ocular toxoplasmosis

Congenital transmission has been reported in mice and hamsters more than 50 years ago [63, 64, 65]. Due to the similarities in transmission of the parasite, detailed investigation of ocular toxoplasmosis following congenital transmission was performed in mice by Hay and colleagues [66, 67]. Using subcutaneous inoculation of cysts, these authors reported the development of bilateral or unilateral cataracts. Shortly thereafter, ultrastructural studies revealed that the parasite was located in the inner retina, particularly the ganglion cell layer, but in no other ocular tissue [68], and pathological changes in diseased tissues ranged from low-grade mononuclear cell infiltration in the subretinal space to complete destruction of the outer retina, the retinal pigmented epithelium, and the choroid in the presence of a granulomatous inflammatory reaction [69]. Phagocytosis of photoreceptor outer segments by macrophages was observed, and macrophages and lymphocytes appeared to mediate photoreceptor lysis. Severely affected eyes exhibited vasculitis and inflammatory cell invasion into the vitreous, and a lymphoplasmacytoid cell infiltrate was present in the outer retina and choroid in these eyes.

More recently, congenital infection of mice with type II strains of *T. gondii* has been investigated in more detail; development of retinal lesions was observed 4 weeks after birth [70, 71]. In neonatally infected mice, these authors observed a shift from a proinflammatory Th17 type response in primary infection to a Th1/Th2/Treg immune response in reinfected (challenged) mice [72]. Following congenital infection, upregulation of glial fibrillary acidic protein (GFAP) and vimentin was ob-

served in glial cells in the eye associated with damage to the morphology of the eye, indicating that severe retinal damage and loss of vision observed in human OT may be impacted by reactive gliosis (Müller cell activation and photoreceptor depletion) in a mice model of congenital ocular toxoplasmosis [73].

The efficacy of azithromycin in reducing congenital transmission of *T. gondii* was investigated in large vesper

Table 2. Characteristics of acquired ocular toxoplasmosis in wildtype mice

Mouse strain	Parasite strain, stage, and dose	Inoculation route	Observations	Reference
C57BL/6 and MRL/MpJ	Primary infection: 10 PLK cysts Challenge: 50, 500, 5000, or 50,000 PLK tachyzoites	Peroral Intracameral	More severe ocular pathology in mice with primary vs. challenge infection Dose-dependent outcome in challenged mice 6 and 8 days postinfection	[82]
C57BL/6	5×10^3 ME49 bradyzoites	Instillation vs. intravitreal	Mechanical lesions following intravitreal infection; inflammatory changes, edema, and lacunae formation 7 days after infection independent of route of infection	[47]
C57BL/6, BALB/c, and CBA/J	Immunization: 1×10^5 ts-4 tachyzoites Challenge: 100 RH, PLK, or SAG1$^{-/-}$ RH tachyzoites	Intraperitoneal Intracameral	Severe inflammation in naïve C57BL/6 mice regardless of parasite strain 11 days postinfection; mild to moderate lesions in BALB/c and CBA/J mice following primary infection No or mild inflammatory changes in vaccinated mice	[79]
C57BL/6	50 ME49 cysts	Intraperitoneal	RPE migration 60 days postinfection	[41]
C57BL/6	30 ME49 cysts	Intraperitoneal	Parasites in retinal vessels, vasculitis, inflammatory infiltrates in retinal layers, lacunae formation by FTR; increases in IFN-γ and TGF-β, lower IL-10 concentrations in aqueous humor 30 days postinfection	[62]
BALB/c C57BL/6	10 to 20 ME49 cysts 4×10^2 -1×10^5 PTG/ME49 tachyzoites	Intraperitoneal or peroral Intraperitoneal	Retinochoroiditis within 2 to 4 weeks postinfection	[54]
C57BL/6	10 Fukaya cysts	Peroral	Increased IFNγ, IL-17A, CCL3, CCL4, CCL5, CXCL1, CXCL2, CXCL10, CCR5, CCR7, CXCR2, CXCR3, and ICAM-1concentrations in retinas	[89]
C57BL/6	10^6 RH tachyzoites	Intravitreal	Neutrophil- and mast cell retinochoroidal and vitreal inflammatory infiltrates as well as RPE vacuolization 24, 48, and 72 h postinfection; increased annexin A1 in RPE	[35]
C57BL/6	20 ME49 cysts	Intraperitoneal	Chronic ocular infection within 21 to 28 days postinfection; highly activated T cells, increased IFNγ, CXCL9, CXCL10, and CXCR3 expression	[95]
C57BL/6	5, 10, 20 ME49 cysts	Peroral	Unilateral or bilateral vitreitis, perivascular inflammatory infiltrates, inflammatory cells in ganglion cell layer, as well as RPE migration at 21 and 41 days postinfection; cysts in 50% of mice infected with 5 cysts, cysts in 80% of mice infected with 10 cysts	Current study
NMRI	20, 100 ME49 cysts	Peroral	Bilateral ocular toxoplasmosis 14 and 21 days postinfection, vitreal and retinal inflammation mostly in the ganglion cell layer, migration of RPE, cone formation, alteration of the retinal architecture; cysts in a small portion of histologic material	Current study

mice *(Calomys callosus)* [74] following peroral infection. Parasites were detected in fetuses' eyes in control mice and mice treated with pyrimethamine and sulfadiazine plus folinic acid but not in fetuses treated with azithromycin, indicating that azithromycin may represent an alternative treatment for toxoplasmosis during pregnancy.

Investigation of congenital toxoplasmosis in experimental models other than rodents is only rarely performed. Schoondermark et al. infected rhesus monkeys in the second (d90) or third trimester (d130) of pregnancy to study congenital transmission and the prenatal diagnosis of infection [75]. Transmission of *T. gondii* was proven in 44% of fetuses in the second trimester and in 33% of fetuses in the third trimester of gestation, and suspected in four additional fetuses (overall transmission rate 61%, similar to humans). Unfortunately, no reference was made to findings in the eye. Tedesco et al. [76] investigated fetal eyes following infection of pregnant C57BL/6 mice. While no parasite infestation was observed, histological analysis of prenatal fetal eyes from infected female mice revealed alterations in the outer and inner nuclear layer of the retina including edema characterized by the increase of interstitial spaces forming lacunae and a net of vessels associated with intense inflammatory infiltrates. These histological observations suggest that ocular lesions are not delayed manifestations of toxoplasmosis but develop early after congenital infection.

Models of acquired infection in immunocompetent mice

OT in mice successfully mimics the hallmarks of the disease in humans. Retinochoroidal lesions, cellular inflammatory infiltrates in the retina, choroid, and vitreous humor, as well as vasculitis, are consistently described in murine models of the disease [41, 47, 49, 50, 62, 77]. Usually, *T. gondii* cysts are found only in a small portion of the histologic material, mainly in the inner retinal layers, and are not associated with inflammatory infiltrates [49]. On the contrary, tachyzoites in the blood vessels, if present, are often accompanied by perivasculitis [41]. Migration of retinal pigmented epithelium towards the inner retinal layers is commonly described in the eyes of perorally [53], intraperitoneally [41, 49, 62], and intravitreally [47] infected mice. Other common features of ocular toxoplasmosis, such as alteration in the architecture of retinal layers [41, 62], cone formation by the photoreceptor layer and an increase in the interstitial spaces between the retinal cells have been noted [41]. Focal photoreceptor damage characterized by narrowing of the photoreceptor layer was also observed [49, 53]. Additionally, electroretinograms and fluorescein angiography showed no abnormalities after peroral infection of wildtype mice [53, 77].

For a number of reasons including ease of injection and direct infection of ocular cells, the intraperitoneal and intraocular routes of infection have most frequently been used to induce OT whereas the natural, peroral route has been rarely applied in mice so far. Characteristics of acquired toxoplasmosis in wildtype mice are described in *Table 2*.

Differences in susceptibility of various mouse strains to *T. gondii* infection have been shown. This is of importance, as even inoculation of a "nonvirulent" parasite strain may lead to mortality in inbred mice; mice considered susceptible to infection such as those on the H2-b background including C57BL/6 mice [78] will succumb to infection prior to development of ocular pathology depending on the numbers of parasites/cysts used for infection. On the contrary, inbred mice on the H2-d background such as BALB/c and all known outbred mice including the commonly used NMRI strain are considered resistant to the infection and do not develop apparent signs or symptoms of toxoplasmosis [79]. However, it must be noted that the diagnosis of migration of retinal pigmented epithelium is more difficult in albino (NMRI, BALB/c) mice.

Usually, type I (RH) and type II parasite strains (ME49, Beverly, Fukaya) are used to evoke OT in experimental models of toxoplasmosis.

As there is only limited immunological data on reactivating OT, Rochet et al. established a model of direct intravitreal injection of parasites in previously infected mice with a homologous type II strain to describe protective and deleterious response patterns [80].

In Swiss-Webster mice (relatively resistant to primary infection), parasite loads did not peak upon reinfection. In contrast, susceptible C57BL/6 mice showed high parasite loads 7 days after reinfection associated with marked deterioration of retinal architecture. Immune responses were characteristic for a Th2 response in resistant compared to a strong Th1/Th17 response in susceptible mice. Neutralization experiments confirmed the protective and deleterious roles of IFN-γ and IL-6, respectively, for parasite control and retinal integrity during reinfection.

Table 3. Study design to investigate OT in mice following oral infection

Mouse type	No. of mice infected	Parasite dose, strain, and stage	Dissection days (postinfection)
C57BL/6	16	5 ME49 cysts	14, 21
	32	10 ME49 cysts	13, 21, 25, 41, 59
	10	20 ME49 cysts	10
NMRI	5	20 ME49 cysts	14, 21
	5	100 ME49 cysts	14, 21

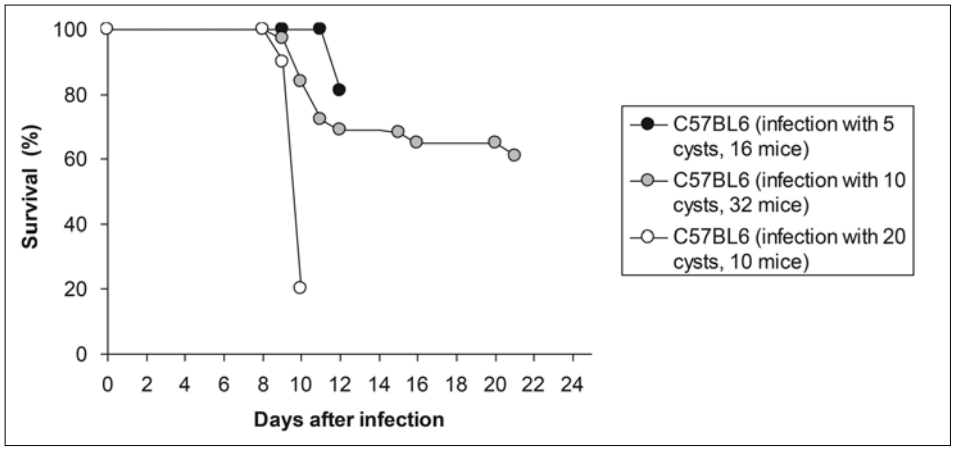

Fig. 2. Survival of C57BL/6 mice infected perorally with 5, 10, and 20 cysts (ME49) of *T. gondii*

Peroral infection of C57BL/6 and NMRI mice with ME49 T. gondii *cysts*

To further investigate the use of the natural peroral route of infection in mice, we perorally infected C57BL/6 mice with 5, 10, or 20 ME49 cysts and compared results with experiments in NMRI mice infected with 20 and 100 ME49 cysts perorally. Mice were sacrificed 14 to 59 days postinfection as shown in *Table 3*.

Using a protocol described elsewhere [81], we confirmed the presence of anti-*T. gondii* IgG antibodies in sera of all mice. Susceptibility of mice to infection differed depending on the mouse strain and inoculum. NMRI mice all survived without signs and symptoms of infection whereas susceptibility of C57BL/6 mice depended on parasite inoculum. Survival of C57BL/6 mice following peroral infection is presented in *Fig. 2*. Of interest, the 20% mortality observed in mice infected with only five cysts of the ME49 strain points towards a rather virulent ME49 strain passaged in our laboratory in latently infected BALB/c mice. Decreasing survival was observed with increasing inoculum, and only 20% of C57BL/6 mice infected with 20 cysts survived the acute stage of infection. These results are not surprising as the correlation of mu-

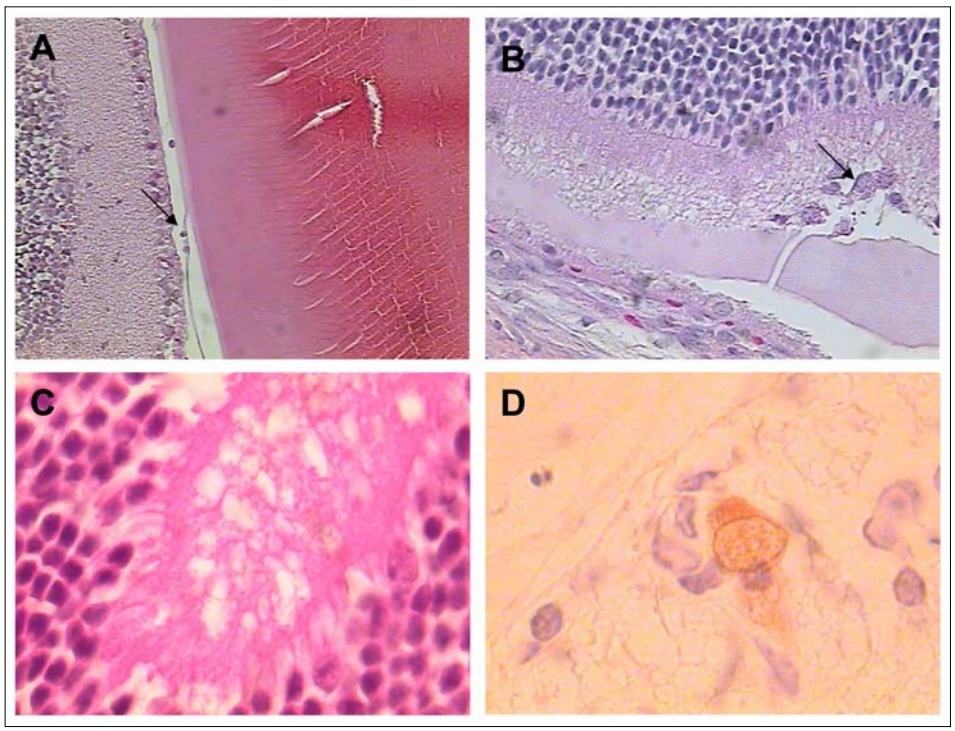

Fig. 3. Ocular histopathology in the eyes of NMRI mice following peroral infection with *T. gondii* cysts: (A) Mild inflammatory infiltrate in the vitreous humor of a mouse infected with 20 cysts 21 days postinfection (magnification ×20); (B) RPE migration to the photoreceptor layer (arrow) in a mouse infected with 20 cysts 21 days postinfection (magnification ×20); (C) cone formation in the eye of a mouse infected with 100 cysts 21 days postinfection (magnification ×100); (D) *T. gondii* cyst at the boarder of a ganglion cell layer and internal plexiforme layer in the eye of an NMRI mouse infected with 100 cysts 21 days postinfection (PAP stain [84], magnification ×100)

rine survival and parasite inoculum was already described in a model based on intraocular [82] and peroral *T. gondii* infection [83].

All NMRI mice infected with 20 and 100 ME49 cysts demonstrated bilateral OT 14 and 21 days postinfection. Ocular involvement was characterized by vitreal and retinal inflammatory infiltrates (mostly in the ganglion cell layer), migration of retinal pigmented epithelium, cone formation, and alteration of the retinal architecture. Cysts were found in a small portion of histologic material. Among mice infected with 20 cysts, one cyst was observed in the retina 14 days postinfection. Among mice infected with 100 cysts, one single mouse presented three unilateral cysts at the boarder between outer plexiforme and inner nuclear layer 14 days postinfection, and two mice showed unilateral individual cysts in the ganglion cell layer 21 days postinfection *(Fig. 3A–D)*.

Among C57BL/6 mice, the severity of ocular toxoplasmosis varied ranging from unaffected retina to multiple changes characteristic of retinochoroiditis. All mice infected with 5 and 10 cysts presented with retinitis at 21 to 41 days postinfection. Unilateral or bilateral vitreitis, perivascular inflammatory infiltrates, and inflammatory cells within ganglion cell layer, as well as RPE migration, were the most common changes. Cysts were observed in 50% of mice infected with five cysts and in 80% of mice infected with ten cysts at 21 days postinfection; cysts were mostly observed in the ganglion cell layer *(Fig. 4A–D)*.

Results of our study confirm that peroral infection of mice with *T. gondii* cysts leads to development of ocular pathology as previously demonstrated *(Table 2)*. Histological changes in NMRI and C57BL/6 mice were similar. However, due to the lack of pigment in NMRI mice, RPE migration was easier to observe in C57BL/6 mice. On the other hand, NMRI mice showed improved survival compared to C57BL/6 mice. To study immunological aspects of OT, the C57BL/6 mouse model should be used as specific tools for analyses including monoclonal antibodies and gene-deficient mice are not available for NMRI mice.

Models of acquired infection in immunocompromised mice

OT in immunocompromised mice mimics the disease in immunocompromised patients [34]. Immunodeficiency is established in gene-deficient mice or can be induced by immunosuppression via cell depletion or cytokine neutralization. Depletion of CD8+ T cells or B cell results in exacerbation of ocular inflammation and increased parasite load in ocular tissues following *T. gondii* infection indicating that CD8+ T cells contribute to control of parasite replication and inflammatory responses [49, 85]. In contrast,

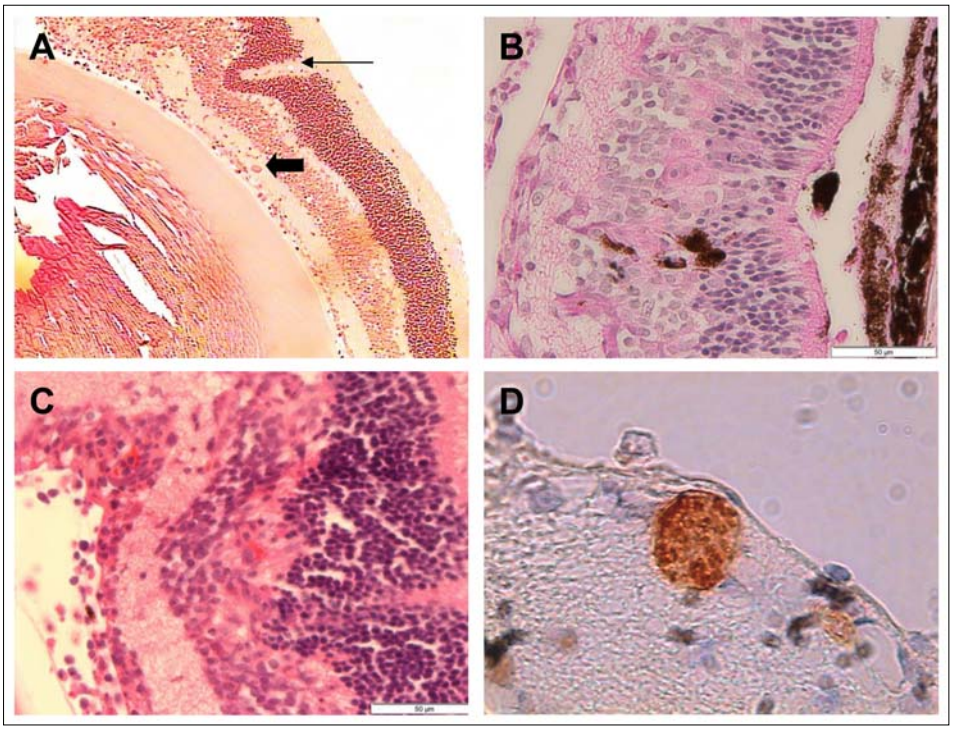

Fig. 4. Ocular histopathology in the eyes of C57BL/6 mice following peroral infection with *T. gondii* cysts: (A) Cone formation (arrow) and a *T. gondii* cyst in the internal plexiforme layer (block arrow) in a C57BL/6 mouse infected perorally with 5 ME49 cysts 21 days postinfection (magnification ×10); (B) RPE migration to the outer nuclear layer, outer plexiforme layer and internal nuclear layer in the eye of a mouse infected with 10 cysts 41 days postinfection; (C) cone formation and architectural changes characterized by alteration in the disposition of internal nuclear layer, outer plexiforme layer and outer nuclear layer; inflammatory infiltrate in the vitreous humor, ganglion cell layer and around a retinal vessel in a mouse infected with 10 cysts 21 days postinfection; (D) a *T. gondii* cyst in the eye tissue of a mouse infected with 10 cysts 21 days postinfection (PAP stain [84], magnification ×100)

depletion of CD4+ T cells leads to a reduction in severity of inflammatory changes and necrosis associated with higher parasite loads, indicating that CD4+ T cells contribute to parasite control as do CD8+ T cells but also contribute to inflammation [85, 86]. Mice deprived of IFN-γ [49, 77, 87], TNF-α [49], or IL-6 [50] developed more severe retinochoroiditis most likely since they were unable to control parasite replication and subsequent inflammation. IFN-γ-deficient mice show retinal vasculitis in fluorescein angiography [77], and electroretinograms performed in mice lacking IFN-γ show deterioration of visual function postinfection [53]. IL-17A, a key cytokine of proinflammatory Th17-responses, was found to be upregulated in the aqueous humor of OT patients [88] and in the retinas of mice infected perorally with *T. gondii* [89]. Intraocular injection of IL-17A-antibodies together with tachyzoites led to less severe ocular inflammation and delayed parasite replication in comparison with infected but untreated mice. Thus, IL-17 plays a detrimental role in OT. Of interest, simultaneous anti-IFN-γ treatment reversed the effect of IL-17A neutralization [88]. In line with these results, mice lacking anti-inflammatory mediators such as IL-10 develop more severe ocular involvement compared to immunocompetent mice [90, 91]. The role of nitric oxide in infection with *T. gondii* appears to be dependent on the infection model [92]. While playing a protective role in acute low dose infection with *T. gondii* [93], the same molecule mediates hyperinflammation following high-dose infection [92, 94]. However, in *T. gondii*-infected mice treated 4–6 weeks after infection with L-omega-nitro-L-arginine-methyl ester (L-NAME) to inhibit nitric oxide production, exacerbated ocular inflammation was observed, thus, indicating a protective role for nitric oxide in the control of (chronic) ocular infection [90].

Recent studies have focused on the role of chemokines in the course of ocular toxoplasmosis. CXCL10 is a chemokine that promotes T cell trafficking to the retina post *T. gondii* infection. Mice treated with anti-CXCL10 antibodies showed more severe retinochoroidal inflammation, a greater disorganization of retinal architecture and a higher parasite burden in comparison with immunocompetent mice following intraperitoneal parasite inoculation [95]. These results obtained in the local environment of the eye in response to infection with the parasite are in line with findings in the systemic circulation [83, 96]. It is well known that infection with *T. gondii* elicits an immune response characterized by a tight balance between proinflammatory signals to control parasite replication vs. anti-inflammatory signals in control of a tissue-damaging inflammatory response, i.e., in immunocompromised organs such as the eye and brain.

Concluding remarks and outlook

OT continues to be a major health problem. Appropriate experimental models to study different aspects of the disease are warranted. An *in vivo* model of the disease should consist of key features of OT in humans. First, it should show similarities with the anatomy of the human visual organ. Second, it should mirror as closely as possible the course of infection in humans; preferably, it should be based on peroral infection of animals. The ocular pathology and immune response should be similar to the immunopathology in humans. Furthermore, the animal model should allow *in vivo* investigations. Knock-out animals and specific immunologic reagents ought to be available to study immunopathology of OT and to mimic the course of infection in immunocompromized patients.

Technological advances including electroretinograms and fluorescein angiography should allow more in depth investigations in *in vivo* models.

In the absence of appropriate animal models, investigators will rely on *in vitro* models to address key questions. It will be interesting to see whether more advanced *in vitro* models of retinal tissue such as three-dimensional retina-like tissues (sphere-like) from mammalian retina [97] will allow detailed investigations of certain aspects of human OT.

Lastly, the parasite–host interaction in ocular pathology deserves a closer look. It has been hypothesized that both the parasite itself and the host response contribute to disease. The results of recent studies implicate that differences in parasite strains impact the course of OT [9]. As recent analyses of parasite antigens point toward preferential infections with atypical strains in OT patients [9, 11, 98], it will be of interest to use these strains in murine models.

Since prospective randomized studies on treatment of OT are scarce [99, 100], advances in our understanding of OT based on experimental models should ultimately pave the way to develop improved treatment regimens. At present, laboratory mice continue to be the experimental model of choice for the investigation of most aspects of OT.

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