Ocular Toxoplasmosis: Recent Aspects of Pathophysiology and Clinical Implications

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\textbf{Introduction}

The obligate intracellular protozoan parasite \textit{Toxoplasma gondii} is an important opportunistic agent that infects all warm-blooded vertebrates including humans. Two major routes of infection exist. First, humans may become infected by oral ingestion of \textit{Toxoplasma} oocysts, which are produced in the intestine of its specific host, the cat and other Felidae, and released by their feces into the environment. Second, infection occurs by oral uptake of \textit{Toxoplasma} tissue cysts, which persist in skeletal muscles of intermediate hosts including pigs and sheep. Primary infection during pregnancy may result in fetal infection with fetal death, severe congenital malformation or, especially with infection at later stages of gestation, mild infection of neuronal tissue including the retina. However, more frequently, the pathogen is acquired postnatally, which also results in infection of neuronal tissues and, in most cases, takes a clinically asymptomatic course. It is considered the most frequent foodborne parasitic infection globally [1]. Importantly, ocular involvement is a major pathology following both routes of infection and may cause legal blindness. Worldwide ocular toxoplasmosis is considered the most frequent cause of infectious posterior uveitis.
Epidemiology

Postnatally acquired ocular toxoplasmosis occurs in approximately 2 out of 100 seropositive individuals, suggesting that 1 in 400 persons across the world will have posterior uveitis due to *T. gondii* [1]. The large burden on health care systems as a result of ocular toxoplasmosis is illustrated e.g. by an estimated 250,000 patient visits to ophthalmologists in the USA alone [2].

Since ocular toxoplasmosis is a preventable cause of blindness, it is necessary to assess factors that have an impact on human infection. Based on current observations, the risk of acquiring an infection varies geographically and largely depends on control of the release and distribution of oocysts into the environment, the animal reservoir, meat consumption, personal habits and climatic conditions. In many countries, the prevalence of *T. gondii* cysts in livestock and the consumption of their contaminated meat are major factors influencing the rate of human infections.

The risk of infection by tissue cyst-containing meat is dependent on the animal species as well as the type of meat and its preparation and varies from country to country. In general, cysts survive in raw and undercooked but not in well-done prepared meat. With respect to the animal species, pigs and sheep are the dominant sources of *T. gondii* infections (fig. 1). As a consequence of industrialized meet production keeping livestock indoor, feeding sterilized food and keeping stables free of contact with rodents and cats, the prevalence of *T. gondii* in pigs is below 5% in Western countries [3]. Therefore, the risk of infection and seroconversion has declined in most countries in which less meat was consumed and hygiene standards were increased [4].

A significant decline in seroprevalence has been observed in Europe – e.g. in the Netherlands, where it dropped from 35.2% in 1996 to 18.5% in 2006 [5]. Of particular importance is the seroprevalence in childbearing women. In France, the proportion of seropositive pregnant women declined from 80% in the 1960s to 44% in 2003 [6], indicating a significantly lower immune-mediated protection for the unborn child.

There is concern that the decline in exposure to *T. gondii* may be reversed in the future. Improved animal welfare and animal-friendly meat 'production' with outdoor containment are likely to increase the presence of *T. gondii* in meat products and will subsequently put consumers at higher risk of contracting toxoplasmosis [7].

Whereas in Western countries, a decrease in *T. gondii* seroprevalence was observed, the opposite may be true in regions with strong population growth and urbanization trends, i.e. large parts of Asia [8]. The risk of foodborne infection seems much higher e.g. in China, where an average of 31% seroprevalence of *T. gondii* was found in slaughter pigs [9]. Large changes are expected for the future in the light of increased meat consumption in developing countries. A significantly higher risk of acquiring a *T. gondii* infection has also been observed in Russia, China and Indonesia [9–11].

Also, exposure to other reservoirs containing *T. gondii*, such as fresh water, may become an increasing source of infection. The quality of water is known as an important risk factor in human infection with oocysts. Contaminated water has repeatedly been a source of epidemics with ocular toxoplasmosis [12, 13]. Drinking unfiltered surface water bears a high risk of infection especially in countries with humid weather conditions.

Since global climate changes are predicted in coming years, this may have an impact on the *T. gondii* prevalence in humans [14]. Interestingly, also seawater and seafood such as mussels or oysters are frequently (45–100%) contaminated with *T. gondii* oocysts and may account for a still underestimated source of human infections [15].

Pathophysiology

How and Why Does the Eye Become Affected?

It is probably not by chance that the three main immune-privileged areas of the body, the placenta, the brain
and the eye, are major targets of pathology in humans. The unique immunological milieu (e.g. high levels of TGF-β) may provide preconditions for a specific balance between parasite invasion and host resistance. Following primary infection of intestinal epithelial cells, *T. gondii* disseminates via the bloodstream throughout the host and has the ability to cross vascular barriers, e.g. the blood-brain barrier, and to form local cysts [16, 17]. Although free tachyzoites have recently been observed in human blood samples, it is unlikely that these parasites are able to infect ocular tissue directly. It is the current understanding that dendritic cells and macrophages serve as "Trojan horses" to guide the parasite throughout the body into the target organs [17, 18].

Invasion of the host cell differs from other microorganisms and is mainly an active parasite-driven process, based on the interaction of several parasite-host surface ligands. Following attachment, unknown triggers activate calcium-dependent protein kinases, which in turn regulate motility and parasite invasion (fig. 2) [19]. In the host cell, the parasite protects itself from toxic host molecules in a parasitophorous vacuole. During invasion and intracellular infection, the host cell remains astoundingly passive, with little change of the actin cytoskeleton or protein phosphorylation. This finding is considered to be due to the manipulation of intracellular signaling by the parasite, which secretes immune modulators (e.g. ROP and GRA proteins) into the cytoplasm of the host cell. Notably, the parasite simultaneously provokes the production of proinflammatory IFN-γ and IL-12, but at the same time *T. gondii* suppresses a strong Th1 immune response. This balanced immune response allows the immunologi-
cal control of the parasite and, in parallel, prevents an immunopathology. As an important regulatory component, Th17 cells have been identified as key contributors to this balance of immune pathological response in the eye (fig. 3). Th17 cells are characterized by the production of IL-17 mediated by IL-23 from dendritic cells and probably may have both protective and proinflammatory effects. Different roles in infectious and inflammatory events are likely to be related to the local (cytokine) environment and the stage of the pathological process [20–22].

Recent clinical and experimental studies indicate intraocular overexpression of IL-17A in active ocular toxoplasmosis. Interestingly, in one study, the production of this signature cytokine occurred early in the course of infection and was predominantly caused by resident retinal cells rather than infiltrating T cells [23]. Since IL-17A is a well-known inducer of proinflammatory responses and autoimmune diseases, this may have direct pathogenic and therapeutic implications. In contrast, however, IL-17 demonstrated strong neuroprotective properties by inhibiting intracellular calcium, maintenance of homeostasis and prevention from apoptosis in active uveitis [24]. The exact role of IL-17A in infectious diseases is therefore ambiguous, varying between antipathogenic activity and tissue destruction.

It is increasingly clear that parasite- as well as host-specific factors are important determinants of whether an infection results in ocular manifestation. This is likely the key to answering the question why some individuals develop ocular disease, whereas others remain in an asymptomatic stage.

**Parasite-Related Factors: Do *T. gondii* Strains Influence Clinical Features?**

There is an ongoing discussion whether the infection and the severity of ocular toxoplasmosis are influenced by genotypic differences between infecting parasites. *T. gondii* exists in three main clonal lineages (strains I, II and III), with type I strains being highly virulent and often lethal in mouse models of infection. Type II and III strains are only moderately virulent under identical experimental conditions [25]. These differences observed in animals have a genetic basis and are linked to certain gene loci coding for rhoptry proteins (ROP18, ROP5 and ROP16) [25, 26].

Also in humans, type I strains have been reported to cause severe postnatally acquired ocular toxoplasmosis in Brazil [27]. Further evidence for severe fulminant retinitis caused by *T. gondii* type I strains derives from vitreous humor samples of patients who underwent vitrectomy [28]. Based on these studies, two subgroups of patients could be distinguished. All patients affected by type I strains were otherwise healthy and immune competent, whereas patients infected with type II and III strains were immune deficient [28]. From these observations, it might be concluded that in immune-competent patients, the genotype of parasite dominates the clinical course of ocular toxoplasmosis, whereas in immune-deficient patients, host factors are more important and severe ocular toxoplasmosis can be caused by any parasite type.

However, sexual recombination allows much larger parasite diversity, and currently more than 130 ‘atypical’ genotypes have been characterized [29]. Differences in the virulence of these strains have again been shown for...
mice [30], but the role in humans is more difficult to establish. Unfortunately, it is difficult to detect these differences in patients, since intraocular parasite DNA in clinical specimens from patients with ocular toxoplasmosis is often limited and frequently not detected. To partly overcome this problem, ELISA methods have been developed that allow serotyping of parasites based on patients’ antibody repertoire directed against certain allelic peptide motifs [31]. This serotyping approach has the advantage that it can be extended to a healthy seroconverted population and no parasite isolation is needed. By serotyping, a dominance of type II-specific antibody response was observed in positive but clinically asymptomatic Europeans [32–35]. Interestingly, using this technique, an ‘atypical’ nonreactive serotype was significantly more frequently detected in sera of patients with ocular toxoplasmosis than in seroconverted individuals without ocular involvement (p < 0.0001). Among ocular toxoplasmosis patients, those with this serotype experienced more frequent recurrences (p = 0.037) [36]. In line with these findings are results from a cohort of 193 patients with congenital toxoplasmosis in North America. Using the same detection method, the nonreactive serotype was associated with prematurity (p = 0.03) and severe disease at birth (p < 0.01) [37].

**Host Genetic Factors: Do They Play a Role in the Susceptibility to and Severity of Ocular Toxoplasmosis?**

Whether susceptibility to ocular toxoplasmosis differs between individuals remains an important question. At least five genes at the MHC locus have been associated with protection and resistance to otherwise lethal *T. gondii* infection in experimental rodent models [38]. With respect to humans, early studies have shown a significant association of the HLA-DQ3 genotype with congenital *T. gondii* encephalitis and hydrocephalus [39] but not with ocular involvement. However, more recent observations mainly focusing on congenital toxoplasmosis imply that a number of gene polymorphisms are linked to susceptibility.

Both encephalitis and retinitis could be linked to the ABCA4-encoding genes that are selectively expressed in the choroid plexus throughout the development of the eye and brain and are closely related to hereditary retina dystrophies. This may suggest a possible role for ABCA4 in determining the simultaneous pathology in the brain and eye, as often seen in congenital toxoplasmosis [40, 41].

In addition, Toll-like receptors (TLR) are important transmembrane proteins that recognize microbial components and orchestrate an early immune defense, leading to the production of proinflammatory cytokines. Supported by a small family-based study in children with congenital ocular toxoplasmosis, a significant association between gene polymorphisms of TLR (TLR2, TLR5 and TLR9) was reported [42].

Probably not unexpected, host cytokine gene polymorphisms have been a focus of interest in toxoplasmic retinitis. Cytokines, in particular IFN-γ and TNF-α, play an essential role in resistance to *T. gondii* infections (fig. 2). These cytokines activate macrophages, a major first defense line. Polymorphisms in genes encoding various cytokines have been shown to be connected with susceptibility to parasitic diseases. Indeed, individuals homozygous for the A allele (+874T/A) of the IFN-γ gene had a higher risk of ocular toxoplasmosis if they possessed the A/A genotype as compared to a negative control group [43]. In addition, experimental data have demonstrated a relevant role for the anti-inflammatory cytokine IL-10 in modulating acute ocular toxoplasmosis. An IL-10 gene polymorphism (IL-10 –1082 A allele, AA+AG genotypes) could be associated with the occurrence of ocular toxoplasmosis. More recently, a study conducted by Cordeiro et al. [44] similarly identified and associated an IL-6 polymorphism (–174 G/C) with the occurrence but not recurrence of ocular toxoplasmosis in Brazilian patients.

An interesting finding bridges autoinflammatory and immunoregulatory mechanisms in patients with toxoplasmic retinochoroiditis. In children with congenital ocular toxoplasmosis, an association with polymorphisms in the NOD2 gene, an intracellular pattern recognition receptor, could be detected [45]. Of note, the results further suggested that NOD2 influences the production of IL-17A by CD4+ T lymphocytes and likely contributed to the development of ocular toxoplasmosis.

Taken together, a variety of gene polymorphisms are involved in (ocular) toxoplasmosis and may relate to an individual risk profile for a given patient. This may hopefully also open future avenues for studying host-parasite interaction and allow more specific preventive/therapeutic modulation.

**Other Factors**

A variety of other factors may influence the susceptibility to and severity of ocular toxoplasmosis.
Patients’ Age

The role of patients’ age has been debated for decades. It is commonly observed that ocular toxoplasmosis is more prevalent and active in younger individuals and becomes initially manifest between the ages of 25 and 31 years. This may suggest a higher risk at younger age; however, given the natural, steadily increasing seroconversion over decades, this distribution may not be true. Instead, a critical evaluation identifies age at the extreme ends as a risk for the clinical manifestation of ocular toxoplasmosis. Not only patients with congenitally acquired infection but also those at older age seem to be at higher risk for the clinical manifestation of ocular toxoplasmosis. Patients whose ocular toxoplasmosis was first diagnosed and presented recent seroconversion were substantially older (mean age: 50.6 years) than patients with a first manifestation of ocular toxoplasmosis with serologic evidence of an infection some time ago (mean age: 29.9 years) [46].

Interestingly, the more advanced age of patients at first manifestation had an impact on the risk of recurrences as well. The relative risk for individuals aged ≥ 40 years was significantly increased (p < 0.03) and presumably related to the waning immune defense in the aging host [47]. The overall recurrence rate in Europe is up to 80% within 5 years, with the highest rate during the first year following an active episode of retinochoroiditis [46–49]. It has been postulated that recurrences are associated with the proliferation of organisms that emerge from retinal tissue cysts. Over time, the viability of tissue cysts decreases and they eventually die, reducing the pool of organisms and risk of reactivation. Other factors that have been considered to influence recurrences are changes in tissue cysts with reduced release of parasites or antigens, trauma, endocrine fluctuations and transient humoral or cellular immunoreactivity [50, 51]. However, none of these putative factors could be substantiated. Most notably, no association between recurrence and treatment, congenital infections versus postnatally acquired infections, primary lesions versus recurrent lesions and the size of lesions or antibody levels could be established (for a review, see Mänz et al. [52]).

Patients’ Immune Status: Disease in Immunocompromised Individuals

Given the eminent role of the host immune system in ocular toxoplasmosis, an impact on the disease course can be expected in immunocompromised, e.g. HIV coinfected, individuals. Whether AIDS patients per se are at higher risk of primary acquired ocular toxoplasmosis is not clearly documented. Before the introduction of highly active antiretroviral treatment, and even today without adequate treatment, toxoplasmic encephalitis remains an initial AIDS-defining illness in up to 33% of all patients [53]. It remains an important cause of neurological disorders, leading to severe pathology including lethal consequence [54]. Also ocular involvement is far more severe, even when compared to other opportunistic infections of the retina in AIDS patients, e.g. cytomegalovirus retinitis. The essential role of the host immune response is underlined by the fact that patients are at particular risk when CD4+ T cell numbers are reduced below 200 cells/mm³ and, therefore, subsequent monitoring is advised [55]. Often these individuals demonstrate an atypical fulminating clinical course of ocular toxoplasmosis and provide a great diagnostic challenge. Similar problems and atypical clinical presentations of ocular toxoplasmosis can be seen in patients receiving immunosuppressive drug therapy, e.g. following organ or bone marrow transplantation [56]. The prevalence of ocular toxoplasmosis in this population at risk is not known, but careful monitoring of infections in this increasing population is advised [57].

Conclusions

Since a reliable animal model of ocular toxoplasmosis is still missing and will be hard to establish, the research focus on ocular toxoplasmosis is likely to remain clinical. Many questions concerning not only the epidemiology, impact of parasite strains and role of protective and immunopathological immune response but also therapeutic approaches are still unresolved. Close cooperation of ophthalmologists with parasitologists, microbiologists and immunologists are mandatory.

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References


