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Invited Review

New clinical and experimental insights into Old World and neotropical ocular toxoplasmosis

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ABSTRACT

Retinal lesions or other ocular manifestations are serious consequences of infection with the protozoan parasite *Toxoplasma gondii*. Whilst classically considered a consequence of congenital transmission, recent screening studies estimated that 2% of *T. gondii* seropositive persons in Europe and North America have retinal lesions, most of them persisting unnoticed. The situation is more dramatic in South America, probably due to the predominance of virulent strains. Some of these strains seem to exhibit ocular or neuronal tropism and are responsible for severe ocular lesions. Despite the medical importance, the physiopathological mechanisms have only recently begun to be elucidated. The particular immune-privileged situation in the eye has to be considered. Studies on French patients showed low or undetectable ocular parasite loads, but a clear Th1/Th17 type immune reaction. Suitable mouse models have appeared in the last few years. Using such a model, IL-17A proved to impair parasite control and induce pathology. In contrast, in South American patients, the parasite seems to be much less efficiently controlled through a Th2 type or suppressive immune response that favors parasite replication. Finally, several host genetic markers controlling immune response factors have been associated with ocular involvement of *T. gondii* infection, mainly in South America.

1. Introduction

While the apicomplexan parasite *Toxoplasma gondii* infects approximately one-third of the world's population, transmission frequency is very variable, owing to temperature and humidity variation, as well as local eating habits (Montoya and Liesenfeld, 2004). Following a multiplication phase, where the parasites disseminate throughout the body, the host's immune system takes control and eliminates most of the parasites, mainly by cellular, IFN-γ driven Th1 type responses (Pifer and Yarovinsky, 2011). However, *T. gondii* persists in cysts, mostly in muscles and the CNS. These cysts can reactivate when immunity weakens. Consequently, reactivation of cerebral cysts was a major cause of mortality in AIDS patients before the introduction of effective anti-viral therapies. The retina has also been identified as the location of dormant cyst forms in mice (Lahmar et al., 2010). Until recently, the presence of *T. gondii* in eye tissues was not considered to be a threat to health in immunocompetent persons, with the notable exception of congenital infection. However, thorough investigation of *T. gondii* seropositive individuals revealed a non-negligible prevalence of retinal lesions, with a life-long risk of recurrence, i.e. the appearance of new lesions (Delair et al., 2008). Despite this apparent medical importance, the physiopathology is still not well understood, which also thus far prevented the introduction of an efficient treatment (Holland, 2004). This review summarises the current knowledge, the active fields of research and the ideal therapeutic strategy.

2. Epidemiology

Toxoplasmic retinochoroiditis is the commonest form of posterior uveitis in many countries. Prevalence and incidence of ocular symptoms after infection depend on socio-economic factors and the circulating parasite genotypes (Holland, 2003; Furtado et al., 2013). Ocular toxoplasmosis (OT) is more common in South and central America, the Caribbean and parts of tropical Africa, compared with Europe and Northern America, and is quite rare in China. Ocular disease in South America is more severe than in other continents due to the presence of extremely virulent genotypes of the parasite (Petersen et al., 2012). The results obtained in a

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study comparing OT in Europe, North America and South America suggest that disease characteristics also vary in different areas of the world (Dodds et al., 2008), which obviously has fundamental consequences for treatment strategies (Sauer et al., 2011).

2.1. Europe and North America

There are few studies on the prevalence of OT. It is usually estimated through funduscopic screening by discovering chorioretinal scars, suspected to be toxoplasmic, in the general population, as the concerned individuals are often unaware of the presence of scars. A large retrospective study in a United States (US) medical center identified OT as the most common form of posterior uveitis in the 1990s (Rodriguez et al., 1996), which was confirmed for various countries. Generally, it is estimated that approximately 2% of *T. gondii* seropositive persons will develop retinal lesions (Holland, 2003). This led to the estimation that in 2009, 1,075,242 persons became infected in the US, resulting in 21,505 new cases of retinal lesions, of which 4,839 were symptomatic (Jones and Holland, 2010).

In Europe, Gilbert et al. (1999) placed the incidence of symptomatic OT at 0.8/100,000 persons per year, and the lifetime risk (to 60 years of age) at 18/100,000 British born individuals. *Toxoplasma gondii* infection was the main cause of posterior uveitis in 1,064 consecutive patients at a national uveitis referral center in Italy between 2002 and 2008, accounting for 6.9% of all uveitis cases (Cimino et al., 2010). A French multi-center study showed that retinal toxoplasmic lesions could more often attribute to acquired than to congenital infection (Delair et al., 2008). In Germany, a survey of 1,916 patients seen in a similar setting and almost concurrently, also found OT to be the most frequent diagnosis in patients with posterior uveitis and the cause of 4.2% of uveitis cases (Jakob et al., 2009). Acquired infections also may be complicated by recurrent retinochoroiditis, with recurrences being most common close to the time of acquisition (Delair et al., 2011).

The incidence of congenital infections varies with the geographical origin, in parallel with overall seroprevalence. A large retrospective study in the US estimated the number at approximately one in 10,000 live births (Guerina et al., 1994), whereas three in 10,000 live births were observed in France (Villena et al., 2010). A prospective cohort study on European children with confirmed congenital toxoplasmosis found retinal lesions in one of six of these children, who received treatment for at least 1 year, after the first 4 years of life (Tan et al., 2007). Curiously, some North American studies found retinal lesions in more than 70% of congenitally infected and untreated, and 58% of treated children (Mets et al., 1996; Phan et al., 2008). These discrepancies might be due to referral bias or divergent criteria for proven toxoplasmic lesions. In any case, even in countries with low *T. gondii* seroprevalence, such as the Netherlands, congenital toxoplasmosis causes considerable morbidity, with retinal lesions playing an important part (Havelaar et al., 2007).

2.2. South America

The enormous impact of toxoplasmosis on public health is best demonstrated by the incidence numbers of congenital OT. The estimated number of one case of congenital toxoplasmosis in 770 live births in Brazil (Vasconcelos-Santos et al., 2009) is 5–15-fold higher than what is seen in Europe and North America. A comparative prospective cohort study of congenitally infected children in Brazil and Europe showed that Brazilian children were at a five-times higher risk than European children of developing eye lesions. Two-thirds of Brazilian children infected with congenital toxoplasmosis had eye lesions by 4 years of age compared with one in six in Europe (Gilbert et al., 2008).

The burden of OT in South America is impressive not only in congenitally infected children, but also in adolescents and adults, most of whom have presumably acquired infection postnatally (Ajzenberg, 2011). Population-based studies of this age group showed that the prevalence of OT is higher in South America compared with North America. Initial studies found an OT prevalence as high as 17.7% in the Erechim region in southern Brazil (Glasner et al., 1992). However, the situation within South America seems to be much more heterogeneous than in Europe or North America. A survey of university students and employees in the Colombian town of Armenia (Quindio region) diagnosed OT in 6% of the study group, 20% of which had visual impairment. (De-la-Torre et al., 2007). The prevalence of congenital toxoplasmosis in this region was estimated at 0.5%. Although the academic study group might not be altogether representative of the overall population, this study suggests a predominance of postnatally acquired OT. The incidence of OT has been estimated to be three new episodes per 100,000 inhabitants per year (De-la-Torre et al., 2009), compared with 0.4 cases per 100,000 persons in British-born patients (Gilbert et al., 1999). Additionally, striking differences are seen even within Colombia. In military personnel operating in the jungle, *T. gondii* seropositvity was significantly higher than in those serving in Bogota, after only 1 year of service (80% versus 45%), but characteristic toxoplasmic chorioretinal lesions were only found in four soldiers that operated in the jungle (0.8%) and in one urban soldier (0.19%) (Gomez-Marin et al., 2012). Consequently, *T. gondii* strain distribution and OT frequency may vary considerably.

Assuming that half of the 41 million inhabitants of Colombia are chronically infected with *T. gondii*, we can estimate that 1 million people live with retinochoroidal scars and at least 200,000 suffer from unilateral legal blindness due to this infection in this country. If we transpose this scenario to the whole population living in tropical parts of South America, especially in Brazil, we have to become aware that the neglected tropical disease OT is in fact a leading cause of blindness in South America (De-la-Torre et al., 2007; Ajzenberg, 2011).

Some studies estimated the proportion of seropositive patients who will eventually develop retinal lesions. In Southern Brazil, 383 persons were reexamined to determine the rates of seroconversion and the incidence of toxoplasmic retinal lesions in individuals who were seronegative for *T. gondii* infection. In this series, 11 (8.3%) of 131 individuals who were seropositive without ocular lesions in 1990 were found to have typical lesions by 1997 (Silveira et al., 2001). The above-mentioned Colombian study (De-la-Torre et al., 2007) suggests that 11% of people with acquired infection develop ocular lesions.

3. Clinical appearance

3.1. Europe and North America

In young children, OT may be asymptomatic. Children who are able to vocalise may complain of decreased vision or ocular pain, while parents may note leukocoria or strabismus. Adults often present with floaters, which may be associated with altered vision. The ‘classic’ sign of infection includes retinal scars, white-appearing lesions in the active phase often associated with vitritis (Holland, 2000, 2004; Butler et al., 2013). Depending on the size and thickness of involved retina, the overlying vitreous and subjacent choroid are variably involved. Spontaneous resolution of active retinochoroiditis is the rule in immunocompetent patients, resulting in an atrophic, well-defined scar. Complications may include fibrous bands, secondary serous or rhegmatogenous retinal

detachments, optic neuritis and neuropathy, cataracts, increased intraocular pressure during active infection, and choroidal neovascular membranes (Vasconcelos-Santos, 2012; Butler et al., 2013).

Interestingly, Bosch-Driessen et al. (2002) found a significantly increased likelihood of macular lesions (i.e. 46% versus 16%), as well as bilateral disease (i.e. 85% versus 28%), in congenital versus postnatal infections, respectively. Mets et al. (1996) reported macular involvement in 55% and bilateral involvement in 51% of 94 patients with confirmed congenital OT. Congenital infections are not necessarily more severe than postnatal cases, but given the higher incidence of macula involvement, congenital infection carries an increased risk of legal blindness (Bosch-Driessen et al., 2002; Holland, 2004; Butler et al., 2013). Recently, Holland (2009) reported an unadjusted rate of recurrence of 0.2 episodes/year in a cohort of 143 Dutch patients followed for up to 41 years. They noted the recurrence risk decreased with increasing disease-free intervals and increasing age at first clinical episode (Holland, 2009). Recurrences of active retinochoroiditis have been reported to occur in 79% of 76 patients followed for over 5 years, predominantly along the scar border (Bosch-Driessen et al., 2002). In immunocompromised patients, recurrence is the rule in the absence of long-term anti-parasitic therapy (Pivetti-Pezzi et al., 1994; Hodge et al., 1998).

Recurrences in untreated congenital toxoplasmosis occur during teenage years. Manifestations at birth are less severe and recurrences are fewer in those who were treated promptly, early in the course of their disease in utero and in the first year of life. European studies suggested that up to 9% of children with retinal lesions due to congenital toxoplasmosis have significant bilateral vision impairment (Tan et al., 2007).

3.2. South America

Ocular disease in South America is not only more frequent but also more severe than in Europe and North America. Congenital toxoplasmosis caused by atypical genotypes is often more severe than that caused by the canonical strains (Dodds et al., 2008; Lindsay and Dubey, 2011). Comparison of cohorts of congenitally infected children from different continents showed that congenital toxoplasmosis is more often symptomatic in South America than in Europe, with different studies showing that approximately 50% of children will develop ocular lesions during the first year of life (Thiebaut et al., 2007; Gilbert et al., 2008). Additionally, lesions are larger, more numerous, more recurrent and more likely to impair vision. In Colombia, the lethality rate in congenitally infected children in the absence of prenatal treatment is as high as 25% (Gomez-Marin et al., 2011).

Recurrences in OT patients have been reported to have a frequency of two episodes each 11 years in a Colombian study, with recurrences clustering soon after an active attack (De-la-Torre et al., 2009). Regarding all of these elements, it becomes evident that quality of life in South American OT patients is significantly affected, especially if they have bilateral lesions and frequent recurrences (De-la-Torre et al., 2011).

4. Immunological aspects

4.1. Ocular immune response

Given the immune privileged ocular environment, we first outline the principal particularities of specific immunological features in the eye. Crucially, this system controls the development of anti-retinal immune reactions in multiple ways, well beyond a simple physical separation of the ocular compartment (Streilein, 2003). It has long been realised that the intraocular environment diminishes cellular activation (Streilein, 1993). Retinal pigmented epithelial (RPE) cells have been shown to secrete TGF-β and other immunosuppressive mediators (Sugita et al., 2006) and to inhibit T-cell development in a contact-dependent manner (Sugita et al., 2008). This explains, at least in part, the absence of peripheral T-cell reactivity against antigens encountered within the eye. Additionally, this efficient exclusion of anti-ocular T-cell responses has another downside: the increased likelihood of these hidden antigens to induce autoimmune reactions. Indeed when, for example through pathogen-induced injury, the blood-retinal barrier is breached, T-cells might encounter these 'unknown' antigens which suddenly appear in the periphery, as 'non-self' and initiate a detrimental reaction cascade (Caspi, 2006). Many systemic human autoimmune diseases affect the eye, demonstrating the vulnerability of this organ to pathological self-attack (Barisani-Asenbauer et al., 2012). This condition has been modelled by the inducible mouse disease, experimental autoimmune uveitis, and thoroughly immunologically characterised (Horai and Caspi, 2011). Interestingly, while a Th17 response seems to be responsible for pathology upon retinal antigen administration, injection of antigen-pulsed dendritic cells induces a Th1-driven uveitis (Caspi, 2008). Further studies showed that the cytokines IL-17A and IL-17F activate RPE cells and compromise their barrier function (Chen et al., 2011). This very likely leads to an enhanced influx of inflammatory cells and retinal damage, and demonstrated again the detrimental role of an ocular Th17 type reaction during inflammatory processes.

The retina also possesses specialised cell types which often assume dual functions: preserving the structural and functional integrity of this organ and maintaining the metabolic homeostasis of the fragile neurons. The RPE cells are certainly the best known example, as indicated above. Moreover CD-40 stimulated RPE cells eliminate *T. gondii* through an autophagic process (Van Grol et al., 2013). However, the diverse types of glial cells also actively participate in the immune equilibrium. Muller cells, which span the entire thickness of the retina, have been identified as guardians of neuron integrity in the healthy and diseased retina (Bringmann et al., 2006). When infected with *T. gondii* in vitro, Muller cells secrete a large panel of immune mediators (Knight et al., 2006). However, it is not yet known whether this activation is protective or detrimental to the adjacent neuronal cells. As a self-protective mechanism, CD40-associated autophagy was recently described to protect against photoreceptor degeneration (Chen et al., 2013).

4.2. Studies on human OT

Due to the very limited access to ocular tissue, pathophysiological studies on humans are rare. Some post-mortem examinations described histopathological features (Butler et al., 2013), but immunological investigations usually looked at immune mediators in the peripheral blood or genetic markers (see below). Therefore, we assessed cytokine concentrations in aqueous humor, taken by puncture at the same time as the diagnosis, as ocular fluids are the most reliable samples to test for the presence of *Toxoplasma* DNA and/or local specific antibody production (Villard et al., 2003). This allowed the study of the local immune response to *Toxoplasma* in biologically confirmed OT cases. Furthermore, the Bio-Plex® technology allowed the simultaneous evaluation of more than 20 markers in the small available volumes. Interestingly, our retrospective study of patients with toxoplasmic, viral and intermediate uveitis showed a marked expression of IL-17A in the aqueous humor of most patients with OT, but not viral uveitis (Lahmar et al., 2009). It was also observed that Th1 cytokines (IL-2, IFN-γ) as well as inflammatory (IL-6, IL-17, MCP-1) and downregulating cytokines (IL-10) were strongly upregulated in aqueous humor of patients with confirmed OT. The Th2 cytokine IL-13 was only weakly upregulated. Interestingly, TNF-α levels remained

unchanged (Lahmar et al., 2009; Sauer et al., 2012). This inflammatory pattern implicating a Th17 type response and the self-limiting nature of inflammation is similar to the previously described autoimmune diseases, which indicates the direction of further investigation. However, it has to be kept in mind that there is no evidence of an autoimmune component in the development of OT, and treatment strategies have to consider the infectious nature of this condition.

As the epidemiology and clinical course of South American infections are so different, a study to compare the cytokinome as well as the clinical characteristics of French and Colombian OT patients has been conducted. Colombian patients show a more suppressive immune reaction with lowered IFN-γ and IL-17A levels associated with drastically higher local parasite proliferation. Paradoxically, IL-6 levels are significantly elevated in OT patients (De-la-Torre et al., 2013).

4.3. Modeling physiopathology in animals

Thorough insight into the parasitological and immunological dynamics of retinal infection requires adapted animal models, especially in the mouse. Great progress towards establishment of such models was made in recent years, which will increase our understanding of the immunological mechanisms regulating parasite proliferation and the cellular actors involved in the immune response, as well as the formation of retinal lesions. In the longer term, this modelling will allow the development of new therapeutic tools through the identification of specific targets.

The first described animal models used oral or i.p. infection of adult or pregnant mice in order to mimic natural infection, which identified the roles of some key cytokines (Jones et al., 2006). The majority of mice developed minor uveitis and retinal vasculitis. The uveitis is characterised by an infiltration of CD4+ lymphocytes and macrophages into the retina and by IFN-γ and TNF-α transcription in retinal lymphocytes. Chemokines such as CXCL10 are important in this protective response (Norose et al., 2011). Parasites have rarely been detected in situ in these mice. Treating mice with anti-CD4+ or anti-CD8+ antibodies provoked an increase in ocular cyst numbers, whereas treatment with anti-IFN-γ or anti-TNF-α antibodies produced lesions containing tachyzoites (Gazzinelli et al., 1994; Pavesio et al., 1995; Gormley et al., 1999; Sauer et al., 2009). A recent publication confirmed the up-regulation of IL-17A in the retina and the pivotal role of IFN-γ using knockout (KO) mice (Kikumura et al., 2012). Of note, the histopathological characteristics of KO mice or mice treated with neutralising antibodies resemble those seen in immunodepressed patients, rather than the normal course of infection in immunocompetent individuals. The main problem with this infection protocol is the inconsistent rate and slow kinetics of lesion formation, making detailed immunological studies difficult to interpret. Moreover, these experiments could not distinguish between systemic and local effects of cytokines.

Several injection routes close to the eye were tested but proved less than ideal. Subconjunctival injection in guinea pigs did not result in any retinal effects (Skorich et al., 1988). The injection via the right carotid in cats reproduced chorioretinitis lesions. However, this model induced vasculitis and rather non-reproducible ocular lesions (Davidson et al., 1993; Sauer et al., 2009). The eye drop instillation technique was also tested, showing the same pattern of infection as intravitreal infection, with a lower inflammatory infiltrate and the advantage of not causing mechanical damage (Tedesco et al., 2005).

The model of OT using intravitreal tachyzoite injection reproduces key features of the human disease with much higher success rates than systemic infection. It has already proven its effectiveness in a non-human primates (Holland et al., 1988) and rabbits (Garweg et al., 1998). This intravitreal injection in the rabbit model was also combined with a previous systemic infection to test the hypothesis of an autoimmune component in OT. However, their results did not indicate the stimulation of a reaction against retinal antigens by *T. gondii* presence in the eye (Garweg et al., 2009). More recently, intravitreal injection has been introduced in the mouse model (Lu et al., 2005; Charles et al., 2007). The use of very fine (30 Gauge) needles allows modelling of the characteristics of human OT with little or no post-injection lesions. This model was used to test the role of SAG1 in ocular infection, and to demonstrate that immune suppressing properties of retinal cells are induced by local *T. gondii* infection (Charles et al., 2007, 2010; Mimura et al., 2012). We employed simultaneous intravitreal injection of parasites and neutralising antibodies to characterise the intraocular cytokinome following *T. gondii* infection in more detail. We demonstrated that IL-17A was indeed responsible for the retinal pathology, but also for enhanced retinal parasite proliferation, partly by suppression of the protective cytokine IFN-γ (Sauer et al., 2012). Additionally, our recently adapted protocol of systemic infection and intravitreal challenge as an approximate model of OT recurrence will soon permit novel insights in this aspect of OT.

In mouse experiments aimed at the pathological and immunological dynamics of congenital infection, we observed retinal lesions in some eyes 4 weeks after birth. Interestingly, infection rate and parasite load in the eye were always inferior to the brain. We also demonstrated that neonatal infection constitutes a valid and more efficient model for congenital infection (Sauer et al., 2009; Lahmar et al., 2010). Finally, we used the recurrence model in neonatally infected mice to demonstrate a shift from a pathological Th17 type response upon primary infection to a more benign Th1/Th2/Treg response in re-challenged animals following neonatal infection (Sauer et al., 2013). We have to keep in mind, however, that nearly all of these experiments were done with a canonical type II strain of *T. gondii*. The use of atypical strains could shed light on the particular mechanisms at play in South American infections.

4.4. Immunology: outlook

The striking difference between European/North American and South American forms of toxoplasmosis initiated considerable research activity to elucidate physiopathological mechanisms. The few existing immunological studies on OT patients allow us to outline the specific immune response pattern in European and North American patients, in comparison with their South American counterparts (Fig. 1). Further, more detailed studies are necessary, especially in the more heterogeneous South American setting, to investigate more subtle differences such as recurrences and severity of disease.

Beyond pure correlation, the introduction and continuous refinement of suitable animal models gradually opens the way for a thorough mechanistic comprehension of retinal infection and inflammation. This is mainly true for the role of the IL-17 dependent inflammatory response and its relation to the protective IFN-γ driven response (Fig. 1). Many questions remain open to investigation. Th2 cytokines might have a more important role than previously thought in local antibody production, as well as by their immune regulatory properties. Moreover, the regulation of the Th17 type response is central to our understanding of the inflammatory process and should be more thoroughly investigated, for example the role of IL-6 which is involved in Th17 cell polarisation, but was paradoxically shown to protect against retinal pathology (Lyons et al., 2001). Even if

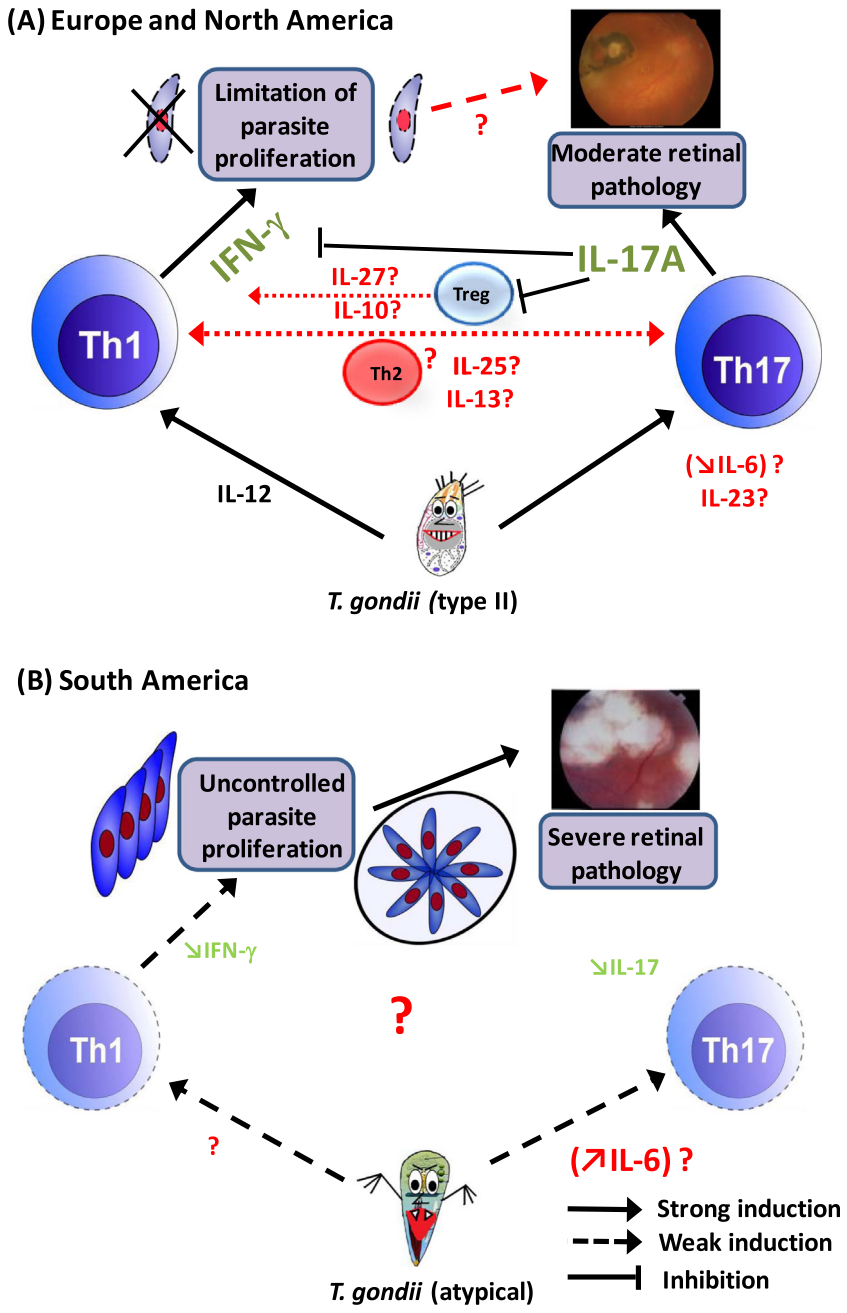

Fig. 1. Proposed scheme of pathology and immune response of ocular toxoplasmosis (OT), according to the data known to date. (A) The type II *Toxoplasma gondii* strain, predominating in Europe and North America, induces both Th1 and Th17 type responses. It seems that IL-17A is responsible for retinal pathology, as well as for suppression of a protective IFN-γ driven response, as neutralisation of this cytokine reverses, at least partially, both effects. This pathological process is usually self-limiting with time, leading to moderate retinal pathology and relatively small lesions. Regulatory T (Treg) cells and perhaps Th2 cells seem to be suppressed by IL-17A, but many details (drawn in red) remain to be elucidated, namely the induction and regulation of IL-17A production (around IL-6 and IL-23), the possible involvement of other IL-17 family members and the exact role of Th2 and/or Treg cells in the interaction between IL-17A and IFN-γ. (B) The atypical and highly variable strains observed in South America, in contrast, induce very little production of both IFN-γ and IL-17A. Curiously, IL-6 is up-regulated in patients. The relative absence of IFN-γ allows uncontrolled parasite replication, which results in severe pathology with numerous, larger lesions. Much less is known about the immunological regulation of this process than in type II infection.

this study used systemic infection and IL-6 KO mice, thus making it difficult to distinguish between local and systemic effects of IL-6, it illustrates the complexity of intraocular inflammation and demonstrates the need to study this process in detail in the process of developing therapeutic intervention. It seems to be clear that a future immune-based intervention will have to take into account the profound geographical differences in OT.

5. Immunogenetics

5.1. Parasite factors

The highly variable clinical expression leads to the question of the respective roles of host or parasite genetic factors. The three canonical European and North American strain types I, II and III

show clear differences in mouse virulence. In contrast, humans are generally less susceptible to *Toxoplasma* infection, and differences between strains are often less clear-cut. However, some *Toxoplasma* outbreaks with unusually severe ocular pathology, e.g. in Canada in 1994–95 (Burnett et al., 1998), have been associated with the mouse-virulent type I parasite. Even more than the differences among the classical genotypes, the discovery of highly variable and often pathogenic strains in South America (Grigg et al., 2001) elicited research with associations between the parasite genome and ocular pathology.

A major obstacle for parasite genotyping is the small quantity of parasites isolated from patients, which often does not allow PCR amplification and sequencing of a sufficient number of loci. Grigg et al. (2001) performed PCR restriction fragment length polymorphism (RFLP) assays for SAG3 (p43) and SAG4 (p18), two single-copy surface antigen genes. Together with strategies for SAG1, SAG2 and B1, multilocus RFLP analyses were performed on PCR-amplified parasite DNA present in 12 clinical specimens from OT patients. Most samples (8/12) were not infected by type II or type III strains. Only one type III and three type II strains were identified, all from immunosuppressed patients. In six otherwise healthy adults and in one immunosuppressed patient, the SAG1 allele associated with type I was amplified. Of 12 samples, three possessed true type I strains; five of 12 had new recombinant genotypes with alleles typical of type I or III strains at all loci examined (Grigg et al., 2001). In Poland, samples taken from peripheral blood of 73 patients with OT identified only type I strains as determined by sequencing *Toxoplasma* non-transcribed spacer 2 (NTR). However, as only one allele was analysed, this result is unlikely to reflect the real genotype in all infections (Switaj et al., 2006). Another multilocus typing study on Brazilian OT patients revealed highly divergent genotypes, mostly of a I/III genotype (Khan et al., 2006). In contrast, direct genotyping of *T. gondii* strains from aqueous or vitreous humor of 20 French OT patients showed a predominance of type II strains, but in this case, multiple microsatellite alleles were analysed (Fekkar et al., 2011). In Colombia, SAG2 genotyping data in humans and animals also suggested a predominance of the type I allele (Gallego et al., 2006). A major breakthrough was the development of serotyping techniques to overcome the problem of insufficient parasite numbers for PCR-based genotyping (Kong et al., 2003). This allowed a comparative study between European and South American infection using large cohorts, which confirmed the homogeneous distribution of serotype II in Europe and of serotypes I/III in South America (Morisset et al., 2008). Of note, these serotype results are based on a few and probably still not very accurate markers. These presumed type I or I/III strains will, in the future, be more precisely characterised. Altogether, these data strongly suggest the existence of distinct European/North American and South American *Toxoplasma* populations. Additionally, it is important to keep in mind that, with the increase in worldwide travel and trade, *T. gondii* can appear in human cases in locations far from its origin. This may explain reports of very severe cases in North America and Europe (Masur et al., 1978; Pomares et al., 2011).

Now that the tools are available, it would be interesting to elucidate the apparent differences in pathology between strains. For example, some of these non-archetypical strains exhibit CNS or ocular tropism, whereas others do not, as seen in local outbreaks with high incidence of retinal affection, or its total absence (de Moura et al., 2006). Mouse studies have shown that monocytes and dendritic cells function as shuttles to transport tachyzoites into the brain, but this has to date only been shown for the canonical strains. Interestingly, RH, but also South American strains are able to migrate through human retinal vascular endothelium as free tachyzoites (Furtado et al., 2012). As for multiplication, avirulent strains show a preference for microglia over astrocytes whereas the virulent strain infects both types of cells with equal efficiency (Fischer et al., 1997). Strain-specific differences in *Toxoplasma* in the modulation of retinal host cell transcription have been identified previously (Knight et al., 2005). Therefore, there is experimental evidence that preferential invasion of nervous and retinal cells may depend of the infecting strain type.

5.2. Host genetic factors

Genetic linkage studies to identify host susceptibility markers are difficult to conduct, due to the low number of cases in Europe and North America. Chances are much better in Brazilian regions with a very high prevalence of OT, and nearly all genetic studies were undertaken in these regions. Obviously, genes coding for known immune mediators or their promoter regions were checked for association with clinically apparent OT. A polymorphism of the extracellular pattern recognition receptor TLR9 was associated with toxoplasmic retinochorioditis in patients originating from the state of Rio de Janeiro, Brazil (Peixoto-Rangel et al., 2009). Recently, another study found an association with the intracellular pattern recognition receptor NOD2 in patients from the same region, as well as from the Belo Horizonte region, Brazil (Dutra et al., 2013).

In recent years, genes coding for immunological factors known to influence the course of *Toxoplasma* infection and the respective promoter regions have been compared between OT patients and controls in endemic Brazilian regions. Thus, the IFN-γ +874T/A gene polymorphism correlated with OT (Albuquerque et al.,

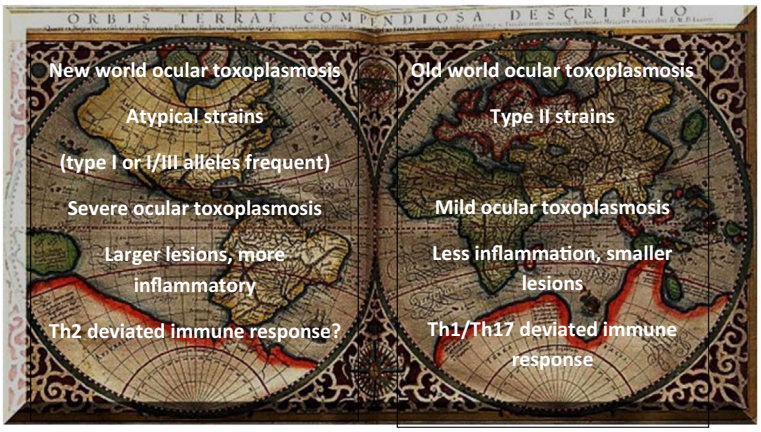

Fig. 2. Geographical divergence of clinical human ocular toxoplasmosis.

2009). While it was not detailed whether this polymorphism changed IFN-γ expression levels, a series of studies from Belo Horizonte University made quantitative assessments. A polymorphism in the IL-1 gene which leads to higher levels of the corresponding protein was positively correlated with recurrence, but not overall OT frequency (Cordeiro et al., 2008c). In contrast, for a polymorphism in the IL-6 promoter (−174 G/C), the variant which leads to lower IL-6 production was associated with enhanced OT frequency (Cordeiro et al., 2013). In another study, the genotypes related with low IL-10 production (−1082 G/A polymorphism) were associated with the occurrence of OT (Cordeiro et al., 2008a). Interestingly, the TNF-α (−308 G/A) polymorphism, which was shown to influence a variety of inflammatory and infectious diseases, could not be correlated with frequency of OT occurrence or recurrence (Cordeiro et al., 2008b). This result corresponds with our observations in human (Lahmar et al., 2009; Sauer et al., 2012) and murine studies (Sauer et al., 2013), which also did not show a change in TNF-α expression.

Jamieson and colleagues looked, in a large multi-center study, at cohorts of mother–child duos in Europe and parent–child trios in North America to identify factors associated with the development or not of ocular disease following congenital infection. Polymorphisms in *COL2A1* and *ABCA4* coding for retinal proteins known for their involvement in genetic retinal disorders indeed correlated with OT expression (Jamieson et al., 2008). Such association was also found, in the North American cohort, for polymorphisms in the gene coding for P2X(7) (Jamieson et al., 2010), a receptor protein known to participate in inflammasome activation.

Together, despite searching only for a restricted numbers of factors, these association studies demonstrate the importance of key immune factors in human OT development and validate results obtained from the above outlined mouse studies.

6. Retinal latency

Toxoplasma gondii remains latent in the retina within cysts. A remarkable feature of retinal cysts is the nearly complete absence of inflammation in the surrounding tissue, except during recurrences, as stated by us and other investigators. The mechanisms which allow its survival and long-term persistence by triggering the down-regulation of a major inflammatory response are still unknown. A clue might be the fact that the intracellular presence of *Toxoplasma* results in efficient dysregulation of the cell cycle (Brunet et al., 2008) and, more generally, the intracellular machinery and transcriptional changes. Targeting regulatory cascades controlling chromatin structure to subvert host cell function allows the parasite to simultaneously down-regulate transcription of several host genes. Transcriptional initiation of many genes requires changes in chromatin structure surrounding the promoter. The most common mechanisms to induce epigenetic changes and control gene expression are DNA methylation and histone modifications by chromatin-remodeling complexes and histone-modifying enzymes. In the last few years, evidence has accumulated that histone modifications and chromatin remodeling are key targets for pathogen manipulation during infection (Gomez-Diaz et al., 2012).

The ability of *T. gondii* to establish chronic infection depends especially on various immune evasion strategies. The parasite has developed epigenetic mechanisms by which it can render the host's immune responses inactive and undergo latency. *Toxoplasma gondii* prevents overinduction of pro-inflammatory cytokine production, a response that enables host survival and allows establishment of persistent infection in the host. Long-term transcriptional silencing by chromatin remodeling of IFN-γ-regulated promoters was found to have an important role in suppression of a host's immune response to *T. gondii* infection (Lang et al., 2012). *Toxoplasma gondii* regulates both inflammatory cytokines such as TNF-α (Leng et al., 2009), as well as anti-inflammatory mediators such as IL-10 (Leng and Denkers, 2009), to optimise its environment.

Histone modification and chromatin remodeling by *T. gondii* infection is an emerging field of study and future work will determine how epigenetic regulation of gene expression by *T. gondii* secreted proteins could be a general mechanism to enhance intracellular survival and reservoir persistence in immune privileged organs, thus maximising its chances of transmission. Finally, this could lead to identification of new potential targets for future development of novel therapeutic intervention strategies.

7. Perspectives

Obviously, OT is not the same disease in Europe and in South America (Fig. 2), with crucial consequences for treatment strategies. The geographical mapping of OT is beginning to take shape. However, there are still considerable discrepancies between some studies, maybe due to the evolution of diagnostic tools. Comparative studies should be undertaken, using the same criteria in diagnosis and strain typing.

Concerning more fundamental research, there are still very few data on the differential infection and proliferation capacity of the different *T. gondii* strains in various retinal cell types. These differential mechanisms are certainly a major factor determining strain-specific virulence. A special focus should be on the molecular mechanisms allowing parasite persistence in retinal cells and the influence of host genetic diversity on primary pathology and recurrence. It is certain that an important part of the answer will be found at the epigenetic level. Finally, from a medical point of view, the reason for ocular tropism of certain strains is of primordial interest, as it could direct prevention and treatment in a more targeted way. Clearly, the actual approach of a monotherapy using steroids is far from ideal (Garweg and Stanford, 2013). More generally, elucidating strain-dependent involvement of the IL-6–IL-23–IL-17 inflammatory cascade should result in targeted treatment according to the infecting strain, the patient's genetic disposition and the severity of the lesions.

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References

Ajzenberg, D., 2011. Unresolved questions about the most successful known parasite. Expert Rev. Anti Infect. Ther. 9, 169–171.

Albuquerque, M.C., Aleixo, A.L., Benchimol, E.I., Leandro, A.C., das Neves, L.B., Vicente, R.T., Bonecini-Almeida Mda, G., Amendoeira, M.R., 2009. The IFN-gamma +874T/A gene polymorphism is associated with retinochoroiditis toxoplasmosis susceptibility. Mem. Inst. Oswaldo Cruz 104, 451–455.

Barisani-Asenbauer, T., Maca, S.M., Mejdoubi, L., Emminger, W., Machold, K., Auer, H., 2012. Uveitis- a rare disease often associated with systemic diseases and infections- a systematic review of 2619 patients. Orphanet J Rare Dis. 7, 57.

Bosch-Driessen, L.E., Berendschot, T.T., Ongkosuwito, J.V., Rothova, A., 2002. Ocular toxoplasmosis: clinical features and prognosis of 154 patients. Ophthalmology 109, 869–878.

Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S.N., Osborne, N.N., Reichenbach, A., 2006. Muller cells in the healthy and diseased retina. Prog. Retin. Eye Res. 25, 397–424.

Brunet, J., Pfaff, A.W., Abidi, A., Unoki, M., Nakamura, Y., Guinard, M., Klein, J.P., Candolfi, E., Mousli, M., 2008. *Toxoplasma gondii* exploits UHRF1 and induces host cell cycle arrest at G2 to enable its proliferation. Cell. Microbiol. 10, 908–920.

Burnett, A.J., Shortt, S.G., Isaac-Renton, J., King, A., Werker, D., Bowie, W.R., 1998. Multiple cases of acquired toxoplasmosis retinitis presenting in an outbreak. Ophthalmology 105, 1032–1037.

Butler, N.J., Furtado, J.M., Winthrop, K.L., Smith, J.R., 2013. Ocular toxoplasmosis II: clinical features, pathology and management. Clin. Exp. Ophthalmol. 41, 95–108.

Caspi, R., 2008. Autoimmunity in the immune privileged eye: pathogenic and regulatory T cells. Immunol. Res. 42, 41–50.

Caspi, R.R., 2006. Ocular autoimmunity: the price of privilege? Immunol. Rev. 213, 23–35.

Charles, E., Callegan, M.C., Blader, I.J., 2007. The SAG1 *Toxoplasma gondii* surface protein is not required for acute ocular toxoplasmosis in mice. Infect. Immun. 75, 2079–2083.

Charles, E., Joshi, S., Ash, J.D., Fox, B.A., Farris, A.D., Bzik, D.J., Lang, M.L., Blader, I.J., 2010. CD4 T-cell suppression by cells from *Toxoplasma gondii*-infected retinas is mediated by surface protein PD-L1. Infect. Immun. 78, 3484–3492.

Chen, Y., Yang, P., Li, F., Kijlstra, A., 2011. The effects of Th17 cytokines on the inflammatory mediator production and barrier function of ARPE-19 cells. PLoS ONE 6, e18139.

Chen, Y., Sawada, O., Kohno, H., Le, Y.Z., Subauste, C., Maeda, T., Maeda, A., 2013. Autophagy protects the retina from light-induced degeneration. J. Biol. Chem. 288, 7506–7518.

Cimino, L., Aldigeri, R., Salvarani, C., Zotti, C.A., Boiardi, L., Parmeggiani, M., Casali, B., Cappuccini, L., 2010. The causes of uveitis in a referral centre of Northern Italy. Int. Ophthalmol. 30, 521–529.

Cordeiro, C.A., Moreira, P.R., Andrade, M.S., Dutra, W.O., Campos, W.R., Orefice, F., Teixeira, A.L., 2008a. Interleukin-10 gene polymorphism (−1082G/A) is associated with toxoplasmic retinochoroiditis. Invest. Ophthalmol. Vis. Sci. 49, 1979–1982.

Cordeiro, C.A., Moreira, P.R., Costa, G.C., Dutra, W.O., Campos, W.R., Orefice, F., Teixeira, A.L., 2008b. TNF-alpha gene polymorphism (−308G/A) and toxoplasmic retinochoroiditis. Br. J. Ophthalmol. 92, 986–988.

Cordeiro, C.A., Moreira, P.R., Costa, G.C., Dutra, W.O., Campos, W.R., Orefice, F., Teixeira, A.L., 2008c. Interleukin-1 gene polymorphisms and toxoplasmic retinochoroiditis. Mol. Vis. 14, 1845–1849.

Cordeiro, C.A., Moreira, P.R., Bessa, T.F., Costa, G.C., Dutra, W.O., Campos, W.R., Orefice, F., Young, L.H., Teixeira, A.L., 2013. Interleukin-6 gene polymorphism (−174 G/C) is associated with toxoplasmic retinochoroiditis. Acta Ophthalmol. 91, e311–314.

Davidson, M.G., Lappin, M.R., English, R.V., Tompkins, M.B., 1993. A feline model of ocular toxoplasmosis. Invest. Ophthalmol. Vis. Sci. 34, 3653–3660.

De-la-Torre, A., Gonzalez, G., Diaz-Ramirez, J., Gomez-Marin, J.E., 2007. Screening by ophthalmoscopy for *Toxoplasma* retinochoroiditis in Colombia. Am. J. Ophthalmol. 143, 354–356.

De-la-Torre, A., Rios-Cadavid, A.C., Cardozo-Garcia, C.M., Gomez-Marin, J.E., 2009. Frequency and factors associated with recurrences of ocular toxoplasmosis in a referral centre in Colombia. Br. J. Ophthalmol. 93, 1001–1004.

De-la-Torre, A., Gonzalez-Lopez, G., Montoya-Gutierrez, J.M., Marin-Arango, V., Gomez-Marin, J.E., 2011. Quality of life assessment in ocular toxoplasmosis in a Colombian population. Ocul. Immunol. Inflamm. 19, 262–266.

De-la-Torre, A., Sauer, A., Pfaff, A.W., Bourcier, T., Brunet, J., Speeg-Schatz, C., Ballonzoli, L., Villard, O., Ajzenberg, D., Sundar, N., Grigg, M.E., Gomez-Marin, J.E., Candolfi, E., 2013. Severe South American ocular toxoplasmosis is associated with decreased IFN-gamma/IL-17A and increased IL-6/IL-13 intraocular levels. PLoS Negl. Trop. Dis. (in press).

de Moura, L., Bahia-Oliveira, L.M., Wada, M.Y., Jones, J.L., Tuboi, S.H., Carmo, E.H., Ramalho, W.M., Camargo, N.J., Trevisan, R., Graca, R.M., da Silva, A.J., Moura, I., Dubey, J.P., Garrett, D.O., 2006. Waterborne toxoplasmosis, Brazil, from field to gene. Emerg. Infect. Dis. 12, 326–329.

Delair, E., Monnet, D., Grabar, S., Dupouy-Camet, J., Yera, H., Brezin, A.P., 2008. Respective roles of acquired and congenital infections in presumed ocular toxoplasmosis. Am. J. Ophthalmol. 146, 851–855.

Delair, E., Latkany, P., Noble, A.G., Rabiah, P., McLeod, R., Brezin, A., 2011. Clinical manifestations of ocular toxoplasmosis. Ocul. Immunol. Inflamm. 19, 91–102.

Dodds, E.M., Holland, G.N., Stanford, M.R., Yu, F., Siu, W.O., Shah, K.H., Ten Dam-van Loon, N., Muccioli, C., Hovakimyan, A., Barisani-Asenbauer, T., 2008. Intraocular inflammation associated with ocular toxoplasmosis: relationships at initial examination. Am. J. Ophthalmol. 146 (856–865), e852.

Dutra, M.S., Bela, S.R., Peixoto-Rangel, A.L., Fakiola, M., Cruz, A.G., Gazzinelli, A., Quites, H.F., Bahia-Oliveira, L.M., Peixe, R.G., Campos, W.R., Higino-Rocha, A.C., Miller, N.E., Blackwell, J.M., Antonelli, L.R., Gazzinelli, R.T., 2013. Association of a NOD2 Gene Polymorphism and T-Helper 17 Cells With Presumed Ocular Toxoplasmosis. J. Infect. Dis. 207, 152–163.

Fekkar, A., Ajzenberg, D., Bodaghi, B., Touafek, F., Le Hoang, P., Delmas, J., Robert, P.Y., Darde, M.L., Mazier, D., Paris, L., 2011. Direct genotyping of *Toxoplasma gondii* in ocular fluid samples from 20 patients with ocular toxoplasmosis: predominance of type II in France. J. Clin. Microbiol. 49, 1513–1517.

Fischer, H.G., Nitzgen, B., Reichmann, G., Gross, U., Hadding, U., 1997. Host cells of *Toxoplasma gondii* encystation in infected primary culture from mouse brain. Parasitol. Res. 83, 637–641.

Furtado, J.M., Bharadwaj, A.S., Chipps, T.J., Pan, Y., Ashander, L.M., Smith, J.R., 2012. *Toxoplasma gondii* tachyzoites cross retinal endothelium assisted by intercellular adhesion molecule-1 in vitro. Immunol. Cell Biol. 90, 912–915.

Furtado, J.M., Winthrop, K.L., Butler, N.J., Smith, J.R., 2013. Ocular toxoplasmosis I: parasitology, epidemiology and public health. Clin. Exp. Ophthalmol. 41, 82–94.

Gallego, C., Saavedra-Matiz, C., Gomez-Marin, J.E., 2006. Direct genotyping of animal and human isolates of *Toxoplasma gondii* from Colombia (South America). Acta Trop. 97, 161–167.

Garweg, J.G., Kuenzli, H., Boehnke, M., 1998. Experimental ocular toxoplasmosis in naive and primed rabbits. Ophthalmologica 212, 136–141.

Garweg, J.G., de Kozak, Y., Goldenberg, B., Boehnke, M., 2009. Anti-retinal autoantibodies in experimental ocular and systemic toxoplasmosis. Graefes Arch. Clin. Exp. Ophthalmol. 248, 573–584.

Garweg, J.G., Stanford, M.R., 2013. Therapy for Ocular Toxoplasmosis – the Future. Ocul. Immunol. Inflamm. 21, 300–305.

Gazzinelli, R.T., Brezin, A., Li, Q., Nussenblatt, R.B., Chan, C.C., 1994. *Toxoplasma gondii*: acquired ocular toxoplasmosis in the murine model, protective role of TNF-alpha and IFN-gamma. Exp. Parasitol. 78, 217–229.

Gilbert, R.E., Dunn, D.T., Lightman, S., Murray, P.I., Pavesio, C.E., Gormley, P.D., Masters, J., Parker, S.P., Stanford, M.R., 1999. Incidence of symptomatic *Toxoplasma* eye disease: aetiology and public health implications. Epidemiol. Infect. 123, 283–289.

Gilbert, R.E., Freeman, K., Lago, E.G., Bahia-Oliveira, L.M., Tan, H.K., Wallon, M., Buffolano, W., Stanford, M.R., Petersen, E., 2008. Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PLoS Negl. Trop. Dis. 2, e277.

Glasner, P.D., Silveira, C., Kruszon-Moran, D., Martins, M.C., Burnier Junior, M., Silveira, S., Camargo, M.E., Nussenblatt, R.B., Kaslow, R.A., Belfort Junior, R., 1992. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. Am. J. Ophthalmol. 114, 136–144.

Gomez-Diaz, E., Jorda, M., Peinado, M.A., Rivero, A., 2012. Epigenetics of host-pathogen interactions: the road ahead and the road behind. PLoS Pathog. 8, e1003007.

Gomez-Marin, J.E., De-la-Torre, A., Angel-Muller, E., Rubio, J., Arenas, J., Osorio, E., Nunez, L., Pinzon, L., Mendez-Cordoba, L.C., Bustos, A., De-la-Hoz, I., Silva, P., Beltran, M., Chacon, L., Marrugo, M., Manjarres, C., Baquero, H., Lora, F., Torres, E., Zuluaga, O.E., Estrada, M., Moscote, L., Silva, M.T., Rivera, R., Molina, A., Najera, S., Sanabria, A., Ramirez, M.L., Alarcon, C., Restrepo, N., Falla, A., Rodriguez, T., Castano, G., 2011. First Colombian multicentric newborn screening for congenital toxoplasmosis. PLoS Negl. Trop. Dis. 5, e1195.

Gomez-Marin, J.E., De-la-Torre, A., Barrios, P., Cardona, N., Alvarez, C., Herrera, C., 2012. Toxoplasmosis in military personnel involved in jungle operations. Acta Trop. 122, 46–51.

Gormley, P.D., Pavesio, C.E., Luthert, P., Lightman, S., 1999. Retinochoroiditis is induced by oral administration of *Toxoplasma gondii* cysts in the hamster model. Exp. Eye Res. 68, 657–661.

Grigg, M.E., Ganatra, J., Boothroyd, J.C., Margolis, T.P., 2001. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. J. Infect. Dis. 184, 633–639.

Guerina, N.G., Hsu, H.W., Meissner, H.C., Maguire, J.H., Lynfield, R., Stechenberg, B., Abroms, I., Pasternack, M.S., Hoff, R., Eaton, R.B., et al., 1994. Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. The New England Regional Toxoplasma Working Group. N. Engl. J. Med. 330, 1858–1863.

Havelaar, A.H., Kemmeren, J.M., Kortbeek, L.M., 2007. Disease burden of congenital toxoplasmosis. Clin. Infect. Dis. 44, 1467–1474.

Hodge, W.G., Seiff, S.R., Margolis, T.P., 1998. Ocular opportunistic infection incidences among patients who are HIV positive compared to patients who are HIV negative. Ophthalmology 105, 895–900.

Holland, G.N., O'Connor, G.R., Diaz, R.F., Minasi, P., Wara, W.M., 1988. Ocular toxoplasmosis in immunosuppressed nonhuman primates. Invest. Ophthalmol. Vis. Sci. 29, 835–842.

Holland, G.N., 2000. Ocular toxoplasmosis: new directions for clinical investigation. Ocul. Immunol. Inflamm. 8, 1–7.

Holland, G.N., 2003. Ocular toxoplasmosis: a global reassessment. Part I: epidemiology and course of disease. Am. J. Ophthalmol. 136, 973–988.

Holland, G.N., 2004. Ocular toxoplasmosis: a global reassessment. Part II: disease manifestations and management. Am. J. Ophthalmol. 137, 1–17.

Holland, G.N., 2009. Ocular toxoplasmosis: the influence of patient age. Mem. Inst. Oswaldo Cruz 104, 351–357.

Horai, R., Caspi, R.R., 2011. Cytokines in autoimmune uveitis. J. Interferon Cytokine Res. 31, 733–744.

Jakob, E., Reuland, M.S., Mackensen, F., Harsch, N., Fleckenstein, M., Lorenz, H.M., Max, R., Becker, M.D., 2009. Uveitis subtypes in a german interdisciplinary uveitis center–analysis of 1916 patients. J. Rheumatol. 36, 127–136.

Jamieson, S.E., de Roubaix, L.A., Cortina-Borja, M., Tan, H.K., Mui, E.J., Cordell, H.J., Kirisits, M.J., Miller, E.N., Peacock, C.S., Hargrave, A.C., Coyne, J.J., Boyer, K., Bessieres, M.H., Buffolano, W., Ferret, N., Franck, J., Kieffer, F., Meier, P., Nowakowska, D.E., Paul, M., Peyron, F., Stray-Pedersen, B., Prusa, A.R., Thulliez, P., Wallon, M., Petersen, E., McLeod, R., Gilbert, R.E., Blackwell, J.M., 2008. Genetic and epigenetic factors at COL2A1 and ABCA4 influence clinical outcome in congenital toxoplasmosis. PLoS ONE 3, e2285.

Jamieson, S.E., Peixoto-Rangel, A.L., Hargrave, A.C., Roubaix, L.A., Mui, E.J., Boulter, N.R., Miller, E.N., Fuller, S.J., Wiley, J.S., Castellucci, L., Boyer, K., Peixe, R.G., Kirisits, M.J., Elias Lde, S., Coyne, J.J., Correa-Oliveira, R., Sautter, M., Smith, N.C., Lees, M.P., Swisher, C.N., Heydemann, P., Noble, A.G., Patel, D., Bardo, D., Burrowes, D., McLone, D., Roizen, N., Withers, S., Bahia-Oliveira, L.M., McLeod, R., Blackwell, J.M., 2010. Evidence for associations between the purinergic receptor P2X(7) (P2RX7) and toxoplasmosis. Genes Immun. 11, 374–383.

Jones, J.L., Holland, G.N., 2010. Annual burden of ocular toxoplasmosis in the US. Am. J. Trop. Med. Hyg. 82, 464–465.

Jones, L.A., Alexander, J., Roberts, C.W., 2006. Ocular toxoplasmosis: in the storm of the eye. Parasite Immunol. 28, 635–642.

Khan, A., Jordan, C., Muccioli, C., Vallochi, A.L., Rizzo, L.V., Belfort Jr., R., Vitor, R.W., Silveira, C., Sibley, L.D., 2006. Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis, Brazil. Emerg. Infect. Dis. 12, 942–949.

Kikumura, A., Ishikawa, T., Norose, K., 2012. Kinetic analysis of cytokines, chemokines, chemokine receptors and adhesion molecules in murine ocular toxoplasmosis. Br. J. Ophthalmol. 96, 1259–1267.

Knight, B.C., Brunton, C.L., Modi, N.C., Wallace, G.R., Stanford, M.R., 2005. The effect of *Toxoplasma gondii* infection on expression of chemokines by rat retinal vascular endothelial cells. J. Neuroimmunol. 160, 41–47.

Knight, B.C., Kissane, S., Falciani, F., Salmon, M., Stanford, M.R., Wallace, G.R., 2006. Expression analysis of immune response genes of Muller cells infected with *Toxoplasma gondii*. J. Neuroimmunol. 179, 126–131.

Kong, J.T., Grigg, M.E., Uyetake, L., Parmley, S., Boothroyd, J.C., 2003. Serotyping of *Toxoplasma gondii* infections in humans using synthetic peptides. J. Infect. Dis. 187, 1484–1495.

Lahmar, I., Abou-Bacar, A., Abdelrahman, T., Guinard, M., Babba, H., Ben Yahia, S., Kairallah, M., Speeg-Schatz, C., Bourcier, T., Sauer, A., Villard, O., Pfaff, A.W., Mousli, M., Garweg, J.G., Candolfi, E., 2009. Cytokine profiles in toxoplasmic and viral uveitis. J. Infect. Dis. 199, 1239–1249.

Lahmar, I., Guinard, M., Sauer, A., Marcellin, L., Abdelrahman, T., Roux, M., Mousli, M., Moussa, A., Babba, H., Pfaff, A.W., Candolfi, E., 2010. Murine neonatal infection provides an efficient model for congenital ocular toxoplasmosis. Exp. Parasitol. 124, 190–196.

Lang, C., Hildebrandt, A., Brand, F., Opitz, L., Dihazi, H., Luder, C.G., 2012. Impaired chromatin remodelling at STAT1-regulated promoters leads to global unresponsiveness of *Toxoplasma gondii*-Infected macrophages to IFN-gamma. PLoS Pathog. 8, e1002483.

Leng, J., Butcher, B.A., Egan, C.E., Abi Abdallah, D.S., Denkers, E.Y., 2009. *Toxoplasma gondii* prevents chromatin remodeling initiated by TLR-triggered macrophage activation. J. Immunol. 182, 489–497.

Leng, J., Denkers, E.Y., 2009. *Toxoplasma gondii* inhibits covalent modification of histone H3 at the IL-10 promoter in infected macrophages. PLoS ONE 4, e7589.

Lindsay, D.S., Dubey, J.P., 2011. *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. Parasitology, 1–3.

Lu, F., Huang, S., Hu, M.S., Kasper, L.H., 2005. Experimental ocular toxoplasmosis in genetically susceptible and resistant mice. Infect. Immun. 73, 5160–5165.

Lyons, R.E., Anthony, J.P., Ferguson, D.J., Byrne, N., Alexander, J., Roberts, F., Roberts, C.W., 2001. Immunological studies of chronic ocular toxoplasmosis: up-regulation of major histocompatibility complex class I and transforming growth factor beta and a protective role for interleukin-6. Infect. Immun. 69, 2589–2595.

Masur, H., Jones, T.C., Lempert, J.A., Cherubini, T.D., 1978. Outbreak of toxoplasmosis in a family and documentation of acquired retinochoroiditis. Am. J. Med. 64, 396–402.

Mets, M.B., Holfels, E., Boyer, K.M., Swisher, C.N., Roizen, N., Stein, L., Stein, M., Hopkins, J., Withers, S., Mack, D., Luciano, R., Patel, D., Remington, J.S., Meier, P., McLeod, R., 1996. Eye manifestations of congenital toxoplasmosis. Am. J. Ophthalmol. 122, 309–324.

Mimura, K.K., Tedesco, R.C., Calabrese, K.S., Gil, C.D., Oliani, S.M., 2012. The involvement of anti-inflammatory protein, annexin A1, in ocular toxoplasmosis. Mol. Vis. 18, 1583–1593.

Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. Lancet 363, 1965–1976.

Morisset, S., Peyron, F., Lobry, J.R., Garweg, J., Ferrandiz, J., Musset, K., Gomez-Marin, J.E., de la Torre, A., Demar, M., Carme, B., Mercier, C., Garin, J.F., Cesbron-Delauw, M.F., 2008. Serotyping of *Toxoplasma gondii*: striking homogeneous pattern between symptomatic and asymptomatic infections within Europe and South America. Microbes Infect. 10, 742–747.

Norose, K., Kikumura, A., Luster, A.D., Hunter, C.A., Harris, T.H., 2011. CXCL10 is required to maintain T-cell populations and to control parasite replication during chronic ocular toxoplasmosis. Invest. Ophthalmol. Vis. Sci. 52, 389–398.

Pavesio, C.E., Chiappino, M.L., Gormley, P., Setzer, P.Y., Nichols, B.A., 1995. Acquired retinochoroiditis in hamsters inoculated with ME 49 strain *Toxoplasma*. Invest. Ophthalmol. Vis. Sci. 36, 2166–2175.

Peixoto-Rangel, A.L., Miller, E.N., Castellucci, L., Jamieson, S.E., Peixe, R.G., Elias Lde, S., Correa-Oliveira, R., Bahia-Oliveira, L.M., Blackwell, J.M., 2009. Candidate gene analysis of ocular toxoplasmosis in Brazil: evidence for a role for toll-like receptor 9 (TLR9). Mem. Inst. Oswaldo Cruz 104, 1187–1190.

Petersen, E., Kijlstra, A., Stanford, M., 2012. Epidemiology of ocular toxoplasmosis. Ocul. Immunol. Inflamm. 20, 68–75.

Phan, L., Kasza, K., Jalbrzikowski, J., Noble, A.G., Latkany, P., Kuo, A., Mieler, W., Meyers, S., Rabiah, P., Boyer, K., Swisher, C., Mets, M., Roizen, N., Cezar, S., Sautter, M., Remington, J., Meier, P., McLeod, R., 2008. Longitudinal study of new eye lesions in children with toxoplasmosis who were not treated during the first year of life. Am. J. Ophthalmol. 146, 375–384.

Pifer, R., Yarovinsky, F., 2011. Innate responses to *Toxoplasma gondii* in mice and humans. Trends Parasitol. 27, 388–393.

Pivetti-Pezzi, P., Accorinti, M., Tamburi, S., Ciapparoni, V., Abdulaziz, M.A., 1994. Clinical features of toxoplasmic retinochoroiditis in patients with acquired immunodeficiency syndrome. Ann. Ophthalmol. 26, 73–84.

Pomares, C., Ajzenberg, D., Bornard, L., Bernardin, G., Hasseine, L., Darde, M.L., Marty, P., 2011. Toxoplasmosis and horse meat, France. Emerg. Infect. Dis. 17, 1327–1328.

Rodriguez, A., Calonge, M., Pedroza-Seres, M., Akova, Y.A., Messmer, E.M., D'Amico, D.J., Foster, C.S., 1996. Referral patterns of uveitis in a tertiary eye care center. Arch. Ophthalmol. 114, 593–599.

Sauer, A., Lahmar, I., Scholler, M., Villard, O., Speeg-Schatz, C., Brunet, J., Pfaff, A., Bourcier, T., Candolfi, E., 2009. Development of murine models of ocular toxoplasmosis and preliminary results of ocular inflammatory transcriptome. J. Fr. Ophtalmol. 32, 742–749.

Sauer, A., de la Torre, A., Gomez-Marin, J., Bourcier, T., Garweg, J., Speeg-Schatz, C., Candolfi, E., 2011. Prevention of retinochoroiditis in congenital toxoplasmosis: Europe versus South America. Pediatr. Infect. Dis. J. 30, 601–603.

Sauer, A., Pfaff, A.W., Villard, O., Creuzot-Garcher, C., Dalle, F., Chiquet, C., Pelloux, H., Speeg-Schatz, C., Gaucher, D., Prevost, G., Bourcier, T., Candolfi, E., 2012. Interleukin 17A as an effective target for anti-inflammatory and antiparasitic treatment of toxoplasmic uveitis. J. Infect. Dis. 206, 1319–1329.

Sauer, A., Rochet, E., Lahmar, I., Brunet, J., Sabou, M., Bourcier, T., Candolfi, E., Pfaff, A.W., 2013. The local immune response to intraocular *Toxoplasma* re-challenge: less pathology and better parasite control through Treg/Th1/Th2 induction. Int. J. Parasitol. 43, 721–728.

Silveira, C., Belfort Jr., R., Muccioli, C., Abreu, M.T., Martins, M.C., Victora, C., Nussenblatt, R.B., Holland, G.N., 2001. A follow-up study of *Toxoplasma gondii* infection in southern Brazil. Am. J. Ophthalmol. 131, 351–354.

Skorich, D.N., Chiappino, M.L., Nichols, B.A., 1988. Invasion of the guinea pig conjunctiva by *Toxoplasma gondii*. Invest. Ophthalmol. Vis. Sci. 29, 1871–1880.

Streilein, J.W., 1993. Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. Curr. Opin. Immunol. 5, 428–432.

Streilein, J.W., 2003. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. J. Leukoc. Biol. 74, 179–185.

Sugita, S., Futagami, Y., Smith, S.B., Naggar, H., Mochizuki, M., 2006. Retinal and ciliary body pigment epithelium suppress activation of T lymphocytes via transforming growth factor beta. Exp. Eye Res. 83, 1459–1471.

Sugita, S., Horie, S., Nakamura, O., Futagami, Y., Takase, H., Keino, H., Aburatani, H., Katunuma, N., Ishidoh, K., Yamamoto, Y., Mochizuki, M., 2008. Retinal pigment epithelium-derived CTLA-2alpha induces TGFbeta-producing T regulatory cells. J. Immunol. 181, 7525–7536.

Switaj, K., Master, A., Borkowski, P.K., Skrzypczak, M., Wojciechowicz, J., Zaborowski, P., 2006. Association of ocular toxoplasmosis with type I *Toxoplasma gondii* strains: direct genotyping from peripheral blood samples. J. Clin. Microbiol. 44, 4262–4264.

Tan, H.K., Schmidt, D., Stanford, M., Tear-Fahnehjelm, K., Ferret, N., Salt, A., Gilbert, R., 2007. Risk of visual impairment in children with congenital toxoplasmic retinochoroiditis. Am. J. Ophthalmol. 144, 648–653.

Tedesco, R.C., Smith, R.L., Corte-Real, S., Calabrese, K.S., 2005. Ocular toxoplasmosis in mice: comparison of two routes of infection. Parasitology 131, 303–307.

Thiebaut, R., Leproust, S., Chene, G., Gilbert, R., 2007. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet 369, 115–122.

Van Grol, J., Muniz-Feliciano, L., Portillo, J.A., Bonilha, V.L., Subauste, C.S., 2013. CD40 induces anti-*Toxoplasma gondii* activity in nonhematopoietic cells dependent on autophagy proteins. Infect. Immun. 81, 2002–2011.

Vasconcelos-Santos, D.V., Machado Azevedo, D.O., Campos, W.R., Orefice, F., Queiroz-Andrade, G.M., Carellos, E.V., Castro Romanelli, R.M., Januario, J.N., Resende, L.M., Martins-Filho, O.A., de Aguiar Vasconcelos Carneiro, A.C., Almeida Vitor, R.W., Caiaffa, W.T., 2009. Congenital toxoplasmosis in southeastern Brazil: results of early ophthalmologic examination of a large cohort of neonates. Ophthalmology 116 (2199–2205), e2191.

Vasconcelos-Santos, D.V., 2012. Ocular manifestations of systemic disease: toxoplasmosis. Curr. Opin. Ophthalmol. 23, 543–550.

Villard, O., Filisetti, D., Roch-Deries, F., Garweg, J., Flament, J., Candolfi, E., 2003. Comparison of enzyme-linked immunosorbent assay, immunoblotting, and PCR for diagnosis of toxoplasmic chorioretinitis. J. Clin. Microbiol. 41, 3537–3541.

Villena, I., Ancelle, T., Delmas, C., Garcia, P., Brezin, A.P., Thulliez, P., Wallon, M., King, L., Goulet, V., 2010. Congenital toxoplasmosis in France in 2007: first results from a national surveillance system. Euro. Surveill., 15.