Epidemiology, Pathophysiology, Diagnosis, and Management of Cerebral Toxoplasmosis

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SUMMARY *Toxoplasma gondii* is known to infect a considerable number of mammalian and avian species, and a substantial proportion of the world's human population. The parasite has an impressive ability to disseminate within the host's body and employs various tactics to overcome the highly regulatory blood-brain barrier and reside in the brain. In healthy individuals, *T. gondii* infection is largely tolerated without any obvious ill effect. However, primary infection in immunosuppressed patients can result in acute cerebral or systemic disease, and reactivation of latent tissue cysts can lead to a deadly outcome. It is imperative that treatment of the life-threatening toxoplasmic encephalitis is timely and effective. Several therapeutic and prophylactic regimens have been used in clinical practice. Current approaches can control infection caused by the invasive and highly proliferative tachyzoites but cannot eliminate the dormant tissue cysts. Adverse events, and other limitations, are associated with the standard pyrimethamine-based therapy and effective vaccines are unavailable. In this review the epidemiology, economic impact, pathophysiology, diagnosis and management of cerebral toxoplasmosis are discussed, and critical areas for future research are highlighted.

KEYWORDS *Toxoplasma gondii*, toxoplasmic encephalitis, immunocompromised patients, pathophysiology, diagnosis, treatment

INTRODUCTION

The opportunistic organism *Toxoplasma gondii* has reached a global interest due to its public health and socioeconomic impacts. This apicomplexan parasite can infect a large number of domestic and wild animals, and has infected a significant number of people throughout the world (1). People become infected primarily through consuming raw or improperly cooked meat (particularly lamb and pork) containing infectious tissue cysts or via ingestion of sporulated oocysts in vegetables, fruits or water contaminated with feline feces (2). At the initial infection site in the intestine *T. gondii* infects various immune cells and use them to migrate to and infiltrate the brain, where it employs various strategies to overcome the complex cellular structure of the blood-brain-barrier (BBB). In the vast majority of individuals with competent immune responses, primary infection is asymptomatic or may produce a mild, flu-like illness - and the parasite eventually lies dormant within a tissue cyst. However, in less than 10% of infections, a mononucleosis-like syndrome with headache, malaise, fever, cervical lymphadenopathy and fatigue may occur (3). Primary *T. gondii* infection can also cause ocular disease, and in pregnant women, can lead to fetal death or brain damage in congenitally infected children (4-6).

In addition to the three classical clinical forms of toxoplasmosis (ocular, congenital, cerebral), association of latent *T. gondii* infection with a number of behavioral modifications and neuropsychiatric disorders has also been reported (7). Recurrence of toxoplasmosis from latency is a frequent cause of toxoplasmic encephalitis (TE) in people with immunosuppressive conditions - such as advanced HIV infection, organ transplantation, neoplastic disease, or those receiving immunosuppressive therapies (e.g. rituximab). These patients are particularly vulnerable to recrudescence of latent infection, wherein slowly-dividing bradyzoites transform into rapidly-replicating tachyzoites, which can result in fatal consequences (3). Symptoms of TE may include diffuse encephalopathy, headaches, confusion, weakness, numbness, incoordination, and seizures. Extra-cerebral manifestations, such as respiratory and visual problems can also occur.

Here, we outline the epidemiology and pathobiology of *T. gondii* infection as it relates to the cerebral form of toxoplasmosis, and we highlight the mechanisms by which this parasite migrates to, invades, and colonizes the brain to causes neurological dysfunctions. We also discuss diagnostic approaches using molecular methods and neuroimaging techniques to confirm brain involvement in infected individuals and summarize current strategies for the treatment and control of TE, particularly in immunocompromised patients. Finally, we shed light on new targets for future research that may accelerate the discovery of improved methods for managing this condition.

PARASITE BIOLOGY

Life-Cycle Forms and Routes of Transmission

The provenance of the multistage lifecycle of *Toxoplasma gondii* has been well established. During its development, *T. gondii* progresses through 3 main distinctive forms namely: oocyst (containing sporozoites), tachyzoite, and tissue cyst (containing bradyzoites). Oocysts are the final product of sexual reproduction and are formed exclusively in the intestine of infected felines. The obligate intracellular tachyzoite and the bradyzoite represent the two main stages in the parasite's asexual reproductive cycle. Tachyzoites represent the rapidly dividing stage within host cells. Tachyzoites can disseminate to multiple and distant tissues within the host's body and can elicit significant immune responses. Although chemotherapeutic drugs and host immune defenses can limit their growth, some tachyzoites, have the ability to overcome these formidable challenges and transform into slowly replicating bradyzoites. In stark contrast to tachyzoites, bradyzoites divide slowly and remain dormant, protected within a stage-specific cyst, mainly located in the brain and muscle (skeletal and cardiac), presumably due to less rapid parasite elimination caused by reduced cellular turnover in these tissues compared to other organs. The preference of *T. gondii* to infect neurons, and their particular location within neuronal processes, was demonstrated *in vivo* for the first time by using a *T. gondii* reporter strain that secretes Cre into host cells, which enabled the specific identification and direct visualization of infected neurons (8). Bradyzoites are contained within tissue cysts that can evade the host immune responses and facilitate the establishment of a long term persistent infection, without causing overt disease (9). A recent study identified the Myb-like transcription factor (BFD1) as a key regulator for bradyzoite differentiation (10). Infection in humans, commonly occurs via the consumption of food or water contaminated with tissue cysts or oocysts (11). Congenital 'vertical' transmission also occurs wherein

T. gondii tachyzoites are passed from mother to fetus through the placenta (4). Other routes of transmission of *T. gondii* include organ transplantation and blood transfusion (4).

Reproductive Strategies

According to the established paradigm, the lifecycle of *T. gondii* involves sexual and asexual reproductive phases. The sexual reproductive phase, known as 'gametogony', occurs within cats (and other felids), which serve as the exclusive 'definitive' hosts for *T. gondii*. In the intestinal epithelium of the definitive host, *T. gondii* differentiates into male and female gametes that form zygotes, which leave the cat intestine and are excreted with feces as oocysts (12). This is the main source of genetic recombination, should the cat be coinfected with more than one *T. gondii* strains (13). Infected cats shed millions of oocytes for a non-recurring period lasting up to 3 weeks (14). Within 2-3 days following excretion, depending on environmental conditions, oocysts undergo maturation/sporulation to become infectious. Once they become sporulated, oocysts can survive in the environment and maintain their viability for more than a year. The reason that the feline gut epithelium is the only tissue that can accommodate sexual reproduction of this parasite remains largely unknown. In recent years, interest in the development of methods for establishing *in vitro* culturing models of cat intestine has increased and, it is hoped, that these models may help in unraveling the mechanisms that allow this particular location in this particular host to support the sexual development of *T. gondii* (15-17). A recent study, using feline intestinal organoids, has shown that a critical factor in the exclusive occurrence of sexual reproduction and oocyst production, in the feline gut is the intrinsic abundance of linoleic acid in cats when compared to other mammalian (non-felid) hosts (18). Cat's gut is genetically deficient in the enzyme delta-6-desaturase which converts linoleic acid to arachidonic acid. Astonishingly, high levels of linoleic acid together with inhibiting delta-6-desaturase that suppresses metabolic conversion of linoleic acid to arachidonic acid, have also triggered sexual reproduction and oocyst formation in mice (18). Temporal analysis of the transcriptome of cat intestine during the first 96 hours post *T. gondii* infection revealed significant changes in transcripts associated with immune response and metabolic pathways (19). A recent study showed that sexual differentiation in *T. gondii* can be inhibited by histone deacetylase HDAC3, in a process mediated by microrchidia (MORC) protein and the Apetala 2 transcription factor (20).

T. gondii shows a remarkable diversity in the range of vertebrate species that it uses as intermediate hosts. Most warm-blooded mammals, birds and humans can serve as the intermediate host. Although humans are permissive to *T. gondii* infection, they are dead-end hosts, unless they are eaten by a feline species. Mammals and birds serve as proper intermediate hosts and transmit tissue cysts to both the definitive feline host, and to other intermediate hosts. Within humans and other intermediate hosts, *T. gondii* exists in either the more rapidly proliferating tachyzoite stage or the more dormant bradyzoite stage. Ingestion of bradyzoites-containing cysts in raw or poorly cooked meat or infectious oocysts that are excreted in felid fecal material are the major infection sources. Upon entry into the small intestine, bradyzoites within the cysts, or sporozoites within the sporulated oocysts, are released and invade the intestinal epithelial cells where they differentiate into tachyzoites. *T. gondii* tachyzoites have a distinctive ability to migrate to distant body regions including peripheral and immunoprivileged regions, such as the eye, brain and placenta. As they invade, tachyzoites produce a membrane-bound parasitophorous vacuole (PV). Within this protective shelter, *T. gondii* tachyzoites secret many effector molecules, exploit host cell metabolic resources and reproduce asexually in a process known as 'endodyogeny', whereby each parental tachyzoite divides to form two daughter tachyzoites. This reproductive process in the intermediate host continues until the accumulation of newly produced tachyzoites causes rupture of the infected cells. Tachyzoites then continue with further rounds of invasion and proliferation in new cells. Without therapeutic interventions, or a strong immune response, these repeated cycles of intracellular replications will cause severe or even fatal pathologies. In the setting of effective immune response or treatment, *T. gondii* establishes a chronic infection in the infected host as bradyzoites inside cysts, surrounded by a wall, formed by modification of the membrane, that limits the bradyzoites-containing PV (21). Under certain conditions, *T. gondii* undergoes phenotypic transformation from bradyzoites (the hallmark of latent infection) to tachyzoites (associated with acute infection) (22), and this differentiation can lead to adverse, or even life-threatening, consequences in immunocompromised individuals (3, 23).

EPIDEMIOLOGY

Burden, Global Prevalence and Risk Factors of Toxoplasmosis

As a single disease, toxoplasmosis exerts a significant impact on health-care services, individual healthcare costs and health insurance companies. Earlier estimates, published in 2012, suggest that toxoplasmosis accounted for nearly $3 billion of illness-related costs, and approximately 11,000 quality-adjusted life-years (QALYs) lost per year (24, 25). In the USA alone, the total annual incidence of toxoplasmosis was estimated to be 9,832; with ocular (2,169) and cerebral (1,399) toxoplasmosis being the most prevalent forms of disease (26). Another 11-year study (2000–2010) in the USA reported 789 toxoplasmosis-related deaths, predominantly in people from Black and Hispanic backgrounds; with cumulative productivity losses of approximately $815 million (27). The economic cost of food-borne toxoplasmosis in pork was estimated to be $1.9 billion in the USA (28). During 1998-2010, an annual average of 20,258 encephalitis-associated hospitalizations (attributed to *T. gondii* and other infectious and non-infectious causes) were reported in the USA (29). A 9-year Canadian study (2002–2011) reported an overall annual health-care cost of C$1,686,860 attributed to toxoplasmosis (30). In the Netherlands, the toxoplasmosis burden has been estimated to be ~ €44 million in health costs, with the loss of 1,900 Disability Adjusted Life Years (DALYs) annually (31). In Denmark, foodborne congenital toxoplasmosis was estimated to cause the loss of ~100 years of healthy life in 2017 (32). Estimates of the burden of toxoplasmosis in other countries are needed to support country-specific toxoplasmosis control planning.

T. gondii is a highly prevalent parasite in humans worldwide (1). The seroprevalence of *T. gondii* varies substantially between the geographic regions throughout the world. The parasite is particularly more prevalent in Western European, South American and African countries (33). The prevalence of toxoplasmosis can be seen as a proxy reflecting the hygienic and dietary practices of human populations. An increased risk for *T. gondii* infection has been associated with ingesting raw or under cooked meat, particularly pork or lamb meat, or unwashed raw vegetables or fruits. Others factors that can predispose an individual to risk of infection, include age, gender, race, educational level, socioeconomic status, cultural background, level of health literacy, lifestyle, living in rural areas, proximity to cats, contact with soil, scooping cat litter, pregnancy, number of births, frequent travel to endemic areas, immigration, quality and source of drinking water, and *T. gondii* strain genotype/virulence (4, 34). Because a cure for the persistent cystic stage is not currently available, latent toxoplasmosis can be present for decades, leading to considerable individual and societal burdens. All the aforementioned risk factors are, fortunately, readily modifiable, and reducing their impact could potentially reduce the prevalence of *T. gondii* infection. Health-care systems, and current health-management practices, should take all the risk factors into consideration so that the burden of toxoplasmosis, particularly in certain vulnerable groups or populations, can be minimized.

The Particular Impact On Immunocompromised Populations

TE is often reported in immunosuppressed people, such as persons living with HIV (PLWH) (23) and patients who received hematopoietic stem cell or solid organ transplant (1, 35). Also, patients receiving high doses of immunosuppressive chemotherapy or antineoplastic treatment, and patients with an underpinning condition, such as cancer, or connective tissue diseases, are at a greater risk of toxoplasmosis-associated deaths. In these vulnerable groups, TE imposes a tremendous individual and socioeconomic burden. For example in the USA, from 1988 to 1997, toxoplasmosis accounted for over 21,000 hospitalizations with a mean estimated cost of $28,151 per person attributed to TE-associated hospitalization (36). Also, in the USA ~ 3,000 toxoplasmosis-related hospitalizations were reported in PLWH in 2008 (37). In Canada, HIV comorbidity with toxoplasmosis was detected in 40% of clinical cases between 2002 and 2011, which correlated with an increased number of hospitalizations and treatment cost per case, and accounted for 53% of the total toxoplasmosis-related health-care costs (30). In Tanzania, most deaths attributed to toxoplasmosis were highly associated with HIV/AIDS and TE was responsible for 15.4% of toxoplasmosis deaths (38). In West Africa, TE accounted for 10% of deaths due to AIDS (39). Fortunately, the use of appropriate testing, combination antiretroviral therapy (cART) and antimicrobial drugs to prevent opportunistic infection by *T. gondii* and *Pneumocystis jirovecii* pneumonia, have significantly helped to reduce the incidence of reactivation of latent infection and toxoplasmosis-associated deaths in PLWH (37). Implementation of an early prophylaxis treatment

using trimethoprim–sulfamethoxazole (TMP–SMX) starting on the day of engraftment in *T. gondii*-seropositive patients, can significantly reduce the rate of parasite reactivation in stem-cell recipients (40).

The findings of a comprehensive review of 72 studies revealed a higher *T. gondii* infection rate in immunocompromised patients vs. control (35.9 vs. 24.7%; $p < 0.001$), and in PLWH with advanced HIV infection vs. control (42.1 vs. 32.0%; $p < 0.05$), in cancer patients vs. control (26.0 vs 12.1%; $p < 0.001$), and in organ transplant recipients vs control (42.1 vs. 34.5%; $p > 0.05$) (41). Therefore, prevention and treatment of toxoplasmosis should aim to include both HIV and non-HIV immunocompromised populations. Emerging epidemiologic evidence based on meta-analysis of 74 studies, in particular, regarding concurrent infections by *T. gondii* and HIV from 34 countries revealed a worldwide pooled seroprevalence of 35.8%. The prevalence in Asia and the Pacific was 25.1%, sub-Saharan Africa (44.9%), South American and Caribbean countries (49.1%), and North Africa and Middle East (60.7%). As expected, populations in developing countries exhibited higher comorbidity (54.7%) compared to those in middle-income (34.2%,) and high-income (26.3%) countries. The particularly high burden (87.1%) in sub-Saharan Africa was attributed to the lack of resources, poor dietary and sanitary conditions, poor health literacy, limited healthcare capacities and limited access to safe water, all of which may have increased odds of infection (42). In these resource-limited settings TE can represent a particularly high risk for PLWH patients, particularly those with less than 200 peripheral blood $CD4^+$ T cells per microliter. A more recent systematic review analyzing 111 studies from 37 countries reported a pooled prevalence in PLWH of *T. gondii* of 44.22% by IgG, which was higher than the prevalence obtained based on IgM analysis (3.24%) and molecular methods (26.22%), highlighting the high *T. gondii* infection rate in PLWH (43). The same study also detected a correlation between *T. gondii* positivity and a number of variables, such as gender, consumption of raw meat, proximity to cat and awareness of toxoplasmosis, suggesting that risk factors for toxoplasmosis are the same regardless of the individual's immune status. Given that toxoplasmosis results in significant illness in immunosuppressed persons, these highly vulnerable groups should be advised by their healthcare providers to avoid all behaviors that can put them at risk of serious disease. A greater understanding of the levels of *T. gondii* infection in PLWH, is also important in order to inform policies on allocation of resources and to guide early detection of seroconversion.

MOLECULAR PATHOGENESIS

Marching to The Brain

To cause encephalitis, *T. gondii* must migrate to and enter the central nervous system (CNS) and establish a persistent infection in neural and other brain cells. Following ingestion of the infective stage, either oocysts or tissue cysts, the parasite develops into rapidly-proliferating tachyzoites, which invade and proliferate within the intestinal epithelium. The tachyzoites then exit and infect dendritic cells (DCs) and other immune system cell types, which are important in protecting against *T. gondii* infection (44). These patrolling immune cells are permissive to *T. gondii* infection and represent an important niche for the parasite's replication. In addition to using immune cells as a replicative niche, *T. gondii* manipulates the functions of these cells to increase their metastatic behavior , which is crucial for the dissemination of *T. gondii* to distant organs particularly the brain (45). The exact molecular mechanisms that promote the hypermigratory behavior of infected cells are not fully, but cellular migration seems to depend on chemokines and their receptors. For example, restructuring of the cytoskeleton, upregulation of the chemokine receptor CCR7, downregulation of CCR5, increasing the secretion of gamma-aminobutyric acid (GABA), induction of GABA-A receptor, and activation of calcium channels and calcium signaling, are all implicated in the migration of infected DCs (46-49). Additionally, the upregulation of tissue inhibitor of metalloproteinases-1 (TIMP-1), through CD63–integrin β1 (ITGB1)–focal adhesion kinase (FAK) signaling promoted the motility of infected DCs (50). An increased velocity of infected DCs and microglia was also mediated by the secretory *T. gondii* 14-3-3 protein (51). Moreover, the secreted kinase ROP17 promoted the mobility and dissemination of *T. gondii*-infected monocytes (52).

Crossing The BBB

T. gondii spreads to various tissues, such as the eye, heart, liver, lung, lymph nodes, muscles; however, this parasite seems to persist in neurons (and muscle) probably due to reduced parasite elimination or cellular turnover in these tissues compared to other organs. The parasite's entry to the brain from the blood through cerebral capillary endothelial cells occurs via paracellular transfer into the brain following BBB damage, invasion of cerebrovascular endothelial cells allowing transcellular transfer, and trafficking within infected immune cells into the brain in so-called 'Trojan horse' attack (Fig. 1A) (53). The accumulation of cell-free *T. gondii* tachyzoites at the intercellular junctions prior to transmigration indicates that crossing of the BBB can occur via breaching intercellular tight junctions (TJs). *T. gondii* adversely affects the resistance of cerebrovascular endothelial cell monolayers and impairs the barrier's function, thus facilitating paracellular migration (54). Although extracellular tachyzoites can overcome the BBB, invasion and replication within brain microvascular endothelium is an alternative pathway by which *T. gondii* enters the CNS (55). Secretion of toxofilin (56) and proteases (57) by *T. gondii* facilitates its traversal of the BBB and may underpin the cellular destruction observed along the BBB, when only focal sites are infected by the parasite. Crossing the BBB by *T. gondii* can also occur via exploitation of DCs and monocytes as vehicles for their protection and transport across the BBB. *T. gondii* preferentially enters the brain within parasitized $CD11c^{-}CD11b^{+}$ monocytes and to a lesser extent $CD11c^{+}$ $CD11b^{+/-}$ DCs. The increased mobilization of *T. gondii*-infected phagocytes involves G_i protein-coupled signalling (57), which promotes diapedesis and extravasation of infected cells from cerebral capillaries into the brain. Migration of murine *T. gondii*-infected macrophages in Matrigel™ correlates with upregulation of matrix metalloproteinases (MMPs), such as MMP-9, MT1-MMP and ADAM10 (58).

The infiltration of infected cells into such an immune-privileged site as the brain is a highly orchestrated process involving interactions with cerebrovascular endothelial cells and is mediated by a number of adhesion molecules and chemokine receptors (59). Through activation of the chemokine receptor CXCR3, the chemokines CXCL9 and CXCL10 promote the chemotactic recruitment of T cells to the brain (60). Through their adhesive function, ICAM-1, and its counterpart VCAM-1, facilitate the binding of leukocytes to the cerebrovascular endothelium. The IL-1 signaling stimulates the expression of VCAM-1 and ICAM-1 during *T. gondii* infection in mice (61). When crossing the BBB, infected leukocytes use their LFA-1 integrin to adhere to ICAM-1. Infected leukocytes also use selectin, and its glycoprotein ligand, to cross the BBB endothelium. Using antibodies that block the ligand for VCAM-1 prevents cell entry, further supporting the important role that adhesion molecules and endothelial cell surface receptors have in the influx of myeloid cells, particularly infected monocytes, into the brain (62). Interestingly, *T. gondii* tachyzoites were found in the choroid plexus (CP) of PLWH (63). However, the role of the CP and blood-cerebrospinal fluid barrier in the parasite's entry into the CNS is unknown. Collectively, these studies have provided significant insights into the tactics employed by *T. gondii* to cross the BBB in animal models or *ex vivo* studies. Although our current understanding of these mechanisms in humans remain largely speculative, it is logical to assume that *T. gondii* does not cross the BBB via one exclusive mechanism in humans.

What Mediates the Course of a Persistent Brain Infection?

The preference of *T. gondii* for neurons is apparent, perhaps because neurons (Fig. 1B), unlike other brain cell types, do not react to inflammatory cytokines and thus do not induce a strong antiparasitic immune response (64). Although neurons are the main host cell type preferred by *T. gondii* (8), other non-neuronal cell types also contribute to TE (65). Despite continuous immune surveillance, neuronal degeneration is rarely observed in immunocompetent hosts infected by *T. gondii*. This is attributed to the effective and balanced proinflammatory (T helper 1 (Th1) antiparasitic) and anti-inflammatory (neuroprotective) immune responses. Maintaining this balance is crucial for containing parasite proliferation by promoting persistent infection and preventing parasite reactivation, while limiting excessive immune pathology.

Homing of immune cells into the brain. Immune cell migration and extravasation through blood vessels, and their homing into infection sites, are important mechanisms for establishing an effective immune response. The chemokines and metalloproteinases are key regulators of these events. During TE, the cross-talks between chemokines and the cerebral immune response involve $CD4^{+}$ and $CD8^{+}$ T cells and is mainly mediated by interferon gamma (IFN-γ). Increased production of T cell chemoattractants, particularly chemokines CXCL9, CXCL10 and CCL5, was detected in the brains of

mice during latent infection, which was dependent on IFN-γ (66). Infection of astrocytes or microglia by *T. gondii* induced the expression of chemokines CCL5, CCL2, CXCL9 and CXCL10 (Fig. 1C), which actively recruit IFN-γ-expressing T cells to control tachyzoite proliferation (59).

The chemokine receptor CCR2 (and its ligand CCL2) contribute to the migration of monocytes and neutrophils into the brain following infection. In CCR2-deficient ($CCR2^{-/-}$) mice, leukocyte trafficking was decreased and immune cells within the brain became less active, leading in turn to an increased parasite burden (67). Astrocytes express CCL21 and CXCL10 to promote brain infiltration of $CD8^+$ T cells (68) and microglia-derived CXCL10 modulates the search behaviour of $CD8^+$ T cells in a way that enhances their ability to detect infection sites in the brain parenchyma (60). The damage signal protein IL-33, expressed by oligodendrocytes, induced the expression of monocyte chemoattractant CCL2 by astrocytes (Fig. 1B), which is required for the recruitment of monocyte-derived myeloid cells, and the expansion of focal myeloid cell-derived inducible nitric oxide synthase (iNOS) - crucial for survival during chronic infection (69). *T. gondii* can also increase the production of the chemokines CCL3 and CCL4 in the brain of C57Bl/10 ScSn mice (70) and in cultured cerebellar neurons of mice (64). Infected neurons increase the production of the chemotactic macrophage inflammatory protein-1 alpha and beta (MIP-1α and MIP-1ß), which have proinflammatory effects, leading to influx of leucocytes at the site of inflammation (Fig. 1B).

The interplay of the TIMP-1 and MMPs also influences the infiltration of T cells and parasite control (Fig. 1D). Astrocytes infected with *T. gondii* produced MMP-2 and MMP-9, possibly to increase extravasation and infiltration of inflammatory cells to the infection sites (71). In a mouse model, MMP-8 increased infiltration of $CD4^+$/$CD8^+$ T cells and MMP-10 increased infiltration of $CD4^+$ T cells into the brain. TIMP-1 in astrocytes and in the infiltrating $CD4^+$/$CD8^+$ T cells decreased the brain parasite load, without the development of adverse pathology. An increase in $CD4^+$ T cells and a significant reduction in parasite load were detected in the brain of mice deficient in TIMP-1 when compared to wild type (WT) controls, without substantial brain injury nor any reduction in the peripheral parasite burden (72). This suggests that production of TIMP-1 may be an attempt by astrocytes to block parasite elimination by restricting MMP-mediated recruitment of lymphocytes. This scenario seems to favour parasite survival and to restrict neuroinflammation and brain damage, which ultimately promotes persistent infection.

The roles of cytokines in containing infection. Following brain invasion, *T. gondii* tachyzoites encounter strong cell-mediated (type 1) immune responses marked by the production of IFN-γ, interleukin [IL]-12 and tumor necrosis factor-α (TNF-α), which clear most of the invading tachyzoites (73). *T. gondii* infection induces the expression of Toll-like receptor (TLR) 11 in astrocytes, neurons, and microglia in mice (74). TLR11 stimulation by parasite's profilin protein induces IL-12 in DCs (75). MyD88 knockout mice rapidly succumbed to acute infection, with a commensurate impairment in IL-12 response - suggesting that mice resistance to toxoplasmosis depends on MyD88-dependent signals regulated by TLRs (76). Many *in vitro* and *in vivo* studies have indicated the fundamental role that IFN-γ plays in protecting the host from severe *T. gondii* infection. IFN-γ controls parasite replication via various mechanisms, such as stimulating the degradation of the PV via the production of immunity-related GTPases (IRGs) and interferon-inducible guanylate-binding proteins (GBPs), increasing expression of major histocompatibility complex (MHC) and induction of iNOS and the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) (77-80). Production of NO by iNOS inhibited *T. gondii* proliferation within macrophages *in vitro* (81) and in mouse brain (82). The procurement of nutrients, such as tryptophan, from the host cells is essential for intracellular *T. gondii* survival. Hence, the breakdown of tryptophan into kynurenine due to IFN-γ-mediated IDO induction can result in inhibition of tachyzoite growth (83). Increased kynurenic acid concentrations during infection acts as an agonist of the immunosuppressive aryl hydrocarbon receptor (AhR), which results in controlling the pathological immune response associated with toxoplasmosis. Mice deficient in AhR ($AhR^{-}/^{-}$) succumbed more readily to illness, but developed fewer brain cysts than WT mice infected by *T. gondii* (84). Additionally, the excessive production of kynurenic acid, which blocks the alpha-7 nicotinic acetylcholine receptor (α7 nAChR) and disrupts other neuroreceptors in infected astrocytes and microglia can lead to abnormal neurotransmission (85), and was hypothesized to lead to cognitive dysfunction (Fig. 1C). In response, *T. gondii* secretes TgIST, an inhibitor of signal transducer and activator of transcription 1 (STAT1) transcriptional activity, which antagonizes IFN-γ-induced IDO1-induced immunity against *T. gondii* in human cells (86). Mice deficient in the lymphotoxin-β receptor (LTβR), which has diverse antimicrobial immunoregulatory roles, had a higher parasite burden and

increased mortality in acute *T. gondii* infection than immune competent control mice. The impaired resistance of LTβR$^{-/-}$ mice was attributed to the deregulation of interferons and interleukins, as well as a reduced upregulation of murine GBP, which is mediated by IFN-γ and is crucial for parasite elimination - further supporting the importance of IFN-γ in controlling *T. gondii* (87). Mice lacking mGBP7 failed to control parasite replication and succumbed to acute infection (88).

The contribution of astrocytes and microglia to the pathogenesis of TE is notable. Inhibition of *T. gondii* replication in astrocytes was attributed to an increased induction of IFN-γ–induced GTPase (IGTP) (89). Genetic deletion of STAT1 in astrocytes promoted the spread of *T. gondii* infection and increased susceptibility to TE (90). The orphan nuclear hormone receptor TLX, expressed in astrocytes and neural stem cells can support resistance to *T. gondii* through enhancing STAT1 activity (91). Mice lacking cytokine receptor gp130 (a protein of IL-6 signalling pathway) in astrocytes showed increased astrocyte apoptosis and inflammation during *T. gondii* infection. Although these mice were able to control parasite numbers, they developed excessive inflammation and succumbed more easily to rapid development of TE (92). Interestingly, microglia did not seem to be the only cells in charge of controlling *T. gondii* in the brain through T cell-derived IFN-γ mechanisms. Mice in which both monocyte-derived macrophages and microglia were made deficient in IFN-γ signalling, succumbed to infection by 19 days post infection. Conversely, mice in which microglia were only deficient in IFN-γ signalling, were able to resist infection (93). Interestingly, a recent study using a microglia reporter mouse model showed that microglia can promote neuroinflammation via the release of alarmin IL-1α, which recruits more immune cells to control chronic brain infection (61).

The cysticidal role of CD8+ cytotoxic T lymphocytes (CTLs). The anti-cyst activity of CD8$^+$ CTLs and the control of TE are mediated by mechanisms that utilize both the IFN-γ (the key effector of protective immunity against tachyzoites) and perforin-mediated immune responses (94). Control of brain cyst burden is mediated largely by CD8$^+$ CTLs that produce perforin and M2 microglia expressing CXCR3, acidic mammalian chitinase (AMCase) and arginase-1 (95, 96). Although M2-like microglia populations act to inhibit neuroinflammation, AMCase-deficient mice can develop a high parasite burden in the brain, indicating a role for M2-like microglia in controlling the parasite cysts. Both perforin and granzyme B underpin the capacity of CD8$^+$ CTLs for direct invasion and elimination of the existing *T. gondii* cysts (97). Adoptive transfer of perforin-competent CD8$^+$ CTLs to immunodeficient mice significantly reduced cyst load and increased the level of CXCR3 and CXCR6, which promote the recruitment of microglia and macrophages to destroy bradyzoites within T cell-attacked cysts (98). The induction of the CD8$^+$ CTLs responses is also mediated by rhoptry protein ROP7 and dense granule protein GRA6 (99, 100). The cysticidal capacity of CD8$^+$ CTLs is related to the amino-terminal region of *T. gondii* GRA6 (GRA6Nt) (101). Markedly, one epitope in the C-terminal region of GRA6 was a strong inducer of IFN-γ-mediated protection against acute toxoplasmosis in mice (99). These results suggest that host immune responses can recognize different GRA6 regions to stimulate CD8$^+$ T cell-based protection against both tachyzoites and tissue cysts. The cysticidal mechanism is initiated by CD8$^+$ CTLs, but the removal of cysts is accomplished by microglia and macrophages, which invade the cysts and destroy the bradyzoites (95). INOS-mediated protection conferred by CD8$^+$ CTLs is important for inhibiting the development of toxoplasmic cysts; but does not play a role in the mechanism required to eradicate the cysts (102).

Immune evasion strategies. *T. gondii* is an exceptionally successful intracellular parasite. Despite being at constant risk of a sustained and efficient immune defensive patrol, it can linger inside the body of the host for a long time. Some tachyzoites can escape destruction from the immune response (or from drugs used to treat toxoplasmosis) and transform into bradyzoites inside quiescent cysts. Paradoxically, the Th1-polarized immune response essential for eradication of acute infection is the same response required to develop latent infection in the brain. *T. gondii* seems to subvert CD4$^+$-mediated immune responses through the inhibition of the MHC II transactivator CIITA, which contributes to its long-term survival in the brain (103). *T. gondii* also produces cyclophilin-18 mimicking chemokine, which, when bound to CCR5 on DCs, evokes a Th1 response (e.g. production of IL-12), required for the establishment of persistent infections (104). The expansion and functional maturation of cerebral DCs contributes to the development of latent infection (105). *T. gondii* possesses secretory organelles that discharge a number of polymorphic effector proteins that seem spatially and temporally controlled, as different effectors are secreted at different stages of host cell invasion and colonization; and effector secretion is targeted to specific locations to selectively modulate a specific function or a regulatory pathway within the host cell (106-108). These effectors are utilized by *T. gondii* to circumvent host

immunity, and to support the establishment of a latent infection (109). For example, GRA23 and GRA17 control the delivery of small metabolites to the PV and promotes the structural stability of the PV (110). More recently, GRA17 and GRA23 have been shown to mediate the growth, virulence, cyst burden, and immunogenicity of the type II PRU strain (111). GRA39 contributes to parasite virulence and tissue cyst burden (108). Additionally, ROP16 serves as a virulence factor that activates STAT3 and STAT6 and inhibits T cell responses (112). ROP5 forms a complex with ROP17 and ROP18 to prevent the recruitment of IFN-γ–mediated IRGs, which are essential for PV destruction and parasite control (113). In addition to inhibiting STAT1 transcriptional activity (86), TgIST binds to the STAT1/STAT2 heterodimer, leading to the inhibition of type I IFN pathway (114).

Striking a balance between friend and foe. The long-term presence of dormant cysts in the host tissues requires a well-coordinated immune arsenal that is sufficiently potent to combat the infection, yet moderate enough to counterbalance hyperinflammation. Mice deficient in 5-lipoxygenase (5-LO) (115) or suppressor of cytokine signaling (SOCS)-2 (116), succumbed to chronic infection and showed increased mortality due to excessive inflammation caused by elevated IL-12 and IFN-γ, regardless of the reduction in the brain cysts. Therefore, the effects of proinflammatory cytokines (e.g. IFN-γ) must be buffered by immunosuppressive effectors (IL-27, IL-10, TGF-β) and receptors (e.g. PD-1, LAG3, TIGIT), without which continued secretion of inflammatory cytokines and homing of T cells into the brain can lead to an exaggerated inflammatory response and encephalitis (117, 118). Therefore, the relative balance between IL-10 and IFN-γ produced by T cells will dictate whether the immune response will eliminate the parasite with limited immunopathology or whether chronic infection will be established. Regulatory T cells (Tregs) are activated by IL-27, secreted by monocytes, to induce IL-10 and T-box transcription factor (T-bet) expression to attenuate the inflammatory responses (119). In the absence of IL-27, *T. gondii* increases the levels of IL-17 and GM-CSF and results in neuroinflammation in mice (118). The reduction of IL-10, by blocking the IL-10 receptor in chronically infected mice led to significant tissue destruction due to the extensive inflammatory response in the brain. The anti-inflammatory role of IL-10 during toxoplasmosis is also evidenced by the fact that mice lacking IL-10 succumbed to fatal CNS inflammation (120, 121). Deletion of the transcription factor Bhlhe40, an IL-10 repressor, resulted in severe *T. gondii* infection in mice, which was attributed to reduced IFN-γ and increased IL-10 production (122).

Although the IL-10, which is commonly produced by Th2 cells, has broad anti-inflammatory properties, and suppresses Th1-cell pro-inflammatory responses (123) via its inhibitory effect on the function of macrophages and DCs (124), Th1 cells secreting IL-10- and IFN-γ were detected in animals infected by *T. gondii* (125, 126). Recent studies have identified a number of immune cells (for example B cells, eosinophils, and $CD8^+$ CTLs, Tregs and antigen-driven regulatory $CD4^+$ T cells), which can produce IL-10 (127). Tregs expressing CXCR3 were also found to express T-bet and Foxp3, and secrete IFN-γ and IL-10. It remains to be completely defined how IL-10 functions to limit tissue damage, while at the same time it promotes persistent infection. More research is required to reveal the extent of dependence between the mechanisms used by different types of cells to secrete IL-10 and to elucidate how and to what magnitude different sources of IL-10 execute the aforementioned distinct activities.

TGF-β signaling in astrocytes is of particular interest for its immunosuppressive role during brain injury (128). Infected astrocytes secrete prostaglandin E2 (PGE_2) to activate microglia which suppress nitric oxide (NO) production (129). TGFβ-1 plays a role in the inhibition of iNOS and NO production by IFN-γ-activated microglia (130). TGF-β signaling in astrocytes controls neuroinflammation and neuronal injury, via the inhibition of NF-κB signaling which, in turn, inhibits CCL5 and the infiltration of T cells and macrophages (131). Thus, lack of a TGF-β signaling in astrocytes, while not necessarily affecting parasite burden, can increase inflammation and neuronal damage.

The role of Tregs in TE. The role of Tregs in toxoplasmosis has yet to be fully understood. However, elucidating of the mechanisms that underpin Tregs' interaction with $CD11c^-$expressing DCs in the meninges and perivascular space is warranted (132). Reduction of the suppressive Tregs during acute toxoplasmosis is commensurate with increased IFN-γ in an IL-10–independent/IL-2–dependent manner (133). In TE, Tregs expressing Th1 response-related molecules, such as T-bet, and CXCR3, and IFN-γ, were detected in the brain, probably to limit neuro-inflammation via IFN-γ-mediated increased IDO expression (134). Studies in KO mice have shown that ICOS signaling promotes inflammation and antibody production - both being important for parasite control - while helping to control inflammation by inhibiting effector T cell proliferation and inducing Tregs in the brain and spleen (135), or by inducing IL-10 production in T cells (136).

What makes the murine model different from humans? Our knowledge of the pathophysiology of toxoplasmosis has mostly been derived from studies in mice, and must be interpreted with caution. Marked differences exist between murine models and humans and their mechanisms of innate recognition and cytokine production. For example, IFN-γ-mediated IRGs' and GBPs' immune responses in mice does not seem to have an equivalent in humans (106). Furthermore, parasite recognition and the initiation of innate immunity in mice, requires stimulation of TLR11 and TLR12 by the parasite profilin for the production of cytokines by myeloid cells (75, 137). The role of TLR11/TLR12 in recognizing the parasite profilin in mice is absent in humans; instead, immune recognition triggered by phagocytosis of the live parasite leads to IL-12 being produced by myeloid cells (138). In contrast, immune cells in mice do not require direct contact with live tachyzoites (139). Human peripheral blood mononuclear cells can produce IL-12 and TNF-α after stimulation with *T. gondii* nucleic acids, suggesting the involvement of TLRs 7, 8, and 9, all of which function to recognize foreign nucleic acids (140). A recent study using human monocytes, has shown that the recognition pathway for *T. gondii* relies on the detection of S100A11, a damage-related molecule secreted from adjacent infected cells (141). Taken together, these results indicate that recognition of the pathogen is not a prerequisite for the induction of an anti-*T. gondii* immune response, because immunity in humans and mice can also arise from sensing active infection. Additional differences include the production of large amounts of IL-12 from mouse DCs, whereas the human response to *T. gondii* mainly involves the expression of CCL2 (141). In mice *T. gondii* induces the production of IL-12 by myeloid cCD2 cells (e.g. CD8α$^+$), which are distinct from the IL-12-producing cDC1 subset (e.g. CD1c$^+$) in humans (138). Differences in cytokine production also exist between human and murine subpopulations of monocytes in response to *T. gondii* stimulation (138). Despite the aforementioned differences, mice remain an important model for the investigation of *T. gondii* infection, and for the development of remedial measures. Work towards developing animal models continues, as illustrated by a recent report using Zebrafish as a model to study *T. gondii* replication, *T. gondii* strain-specific variations in host response and interaction with immune cells (142).

Reactivation of Latent Infection

Dormant tissue cysts encapsulating slow-growing bradyzoites are fundamental for the long-term survival and persistence in the host's brain, due to their ability to evade immune-mediated destruction. The question arises: what advantage does *T. gondii* gain by undergoing phenotypic transformation from dormant bradyzoites protected within cysts, to the more active tachyzoites - which are less able to counter host immune responses? Some studies provide direct evidence supporting a link between immunosuppression and reactivation of latent infection. Indeed, parasite cyst reactivation is a high-frequency occurrence in immunocompromised people - such as individuals who received organ transplantation and PLWH who have AIDS, in particular those who are not on cART, and are not receiving prophylactic TMP–SMX (23, 143, 144). In these groups of patients, phenotypic switching provides opportunity for uncontrolled proliferation of tachyzoites in excessive numbers which can overwhelm the capacity of the host's already-compromised immune system, resulting in life-threatening consequences (53). Therefore, durable cell-mediated immunity to *T. gondii* is essential for protecting the host from reactivation of any latent infection. It remains to be shown whether immune suppression triggers reactivation; or if reactivation continues at a set rate and the immune system, which would normally control any tachyzoites that are released, is suppressed allowing replication without control leading to a clinical reactivation.

As stated above, IFN-γ has a crucial role in the control of latent infection (73, 145). Microglia are a further source of IFN-γ production, and contribute to the protective immune response that maintains the CNS tolerance to *T. gondii* presence, and prevents any recrudescence of latent cerebral infection (146). The production of IFN-γ in microglia is mediated by the N-terminus region of GRA6 (GRA6Nt), which likely serves as a warning signal for neighboring uninfected microglia of the parasite's presence. This would be a preemptive strategy to limit any further growth of tachyzoites in the brain via activation of the protective immune responses, and increasing microglial production of IFN-γ (147). Astrocytic TGF-β signaling exerts an anti-inflammatory and neuroprotective functions during *T. gondii* infection (131). Astrocyte dysfunction during HIV can, therefore, lead to loss of their protective roles and diminish their ability to counter infection (131). CXCL9 recruits and accumulates T cells around the parasite-infected brain areas to prevent any reactivation of latent cerebral infection (148). The influx of T cells into the brain is also mediated by the action of IFN-γ on the cerebrovascular endothelium; IFN-

γ increases VCAM-1/α4β1 integrin interactions, which appears to serve as a host defense mechanism during the early reactivation of dormant cysts, to suppress TE (149, 150).

Pharmacological agents, through their anti-parasitic or immunomodulatory effect can also modulate the reactivation of latent cysts and the development of TE. For example, termination of sulfadiazine treatment resulted in reactivation of latent infection in a murine model (151). The use of biological agents, such as anti-α4 integrin monoclonal antibodies used in the treatment of Crohn's disease or multiple sclerosis can interfere with the VCAM-1/α4β1 integrin interaction and brain homing of $CD4^+$ and $CD8^+$ cells, potentially increasing the odds of reactivation of latent toxoplasmosis (Fig. 1E). In line with this assumption, administration of anti-α4 integrin antibody inhibited key factors that play roles in controlling *T. gondii* growth, such as iNOS, T cells and IFN-γ in the mouse brain (150). In addition to taking into account the effect of using immune-modulatory biologics on the reactivation of tissue cysts, the impact of secondary/opportunistic infections in chronically infected patients should be also considered. The interaction between *T. gondii* and HIV is just one example. While HIV-induced impairment in immune response triggers reactivation of dormant cysts, coinfection with *T. gondii* compromises host immune defenses to HIV-1 and HSV-1 by suppressing plasmacytoid dendritic cell (pDC) activation and inhibition of IFN-α production (152). During latent toxoplasmosis, exhaustion of $CD8^+$ CTLs and induction of programmed cell death 1 (PD-1) rendered $CD8^+$ CTLs liable to destruction via apoptosis, leading to reactivation of latent infection and host mortality (153, 154). Blocking PD-1 and its ligands PD-L1 has a potential therapeutic value as anti-PDL-1 treatment reinvigorated $CD8^+$ CTL's response, which prevented reactivation of tissue cysts and improved survival of chronically infected mice (153). Also, inhibition of PD-1–PD-L1 pathway can decrease expression of caspase 3 on polyfunctional and IFN-γ^+/granzyme B^- memory $CD8^+$ CTLs *in vitro* (154). Additionally, blockade of immune checkpoint inhibitor PD-1 significantly reduced brain cyst burden and increased brain infiltration of $CD8^+$ CTLs, $CD11b^+$ DCs and $CD3^+$ T cells in mice (155). All the afore-mentioned studies indicate that development of strategies to prevent *T. gondii* reactivation is achievable by maintaining enhanced proinflammatory effectors without disproportionate reduction of the anti-inflammatory mediators.

CLINICAL SIGNS AND DIAGNOSIS

Infection of the CNS with *T. gondii*, or TE, can cause severe illness and death in immunocompromised individuals. The worldwide *T. gondii* seroprevalence in PLWH ranges from ~26% in high income countries to ~55% in low income countries (42). The risk factors for TE in PLWH include blood $CD4^+$ T cells less than 200/µl, lack of TMP–SMX prophylaxis, which may be given to specifically prevent TE or *Pneumocystis jiroveci* pneumonia (143, 156), and lack of cART used to control HIV infection (23, 144). Because the greatest current experience with TE is in PLWH, and clinical guidelines are available (157), we focus on this risk group. However, the principles used in the diagnosis, screening and prevention of TE in PLWH are applicable to other at-risk populations.

Clinical Features

TE most commonly presents as a single, or more commonly, multiple brain abscesses with a predilection for the deep gray matter structures or the junction between cortical gray and white matter. However, any part of the brain can be affected. As such, neurological abnormalities can be similar to what might be seen in an individual with one or multiple brain lesions from any cause. More unique to TE is fever. Commonly described neurological abnormalities in PLWH with TE are subacute onset of headache, hemiparesis, cranial nerve palsies, ataxia, change in level of consciousness or seizures (158, 159). Due to involvement of the basal ganglia, abnormal movements, including chorea and ballism, and rigidity may also be observed (158, 160). Uncommonly, patients may present with an encephalitic illness rather than discrete brain abscesses. These individuals may present with fever, meningeal signs, such as headache, stiff neck and photophobia, and encephalopathy that is rapidly fatal (161, 162). Similar clinical manifestations and course have been reported in people with necrotizing ventriculitis due to *T. gondii* (163).

Diagnostic Tests

Serological diagnosis. The Sabin Feldman Dye Test, first reported in 1948, remains the "gold standard" for serological detection of anti-*Toxoplasma* IgG and IgM antibodies (164). However, this

method requires using live tachyzoites, which is not feasible in most laboratories. Enzyme-linked immunosorbent assay (ELISA) is commonly used to detect specific IgG and IgM antibodies against *T. gondii*. IgG titers peak within 1-2 months post infection, and stay high for life, which is why they are a good marker of risk for TE in PLWH, which almost always occurs following reactivation of a pre-existing latent infection. Because the proportion of PLWH with TE who have serum anti-*T. gondii* IgG antibodies can reach 100% (165, 166), identification of PLWH who are IgG seropositive identifies those at risk for the disease. However, empiric diagnosis based on IgG level can be misleading because elevated serum anti-*T. gondii* IgG titers are common in the general population, even in the absence of active illness (167). An avidity assay, which is a modification of the ELISA, incorporates a denaturing agent (e.g. urea) within serum dilutions to test for antibody avidity. The avidity values are lower during acute infection and increase over time, which can be used to estimate when seroconversion has occurred, and are useful in the evaluation of reactivated infection and in pregnant women who acquire infection during gestation (168). IgM antibodies can be detectable for over a year. Thus, the presence of IgM antibodies does not necessarily indicate a recent infection, however, the lack of IgM rules out recent infection. Perhaps unexpectedly, PLWH with TE do not mount an IgM response.

Cerebrospinal fluid (CSF) analysis**.** Mild mononuclear pleocytosis and elevated protein can be detected in the CSF of patients with TE. Diagnostic tests for detection of toxoplasmosis-specific IgG and IgM antibodies in the CSF do not have proven utility beyond an empiric treatment trial. The detection of *T. gondii* DNA in CSF using polymerase chain reaction is specific, but not sensitive, for diagnosis of TE (169, 170), meaning that a positive test confirms the diagnosis, but a negative test does not rule it out. Diagnostic accuracy decreases if CSF is examined after more than a week of TE therapy (170). In a PLWH with a focal brain lesion with mass effect who is not receiving anti-*Toxoplasma* prophylaxis, detection of *T. gondii* DNA in CSF increases the likelihood of TE from 87% to 96% (171). It is important to remember that lumbar puncture for collection of CSF may not be safe in TE due to mass effect, which increases the risk of brain herniation after lumbar puncture.

Histopathology. CNS toxoplasmosis in PLWH can lead to granulomatous reactions with gliosis and microglial nodules and necrotizing encephalitis (172). The detection of tachyzoites either alone or together with tissue cysts is diagnostic. Immunohistochemistry can improve the detection and localization of the parasite (173).

Neuroimaging. Brain imaging is particularly useful for the diagnosis and management of patients with TE. Brain examination using computed tomography (CT) scan revealed hypodense and contrast-enhancing focal brain lesions with mass effect, primarily in basal ganglia, thalami, and cortico-medullary junction in 70-80% of PLWH with TE. Less often, TE in PLWH presents with one lesion or with no lesions on brain CT. Rarely, ventriculitis can be seen on brain CT of PLWH with TE (163, 174, 175). Magnetic resonance imaging (MRI) is the clinical imaging standard used in PLWH with suspected TE (176, 177). Given the better sensitivity of MRI compared to CT, often patients with one lesion or no lesions on CT may have multiple lesions detectable by MRI (Fig. 2). Concentric and eccentric "target sign" enhancement on contrast enhanced MR images has been described (178, 179). Although TE can occasionally cause a single brain lesion on MRI, an alternative diagnosis, such as primary CNS lymphoma, should be considered in these individuals (180). Both TE and primary CNS lymphoma can cause contrast-enhancing lesions with mass effect and thus cannot be easily differentiated based on neuroradiologic criteria. However, the presence of hyper-attenuation on non-enhanced brain CT and subependymal location are more specific for lymphoma (181). Based on the retention of thallium-201, using single-photon emission computed tomography ([201Tl]-SPECT) imaging can reliably differentiate CNS lymphoma (uptake) from TE (no uptake). Indeed, SPECT demonstrated a 92% sensitivity and 85% specificity in distinguishing CNS lymphoma from other focal brain lesions in PLWH (182). The use of cART may decrease the specificity of SPECT in distinguishing between the two diagnoses (183).

How Clinicians Use Laboratory Tests in Their Diagnostic Algorithms

In PLWH, the differential diagnosis for those with brain mass lesions with contrast enhancement and mass effect includes most commonly TE, primary CNS lymphoma and tuberculoma or tuberculous abscess (Table 1). Individuals who are seropositive for anti-*Toxoplasma* IgG, have blood $CD4^+$ T cells < 200/µl, and are not receiving prophylaxis for TE have a high likelihood of TE (23, 184-186), and the approach to these individuals is an empiric treatment trial. As noted above, a positive CSF PCR for *T.*

gondii further increases the likelihood of TE to above 90% (171), but lumbar puncture for collection of CSF is often not safe in patients with mass lesions. Clinical and radiographic improvement after 10-14 days of empiric treatment for TE is used to establish the diagnosis. Although brain biopsy is the gold-standard for diagnosis (187), it is reserved for patients with low probability of TE, for example those who are seronegative, and for individuals who fail to respond to a treatment trial (188). Accurate identification of TE can be challenging in individuals who do not have HIV, including recipients of hematopoietic stem cell or solid organ transplants, and those who received immunomodulatory therapies, in whom a wide variety of bacterial and fungal pathogens are possible. An example of this challenge is an individual with multiple sclerosis (MS) who developed TE during treatment with fingolimod (189). Brain lesions resembled tumefactive MS and prompted intensification of MS treatment. Serological testing was useful in establishing the correct diagnosis of TE.

MANAGEMENT

Pharmacotherapy

Parasite dormancy exhibited by the cystic stage remains the main stumbling block to achieve effective eradication of toxoplasmosis because no drugs can clear tissue cysts of *T. gondii* (190). Current treatments can only manage acute and reactivated infections; both are caused by tachyzoites. If treatment is delayed, mortality can reach a very high level in immunocompromised individuals. The activity of pyrimethamine (PYR) is potentiated when combined with sulfadiazine (SDZ) or clindamycin (CLD) for TE in PLWH (191, 192) and, in combination with folinic acid, is the recommended initial treatment (193). High dose TMP-SMX is an alternative when the preferred regimen is not available (194, 195). Owing to the potential allergy or toxicity that can occur with the use of these drugs, alternative regimens have been used in PLWH, including the macrolide antibiotic azithromycin or antimalarial agent atovaquone. Unfortunately, limited access to first-line drugs for acute TE may oblige physicians to choose an alternative therapy. A lower-priced PYR oral suspension successfully controlled TE in a PLWH (196), providing a new solution to tackle the challenge of affordability of the PYR-based regimen. While the aim of the study is laudable, it highlights concerns beyond issues of efficacy and safety, such as rising drug prices, industry profits, and patient access to crucial drugs, especially uninsured patients. More information about the treatment of toxoplasmosis in immunocompromised patients can be found in other reviews (195, 197).

Prevention

Over the past few decades, knowledge of *T. gondii* biology, epidemiology and ecology has expanded exponentially and has provided the underpinning of the measures currently used to control *T. gondii* infection. Preventative measures focus on limiting the contact with known routes of transmission and reducing exposure to the parasite's infective stages. As mentioned above, humans acquire *T. gondii* by consuming food or water contaminated with oocysts shed in cat feces or by ingesting the parasite cysts in raw or undercooked meat. Also, acquisition of infection can occur via ingestion of raw shellfish (198). Therefore, PLWH who are seronegative should only eat meat that has been thoroughly cooked, avoid eating raw shellfish, thoroughly clean their hands following touching raw meat, avoid gardening or handling soil without gloves, and eat fruits and vegetables that are properly washed. If PLWH have a cat, daily changing of the litter box should be delegated to another person who is not immunocompromised and non-pregnant. Wearing disposable gloves and washing hands thoroughly with antiseptic should be a common practice for individuals who manage the litter box. When possible, cats should stay indoors and not be fed raw or undercooked meat, and seronegative PLWH should not adopt or handle stray cats. The above recommendations for prevention of *T. gondii* infection are also applicable to individuals in other specific at-risk groups. Standardized guidelines recommend that all PLWH be tested for serological evidence of previous *T. gondii* infection soon after HIV diagnosis (157). PLWH who are also seropositive for *T. gondii* with peripheral blood $CD4^+$ T cells <100/µl should receive primary prophylaxis to prevent TE (157). Individuals treated with cART who have more than 200 $CD4^+$ T cells/µl for > 3 months can safely discontinue primary prophylaxis. PLWH who have been successfully treated for TE and are receiving cART can discontinue maintenance treatment when they achieve more than 200 $CD4^+$ T cells/µl for > 6 months (157). It is worth noting that despite these preventive measures, no intervention is capable of completely preventing *T. gondii* infection.

QUALITY OF LIFE

Link to Psychiatric Illness and Cognitive Function

Interest in exploring the connection between infection and behavioral alterations and brain illnesses in humans was sparked following the discovery that *T. gondii* can induce neurologic changes in its intermediate murine hosts in order to make them easier prey for the definitive feline host (199). Despite a considerable amount of data, evidence surrounding the impact of *T. gondii* on neurological functions particularly in regard to behavioral modifications and neurodegenerative disease remains conflicting (200). There is serological evidence, albeit indirect and preliminary, pointing to an association between *T. gondii* infection and psychiatric abnormalities, such as schizophrenia and bipolar disorder (201-203). However, randomized clinical trials of toxoplasmosis treatment have not shown a benefit in individuals with schizophrenia (204). Also, correlations between serological evidence of *T. gondii* infection and poorer neurocognitive function has been reported in some, but not all, studies, including in PLWH (205, 206). Cognitive impairment in individuals with schizophrenia who are also *T. gondii* seropositive was attributed to immune-inflammation mediated by alterations of kynurenine metabolism (207, 208). *T. gondii* can also alter other pathways, such as dopaminergic and GABAergic pathways, which have also been implicated in the neurobiology of schizophrenia (209). To what extent *T. gondii*-related alterations of the aforementioned pathways lead to psychiatric illness and cognitive impairment remains to be clarified.

Prognosis of TE in PLWH

More than half of PLWH who survive TE have residual neurological abnormalities (210). In addition, they are faced with the possibility of HIV progression and cognitive impairment. A prospective study of 205 PLWH and TE showed that initiation of cART within 2 months of TE diagnosis reduced HIV disease progression compared to those who started cART after two months (23). These results are consistent with studies that have shown improved survival of PLWH who develop TE in the cART era (210). PLWH with prior TE may be at greater risk of subsequent cognitive impairment and dementia compared to those with other CNS or non-CNS opportunistic infections (211, 212).

PROGRESS, CHALLENGES AND FUTURE PROSPECTS

New Pharmacological Targets

As mentioned above, PYR and SDZ are the drug of choice. This drug combination synergistically inhibits dihydropteroate synthase and dihydrofolate reductase, both play key roles in folate biosynthesis, which is essential for the growth and proliferation of *T. gondii* (213). Other treatment regimens involve atovaquone, which exerts its effect by inhibiting the mitochondrial cytochrome bc_1, leading to interruption of cellular respiration and suppression of the parasite growth (214). Clindamycin exerts its antimicrobial activity via inhibiting protein synthesis in the apicoplast, a vestigial plastid organelle presents in *T. gondii* and other protozoa in the phylum Apicomplexa (215). Intolerance, poor absorption (191), variations in the susceptibility of *T. gondii* strains to PYR (216), increased pricing (196), and adverse dermatologic and hematologic reactions associated with the use of PYR-based therapy (194), and inability to kill bradyzoites (190), are major challenges that limit the effectiveness of the current first-line interventions. For these reasons, there have been significant interests in the discovery of new formulations to treat TE. An earlier study showed that atovaquone nanosuspensions have improved bioavailability and high therapeutic efficacy against reactivated toxoplasmosis in mice (151). Another study demonstrated that treatment of mice with sodium dodecyl sulfate-coated atovaquone nanosuspensions reduced parasite load and inflammatory reactions in the brain (217).

The last few decades have witnessed significant progress towards exploring novel and better therapeutic agents for TE. Parasite-targeted therapeutics has been the most common approach used to discover new generations of anti-*T. gondii* drugs. This approach involves *in vitro* high throughput screening of libraries of compounds for identification of those novel molecules with a potent toxoplasmicidal activity at nanomolar concentrations against the tachyzoite and/or bradyzoite stage.

The clinical benefit of this approach is limited owing to the need to understand the compound's toxicity and potential side effects, mode of action, and pharmacokinetics. Addressing these issues can be costly and time consuming. An alternative strategy to overcome these hurdles is to test compounds approved to treat other indications (218). Drug repurposing has the added advantage of discovering new leads with novel scaffolds (different from the scaffold of PYR) and exhibit novel mechanism of actions. Screening molecules in the open access Malaria Box (i.e. Medicines for Malaria Venture) has revealed new anti-*T. gondii* drug candidates that seem to hold a promising therapeutic potential (219, 220). Encouraging results have been reported in other repurposing screens of existing drugs where several compounds were found to have a new anti-*T. gondii* indication (218, 221).

Tackling The Treatment Impasse of Latent Infection

Elimination of persistent *T. gondii* cysts is a prerequisite for the success of any prophylactic program that aims to eliminate the risk of TE in vulnerable populations. As noted above, researchers have tested the efficacies of repurposed approved drugs used for other indications. For elimination of persistent *T. gondii* cysts, the anti-malarial endochin-like quinolones (inhibitors of the apicomplexan cytochrome *bc1*) (222), the anti-leishmanial and anti-neoplastic miltefosine (inhibitor of phosphatidylcholine biosynthesis and the PI3K/Akt/PKB pathway) (223, 224), and guanabenz (inhibitor of translational control and protein synthesis) significantly decreased the brain cyst burden in latently infected mice (225). Additional compounds, such as the histone deacetylase inhibitor FR235222 (226), and tanshinone IIA (inhibitor of inflammation and cancer cell cycle) and the antihistaminic hydroxyzine (227) have shown efficacy against *T. gondii* cysts. More validation studies are needed to select the compounds with the most potent cysticidal activity from these and many other approved drugs (195).

Vaccines

Vaccination may be used to prevent *T. gondii* infection or to clear latent infection. The long-term goal to control TE can be achieved by preventing its development in at-risk individuals. Therefore, there is a widely acknowledged need for human vaccines to reinforce the existing arsenal of anti-*T. gondii* therapeutics. Given the obvious importance of the cysts in TE, there is a need to have more in depth understanding of the evolutionary pressure and the molecular pathways that mediate cyst development and the associated immune responses. This is especially important in order to provide well-informed perspectives for a rational development of safe and effective vaccine capable of inducting potent immunity against *T. gondii* cysts. Given the crucial role of $CD8^+$ CTLs in controlling the cysts during chronic infection, GRA6 molecules released from bradyzoites within cysts can be a candidate immunogen for stimulating of $CD8^+$ CTL response (101). Hence, in designing a therapeutic vaccine, inclusion of full-length GRA6 protein or GRA6Nt epitope(s) to induce $CD8^+$ T cell responses can be a good approach to boost immune responses against the persistent cysts. Using an adjuvant to induce IgG2a and switch the cytokine balance towards Th1 immune responses can potentiate the immunogenicity of a vaccine. An alternative preemptive vaccination strategy may involve induction of GRA6Nt-primed $CD8^+$ CTLs in healthy individuals so that when immunized individuals acquire infection, the elicited immune response can thwart the development of new cysts, protecting the vaccinated individuals from developing latent infection. There is evidence that the microenvironment of the cystic stage is more dynamic than what was previously thought because bradyzoites' replication seems to continue within the cyst, though more slowly than in tachyzoites (228).

Several approaches to toxoplasmosis vaccine development have been explored, such as live attenuated strains, nanoparticle-based, exosome-based, and carbohydrate-based vaccination (229). Despite significant progress in vaccine discovery, including many promising proof-of-concept vaccination trials in mice, none of the tested vaccines has been advanced to clinical trials in humans. Future work toward a commercial vaccine requires detailed validation studies in order to optimize the potency and sustainability of the protective immunity, and practicality of administration. When researchers reported in the year 2014 the first application of the gene-editing tool CRISPR/CAS9 in *T. gondii* research (230), they kicked off a new era for the identification of genes with immunogenic potentials. Several genes have since been tested in vaccination studies in mice, which have helped to identify correlates of protection and candidates for generating attenuated strains of the tachyzoite stage (231, 232). On the downside, challenges remain as to prioritization of the most promising candidates from among the many fitness-conferring genes, the relevance of these candidates for the bradyzoite

stage and the difficulty of regulatory approval of live vaccines for human use (229, 233). Therefore, we must remain realistic about how soon we expect a human vaccine. *T. gondii* is an eukaryotic protozoan with a large ~69.35Mb genome and has >8,300 protein-coding genes (234). Also, this parasite has a complex lifecycle, with different antigenically distinct developmental stages that elicit different immune responses. Thus, developing a vaccine targeting several developmental stages will not be straightforward. Although the path to effective vaccine is full of hurdles, we should remain hopeful that vaccines for toxoplasmosis may, in the near future, become available for the control of toxoplasmosis. Finally, while a series of landmark studies have provided important contributions to current understanding of TE, many challenges concerning the pathogenesis and management of TE remain unsolved (Table 2). Overcoming these challenges is critical to successful development and realization of efficient therapeutic interventions.

ACKNOWLEDGMENTS

We wish to thank Kevin Webb and Paul Goodwin from University of Nottingham for helpful comments and valuable suggestions on the manuscript.

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Figure legends:

FIG 1 Schematic illustration of *T. gondii* traversal across the BBB and the mechanisms that underlie the disruption of BBB permeability and brain dysfunction.

A. The different routes of *T. gondii* entry into the brain and the associated alterations in the tight junction (TJ) proteins and adhesion molecules of the cerebrovascular endothelium. The extracellular tachyzoites can directly enter the brain by paracellular or transcellular route or via a 'Trojan horse' mechanism where tachyzoites cross the BBB within infected leukocytes.
B. Due to their unique metabolic and immunological attributes, neurons are often vulnerable to the parasite's attack, which replicates within neurons, causing neuronal injury with production of cytokines and chemokines, resulting in more impairment of the neurological function and disturbance of brain metabolism. Neurons also provide a permissive niche to the development of cysts, which persist in dormancy for a long time within the brain.
C. Following entry to the brain, the tachyzoites activate resident microglia and astrocytes and elicit immune responses to limit the parasite proliferation. The M1 phenotype of activated microglia produces pro-inflammatory cytokines, which exacerbate BBB dysfunction by altering the architecture of TJ proteins ZO-1, claudin-5, and occludin. The effects of M1 microglia are counterbalanced by alternatively activated M2 microglia, which produce anti-inflammatory cytokines. Maintaining this immune-inflammatory equilibrium is key to the establishment of latent infection. Infection of astrocytes and microglia also leads to the disruption of neuroreceptors, such as the α7 nAChR and N-methyl-D-aspartate receptor (NMDAR), which may lead to cognitive dysfunction and neurodegeneration. Activated microglia and astrocytes secrete chemokines (e.g. CXCL9 and CXCL10) which function as ligands for CXCR3 to promote the influx of T cells and myeloid cells (granulocytes and monocytes) into the brain.
D. The matrix metalloproteinases (e.g. MMP-8 and MMP-10) and TIMP-1 also contribute to the regulation of the perivascular accumulation and influx of lymphocytes into the brain to prevent the reactivation of dormant cysts.
E. Reactivation from latency can occur due to various mechanisms, such as reduced expression of VCAM-1/α4β1 integrin, CD3δ, CD4, CD8β, IFN-γ, and iNOS. Reduced levels of CXCL9, CXCL10, MyD88, IL-12, MMP-8 or MMP-10 during reactivated toxoplasmosis decrease the influx of T cells into the brain.

FIG 2 Representative magnetic resonance images from a 68-year-old man living with HIV with *Toxoplasma* encephalitis. Images A and C are T1 FLAIR post contrast, and images B and D are the corresponding FLAIR. Note contrast enhancement of both lesions (A, C; bright white rim around the lesion), the "target sign" of the left temporal lobe lesion (white arrow), and significant mass effect (dark (low) signal on A, C; white (high) signal on B, D).

TABLE 1 Diagnostic pearls in the management of toxoplasma encephalitis

Summary of recommendations regarding testing for toxoplasmic encephalitis (TE)
• A positive serum anti-*T. gondii* IgG suggests that a patient is at risk for TE. Therefore, patients at risk for TE, including PLWH, individuals who will receive organ transplantation, including solid organ or hematopoietic stem cells, or those who will begin immunomodulatory therapies should be screened for serological evidence of latent (IgG antibody) *T. gondii* infection in order to determine whether a patient is at risk of TE.
• Lack of seropositivity for *T. gondii* IgG antibody indicates that TE is unlikely.
• Seropositivity for *T. gondii* IgM antibody has limited utility in establishing a diagnosis of TE.
• Diagnosis of TE is made by demonstrating clinical and radiological improvement to empiric anti-TE therapy. Empiric therapy is most appropriate in PLWH who have $CD4^+$ T cells <200/µl, reactive serum anti-*T. gondii* IgG, and are not receiving prophylaxis for TE.
• CSF PCR may support the diagnosis of TE, but lumbar puncture may not be safe in individuals with TE because of the mass effect.
• Lesions of TE must be differentiated from those of primary CNS lymphoma and tuberculoma or tuberculous abscess in PLWH.
• In individuals receiving solid organ or hematopoietic transplants, or patients receiving immunomodulating therapies, TE is among a variety of potential diagnoses, including bacterial and fungal infections and tumefactive multiple sclerosis.

TABLE 2 Key questions to consider for future research

Topic	Questions
Pathogenesis	• What drives the remarkable diversity of *T. gondii* in regard to its intermediate host range and clinical pathogenicity? • How does the mechanism of crossing the BBB differ among *T. gondii* strains? • To what extent do alterations in the actin cytoskeleton of cerebrovascular endothelial cells contribute to BBB disruption during *T. gondii* infection? • What parasite gene products or effectors (e.g. soluble factors, extracellular exosomes and their contents) induce changes in the organization or expression TJ proteins in endothelial cells? • To what extent does *T. gondii* interaction with non-endothelial cellular components of the BBB (e.g. astrocytes, microglia, pericytes) contribute to the pathogenesis of cerebral toxoplasmosis?
Immunity	• In which ways can trafficking and influx of $CD4^+$ and $CD8^+$ T cells into brain be modulated by MMP agonists or TIMP antagonists? • How do cytokines, MMPs and chemokines orchestrate T cell migration to and recruitment into the brain to combat toxoplasmosis? • How much overlap exists in the role of IL-10 originated from different sources in maintaining brain immune homeostasis during *T. gondii* infection? • How does IL-10 limit *T. gondii* growth while at the same time attenuate excessive immune responses?
Latent infection	• What is the evolutionary advantage to *T. gondii* parasite of infecting the CNS? • Which molecular mechanisms regulate *T. gondii* transition from dormant bradyzoites to actively proliferating tachyzoites, and vice versa? • What are the factors that make neurons the most preferable host cell type and what effects the neural cell microenvironment may have on the latency and reactivation of infection?
Treatment	• What can be done to improve the efficacy, safety and tolerability profiles of existing anti-*T. gondii* therapeutics? • Comparative studies evaluating effectiveness of different treatment strategies for toxoplasmosis are needed, especially for those populations of patients at highest risk of TE.

	• Can host-targeted therapy provide new adjunctive treatments for toxoplasmosis and reduce the development of drug resistance?
Vaccination	• Can vaccine cocktails incorporating multiple antigens induce more effective anti-*T. gondii* immune responses than a single-antigen vaccine? What mechanisms might be involved? • What are other potent immunogenic antigens are involved in *T. gondii* immunopathogenesis and can be targeted for vaccine development? • Would genetically manipulated parasites be accepted by regulatory authorities as a potential live attenuated vaccine?

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