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Title

Persistence of *Toxoplasma gondii* in the central nervous system: a fine tuned balance between the parasite, the brain and the immune system

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Abstract

Upon infection of humans and animals with *Toxoplasma gondii*, the parasites persist as intraneuronal cysts that are controlled, but not eliminated by the immune system. In particular, intracerebral T cells are crucial in the control of *T. gondii* infection and are supported by essential functions from other leukocyte populations. Additionally, brain-resident cells including astrocytes, microglia and neurons contribute to the intracerebral immune response by the production of cytokines, chemokines and expression of immunoregulatory cell surface molecules, such as major histocompatibility (MHC) antigens. However, the *in vivo* behaviour of these individual cell populations, specifically their interaction during cerebral toxoplasmosis, remains to be elucidated. We discuss here what is known about the function of T cells, recruited myeloid cells and brain-resident cells, with particular emphasis on the potential cross-regulation of these cell populations, in governing cerebral toxoplasmosis.

Introduction

The intracellular parasite *Toxoplasma gondii* (*T. gondii*) is one of the most common zoonotic pathogens, infecting a wide range of animals as well as humans. The seroprevalence of *T. gondii* in humans varies between 10-70% worldwide, depending on the region and increases significantly with age. Upon infection, the parasites persist as intraneuronal cysts in the central nervous system (CNS) for the lifetime of the host (1, Fig. 1). Until recently, parasite persistence in healthy individuals was regarded as clinically asymptomatic. However, in the last decade several reports have indicated that chronic cerebral toxoplasmosis may impact on the behaviour of its host (2).

The control of cysts depends on T cell- and NK cell-derived IFN-γ, indicating that ongoing inflammation is required to control *T. gondii* also in chronic cerebral infection. Interestingly, *T. gondii*-seropositive AIDS patients with low CD4$^+$ T cell counts are at high risk of developing life-threatening reactivated cerebral toxoplasmosis (3). In reactivated CNS toxoplasmosis, slow-replicating *T. gondii* bradyzoites within cysts switch to fast replicating tachyzoites, which infect and destroy brain-resident cells.

Although different genotypes of *T. gondii* have been described, the vast majority of human infection is caused by the type II genotype, which accounts for most cases of reactivated cerebral toxoplasmosis in AIDS patients. In contrast, different genotypes of the parasite including atypical strains may cause sight-threatening ocular toxoplasmosis, which preferentially occurs in immunocompetent humans (4).

In this review, we focus on the intracerebral target cells of the parasite and the contribution of brain-resident and recruited immune cells to control the infection within the CNS. The schematic description of the immune response upon *T. gondii* infection in the CNS is depicted in Figure 2.

Murine models of cerebral toxoplasmosis

Since studies on the *in vivo* mechanisms of intracerebral parasite control cannot be performed in humans, most of our knowledge on the interaction of *T. gondii* with brain cells and the immune system derives either from *in vitro* studies or from experimental rodent models. Mice are important host of *T. gondii* and play a central role in the predator-prey life cycle of the parasite, since cats are the definitive host of *T. gondii* (5).

The course of cerebral toxoplasmosis is strongly influenced by the genetic background of the mice. Laboratory mice with the H-2^b (e.g., C57BL/6) or H-2^k (e.g., CBA/Ca) MHC haplotype are susceptible to chronic progressive *Toxoplasma* encephalitis (TE), to which they may eventually succumb. In contrast, outbred mice (e.g. NMRI strain) and inbred mice with the H-2^d haplotype (e.g. BALB/c or B6-C.H2^d) are resistant to the infection and develop less severe latent toxoplasmosis (LT) with marginal inflammation (6). H-2^d mice are therefore useful to study LT, which is the predominant form of cerebral toxoplasmosis in immunocompetent humans. Moreover, we previously developed a murine model of reactivated TE based on infection of mice deficient in Th1 immune responses, where treatment with sulfadiazine allows the timing of cyst reactivation (7). This model is the most analogous to human reactivated TE, where cysts reactivate due to insufficient T cell control. Thus, the chronic stage of cerebral toxoplasmosis can have distinct outcomes, which are represented by corresponding mouse models, as summarized in Box 1.

In addition to the genetic background and immunological status of the host, the parasite genotype and strain has a major impact on the course of toxoplasmosis. Infection with type I strains (e.g. RH strain) induces a lethal acute toxoplasmosis in mice without inducing cyst formation. Infection with *T. gondii* of the type II clonal lineage, which causes most infections in humans, is followed by cyst development in the brains of mice. Adding to the complexity, virulence within these *T. gondii* type II strains diverges. For instance, the commonly used

ME49 strain displays low virulence, whereas DX and B7 strains exhibit rather increased virulence. In addition, the experimentally applied cyst number strongly determines the outcome of the disease ranging from LT to TE. These facts make the comparison of the different studies even more complicated, but allow us to understand varied outcomes of infection with different strains that occur in nature. It is important to note that the aforementioned genetic details on various *T. gondii* strains explain the subtle differences between the use of numerous murine models and highlight the difficulties in comparison studies using models of LT, TE and reactivated TE (Box 1).

Target cells of *T. gondii* in the CNS

In the CNS, *T. gondii* tachyzoites infect microglia, astrocytes and neurons. These cell types can only control the pathogen upon stimulation by the immune system, especially by T cell-derived IFN-γ. Whereas infected astrocytes and microglia can clear the parasites, evading elimination in neurons the parasites convert into cyst-forming bradyzoites (1, 8). It has long been controversial whether cysts remain within astrocytes, but a recent study using immunohistochemical confocal microscopy ruled out cyst formation in astrocytes *in vivo* (9). The mechanisms through which intraneuronal bradyzoites evade elimination by the immune system and the reasons why neurons in particular provide a safe niche for the parasite remain unclear. One possibility is the limited capacity of neurons for antigen processing and presentation, which plays an important role for immune evasion of *T. gondii*. Currently available treatment regimens efficiently eliminate *Toxoplasma* tachyzoites from infected brain-resident cells. However, no therapeutic agent is able to completely eradicate *Toxoplasma* cysts and bradyzoites from the CNS.

T cells

How does the antigenic composition of T. gondii influence the T cell response and parasite load in the chronic stage ?

More than 10 years ago, a comparison of *T. gondii* transfectants expressing the β-galactosidase model antigen with distinct stage-specific expression and trafficking, established that efficient T cell priming requires antigen expression at the tachyzoite stage (as opposed to bradyzoite) and antigen release into the parasitophorous vacuole of the infected host cell (as opposed to constrained within the parasite cytosol) (10). This seminal finding was corroborated by further work with the ovalbumin (OVA) model antigen, which was recognized by T cell receptor-transgenic CD8$^+$ (11) and CD4$^+$ (12) T cells. Moreover, by the identification of 6 natural antigens eliciting T cell responses during infection, which all contain signal peptides (13, 14, 15, 16, 17). Although robust endogenous responses are mounted against third-party model antigens, their impact on the course of chronic toxoplasmosis seems rather modest (10, 18). Furthermore, immunization with the naturally processed 28m and ROP5 antigens conferred no or only partial protection (15, 16).

Resistance to TE was mapped 20 years ago to the single L^d MHC I gene (19, 20). The molecular basis was then elucidated with the discovery of the GRA6 antigen, which gives rise to a decamer epitope presented by L^d and eliciting an immunodominant and protective CD8$^+$ T cell responses (13). Consequently, GRA6 appears to be the only natural antigen associated with strong protection following immunization, but also in a natural infection context (13). Modifying a 'GRA6 epitope-null' *T. gondii* strain to express the GRA6-derived dominant epitope indeed resulted in lower CNS parasite burden during LT (21). The strongly protective potential of the GRA6-specific response is related to the C-terminal position of the epitope within the source antigen (21). Yet other parameters such as the stage-specific pattern of expression, the localization within infected cells and/or the processing efficiency by distinct antigen-presenting cell (APC) populations in the brain could also influence protection

efficacy. Thus, the molecular basis underlying LT 'armed peace' ought to be explored in more depth.

How do T cells enter and how are they maintained in the T. gondii-infected CNS ?

Migration of T cells into the brain is initiated during acute infection. In the case of TE with ongoing inflammation, the permeability of the blood-brain barrier is likely to be higher and accompanied by continuous refueling of brain-infiltrating T cells from the periphery (22). In contrast, within the context of LT, peripheral CD4$^+$ and CD8$^+$ depletion had no effect on intracerebral T cell numbers and the recruitment of T cells to the brain is thought to stop after acute infection (23). In the course of LT, intracerebral CD4$^+$ and CD8$^+$ T cell numbers gradually declined in BALB/c mice due to a low levels of intracerebral T cell apoptosis (23). In LT, the mechanisms of intracerebral T cell survival are unknown, but may include the frequent restimulation of T cells by the persisting *Toxoplasma* antigen.

The mechanisms of CNS entry are reminiscent of those described for autoreactive T cells in multiple sclerosis (24). The integrin receptor vascular cell adhesion molecule (VCAM)-1 is upregulated on vascular endothelium of *T. gondii*-infected mice and its ligand, the α4β1 integrin (VLA-4) is expressed on activated CD4$^+$ and CD8$^+$ T cells isolated from infected animals (25). As shown in the model of reactivated TE, treatment with anti-α4 antibody restricted CNS entry of transferred T cells, which resulted in higher bradyzoite-to-tachyzoite conversion (25). Of note, anti-α4 treatment had a more pronounced effect on CD8$^+$ than CD4$^+$ T cell numbers. These data may appear to conflict with earlier data that reported no involvement of VCAM-1 (26). However, in the latter model residual VCAM-1 expression was noted on the choroid plexus endothelium. This discrepancy could in fact reveal interesting clues as to the portal of entry of T cells into the infected brain.

Data from both TE and LT models support the notion that brain-infiltrating T cells show low or no proliferative capacity in comparison to peripheral T cells. In TE-susceptible B6 mice, anti-VLA-4 treatment applied to prevent transferred T cells from entering the brain, decreased T cell numbers within the brain, suggesting that *in situ* proliferation was unlikely (27). Using BrDU incorporation assays in LT of BALB/c mice, no intracerebral proliferation of T cells was reported either (23). Additionally, as T cells expand in the periphery, it follows that *T. gondii*-derived antigens are not confined within the brain during chronic stage. Yet the nature of the 'peripherally accessible' antigenic material and the mechanisms by which it reaches spleen or lymph nodes have yet to be elucidated.

Which effector activities are exerted by T cells in the brain?

Regarding effector activity during chronic toxoplasmosis, one key issue concerns the relative involvement of $CD4^+$ *vs*. $CD8^+$ T cells. The answer appears largely model-dependent. While $CD4^+$ and $CD8^+$ T cells play a balanced role in controlling TE in $H-2^b$ (B6) mice (22, 15), $CD8^+$ T cells play a more prominent role in $H-2^d$ (BALB/c) mice during LT (28). As discussed above, this finding is thought to be linked to the strong immunodominance and protective function of the L^d-GRA6-specific response (13, 21).

IFN-γ produced by $CD4^+$ and $CD8^+$ T cells is undoubtedly essential for parasite control and resistance against TE. TNF also plays an instrumental role in preventing parasite reactivation during LT (29), although other cellular sources beside $CD8^+$ T cells could produce this cytokine. With respect to perforin-dependent cytotoxicity of $CD8^+$ T cells, it appears that perforin is required for resistance to TE, as perforin-deficient B6 mice have higher cyst loads and lower survival (30). No formal evidence for direct killing of neurons containing cysts by cytotoxic T lymphocytes (CTL) has been reported. New T cell receptor-transgenic or

transnuclear tools (31) as well as multi-photon tracking of T cell function in real-time and *in situ,* should help advance our understanding of effector processes in infected brains.

Interestingly, as initially reported in certain chronic viral infections and in line with the chronicity of infection in the B6 model, brain-infiltrating T cells lose their effector capacity over time and upregulate markers of functional 'exhaustion' such as PD1 (27). This can be counteracted by treatment with anti-PD-L1 antibody, which restores CD8$^+$ T cell function and prevents TE-associated mortality (32). Efficacy of this treatment may be related to the prevention of apoptosis for PD-1$^+$ polyfunctional memory CD8$^+$ T cells (33). So far in the B6 model, IL-12 has been shown to support the development and maintenance of IFN-γ-producing effector memory CD8$^+$ T cells, as well as survival of TE (34). Interestingly, an analysis of systemic immune responses illustrated that IL-12 fosters generation of effector CD8$^+$ T cells, but at a cost to the numbers and function of central memory CD8$^+$ T cells in toxoplasmosis (17). Potentially skewing the balance in favor of effector cells could reduce the parasite load, but compromise the level of long-term protective immunity and contribute to over-reactive immunopathological effector T cell response. How effective memory is generated in the protective H-2^d context remains to be determined.

What are the modalities of antigen presentation to T cells within the brain ?
During acute infection, it is widely known that priming optimal CD8$^+$ T cell responses require direct presentation of parasite antigens by invaded dendritic cells (DCs). This notion is supported by results showing that *ex vivo*-isolated parasite-containing cells (35) or *in vitro*-infected cells (36, 11) efficiently present *T. gondii* antigens and by a more recent study using a flow cytometry-based methodology to sort and separate actively infected cells from cells that have phagocytosed parasites (37).

Does a similar scenario apply within the CNS at chronic stage? So far this central question has been addressed exclusively in the TE-susceptible context, using the OVA model antigen released via dense granules. Using two-photon microscopy to monitor cell dynamics in live brain slices, OVA-specific CD8$^+$ T cells were found in the vicinity of an abundant, inflammation-induced network of fibers acting as cues for T cell migration (27). Although these structures generate second-harmonic signals when imaged by two-photon microscopy, they appear to be different from collagen as one would expect. Cognate T cells were observed slowing and stopping nearby isolated parasites in granuloma-like structures containing CD11b$^+$ and CD11c$^+$ cells, a behavior strongly suggestive of antigen recognition events (18). However, the fact that T cell dynamics did not differ whether they were close or further from the parasite, indicated that the antigen was likely to be distributed throughout the granuloma rather than localized to individual parasite-containing cells (18). This may mean that in contrast to the priming phase during the acute stage, presentation by bystander cells, which internalize dead parasites or debris, is a major process regulating T cell effector activity in the chronic stage. Of note, microglia are the only brain resident cells expressing MHC class II antigens in LT and TE, whereas both microglia and astrocytes express MHC class I$^+$ (6). Since both cell types can also be infected by *T. gondii* and uptake antigens, they may directly interact with *T. gondii*-specific T cells and regulate their function. In fact, parasite-specific CD8$^+$ T cells make transient contacts with both infected and non-infected CD11b$^+$ cells (i.e., macrophages, monocytes, DC, microglia) in TE (18). Thus, microglia and astrocytes may play a significant role as APCs, by fine-tuning T cell function, as wells as determining the exact nature and purpose of brain APCs in promoting protective responses in LT. This represents an exciting avenue for future investigation.

Interestingly, no direct interactions between cognate T cells and cysts or cyst-containing cells have been noted in two studies investigating the behavior of T cells in chronically infected

brains (18, 27). These two studies used *T. gondii* expressing the OVA model antigen to infect B6 mice (leading to TE) and analyzed OVA-specific OTI CD8$^+$ T cells as cognate T cells. Importantly, T cells did not interact with neurons containing cysts, but made transient contacts with infected and non-infected CD11b$^+$ cells (i.e. macrophages/microglia) (18). Since neurons are, in general, MHC class I$^-$ and also MHC class II$^-$, it may well be that T cells induce anti-parasitic effector molecules in cells neighbouring cyst-containing neurons and that these anti-parasitic molecules act on infeced neurons. Such a tripartite model has recently been described for cutaneous *Leishmania* infection where T cells induce nitric oxide (NO) production in phagocytes and diffusion of NO promotes equally effective parasite killing in NO-producing and NO-negative bystander cells (38).

Mononuclear cells are essential contributors to the control of chronic toxoplasmosis in the CNS

While the peripheral innate immune system in acute toxoplasmosis has been extensively studied in the recent years, little is known about the contribution of innate immune cell subsets upon chronic cerebral *T. gondii* infection (39, 40, 41, 42, 43, 44). Early studies highlighted the function of resident microglia cells in TE, describing production of essential pro- and anti-inflammatory cytokines such as IL-1β, IL-10, TNF, IL-12 and IL-15 (45, 46, 47, 48). In terms of chemokine expression, the ubiquitously activated microglia and brain leukocytes produce RANTES, MuMIG and occasionally CXCL10 (49). T cell derived IFN-γ activates the signal transducer and activator of transcription 1 (STAT1) and, thereby, enhance antimicrobial capacity by upregulation of NO, reactive oxygen intermediates (ROIs), immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs), which are essentially involved in parasite clearance in mononuclear cells (50, 51). IFN-γ-activated microglia cells significantly produce NO, which can stop intracellular parasite replication via

blockage of enzymatic activity and direct damage of the parasitic DNA (52). NO is critically important to control *T. gondii* in TE of B6 mice, but may also be involved in the development of pathology (53, 54). Moreover, a current study describes an IFN-γ-independent CD40-autophagy pathway via Beclin-1 that also contributes to the intracellular control of parasite replication (55). In conclusion, during chronic *T. gondii* infection in the inflamed CNS resident activated microglia cells displays fundamental anti-parasitic and immuno-regulatory properties.

However, along with the resident microglia cells other mononuclear cells, such as brain DC are also essential to control cerebral toxoplasmosis. The number of infiltrated CD11c^{+} brain DCs strongly increases in cerebral toxoplasmosis, and these cells home to the inflammatory foci in the infected brain (56). Importantly, recruited brain DCs are the main producers of IL-12, which is crucial for the maintenance of IFN-γ production of T cells in LT (56). A recent report elegantly characterized the phenotype and behavior of DC subsets in TE using multiphoton microscopy (57). This study indicates that CD11c expression is increased on activated microglia cells and that several DC subsets are present in the *T. gondii*-infected brain such as CD11b^{+} CD11c^{+} myeloid DCs, CD8α^{+} CD11c^{+} lymphoid DCs, and PDCA^{+} B220^{+} plasmacytoid DCs. All these DC subpopulations expressed high levels of MHC I, MHC II, CD80 and CD86 molecules, indicating imperative involvement in T cell activation (57).

Recently, mononuclear cell heterogeneity in the periphery as well as in the CNS has been extensively debated, since the availability of new surface markers and fate mapping technologies have led to a paradigm shift describing the behaviour of distinct monocyte-derived macrophages and their differences to tissue-resident mononuclear cells (58, 59). Monocytes are generated from the common macrophage and DC precursors (MDC) in the bone marrow and released to the bloodstream in a CCR2-dependent manner (60, 39). On the

basis of chemokine receptor expression and specific surface molecules, monocytes can be divided into two populations: the inflammatory $CX3CR1^{lo}CCR2^{+}Ly6C^{hi}Gr1^{+}$ and the patrolling $CX3CR1^{hi}CCR2^{-}Ly6C^{lo}Gr1^{-}$ (61). The inflammatory monocytes are actively recruited to sites of infection serving an immediate precursor for antigen-presenting DCs and macrophages. Nance et al. reported the presence of a distinct macrophage subset in the brain of *T. gondii* infected mice, which express $CX3CR1^{+}$, the scavenger receptors MMR, stabilin-1 and arginase-1. This alternatively activated macrophage subset produced chitinase, and was able to destroy the *T. gondii* cyst wall, thus suggesting a novel mechanism for parasite clearance (62).

We previously identified the crucial involvement of $Gr1^{+}$ inflammatory monocytes in the parasite control during the acute phase of *T. gondii* infection in the periphery (39). In extension, our current experiments revealed that $Ly6C^{hi}CCR2^{+}Gr1^{+}$ inflammatory monocytes are recruited to the *T. gondii*-infected brain to perform critical anti-parasitic functions in chronic TE (Dunay et al., unpublished data). Their specific depletion resulted in increased parasite load and decreased survival suggesting central function against *T. gondii*. Upon entering the brain, $Ly6C^{hi}CCR2^{+}$ monocytes produced high levels of pro-inflammatory mediators, such as IL-1α, IL-1β, IL-6, iNOS, TNF-α and ROS, when compared to microglia and thus are essential for parasite control. Interestingly, monocyte derived IL-10 was also detected suggesting their protective capacity. The recruited $Ly6C^{hi}CCR2^{+}$ monocytes further differentiated into $Ly6C^{int}CCR2^{int}CD11c^{+}$ and upregulated MHC $I^{+\ and}$ MHC II^{+} adopting DC properties. In addition, the monocytes differentiated into $Ly6C^{neg}CCR2^{int}F4/80^{hi}$ cells and performed effective phagocytic activity similarly to resident microglia cells. Thus, these results indicate the critical importance of the recruited $Ly6C^{hi}$ monocytes in the CNS upon chronic *T. gondii* infection.

Mechanisms of *T. gondii* control in astrocytes and neurons and their immuno- regulatory function

Similar to microglia, astrocytes and neurons serve both as a target cell for the parasite as well as immunoregulators. In contrast to brain-resident, recruited mononuclear cells and intracerebral lymphocyte populations, astrocytes and neurons cannot be directly isolated from the infected brain for further characterization. Therefore, our knowledge on astrocytes and neurons in toxoplasmosis derives from *in vitro* studies with astrocytes and neurons or from histological analysis of *T. gondii* infected mice. Recently, the generation of astrocyte- and neuron-specific gene-deficient mice added an important new tool for their functional analysis (63, 64).

Upon *in vitro* infection with *T. gondii,* astrocytes produce IL-1, IL-6 and granulocyte/macrophage colony-stimulating factor (48). In TE and LT, they also secrete the chemokines MCP-1 and CXCL10 (49, 65). In cerebral toxoplasmosis, other cells including microglia also produce these protective cyto- and chemokines and, therefore, the relative importance of their production by astrocytes is unknown. Functionally important, CXCL10 boosts protective $CD8^+$ T cell function by retaining $CD8^+$ T cells in the brain and increasing their average migration speed in a generalized Lévy walk pattern (66). The latter is predicted to aid T cells in shortening the average time taken to find *Toxoplasma* antigen presenting cells. In addition, there is good evidence that astrocytes inhibit pathological intracerebral Th17 responses in TE through production of IL-27 (67). Of note, *T. gondii*-infected neurons also produce IL-27 upon *T. gondii* infection (63) and, thus, may also prevent over-reactive T cell responses in TE and LT.

In general, our knowledge on cytokine and chemokine production by neurons in toxoplasmosis is sparse. Infection of cultivated neurons up-regulated production of IL-6, Transforming Growth Factor (TGF)-β1, MIP-1α and MIP-1β (68). Importantly, the effector

molecules inducing a toxoplasmastatic activity in neurons have not been identified so far. For astrocytes it is known that *in vitro* control of tachyzoites is largely dependent on IFN-γ, but not on NO. In murine astrocytes, the IFN-γ-inducible GTPases Irgm3 (IGTP) and IIGP1 effectively inhibit tachyzoite replication by the disruption of the parasitophorous vacuole (69, 70, 71). In human astrocytes, the enzyme indoleamine 2,3-dioxygenase, which degrades tryptophan and causes tryptophan starvation of the parasite, is the key anti-parasitic molecule (72). In contrast to IFN-γ-inducible GTPases, which are mainly expressed in rodents, guanylate binding proteins (GBPs) are expressed in most vertebrates and are highly induced in cells upon stimulation with IFN-γ. Importantly mGBP2 is important to control *T. gondii* in astrocytes and macrophages *in vitro* and mGBP2-deficient B6 mice fail to control *T. gondii* in the brain (73).

Mice with a selective deletion of gp130 in astrocytes demonstrated for the first time a specific and crucial *in vivo* function of these cells in TE (64). Gp130 is the common signal-transducing receptor of cytokines of the IL-6 cytokine family. In TE, astrocyte-specific deletion of gp130 resulted in astrocyte apoptosis and consequently, in an inability to constrain *T. gondii*-associated inflammatory lesions and to prevent parasite spread, resulting in rapid death from TE. In parallel, the selective deletion of gp130 in neurons caused neuronal apoptosis and lethal exacerbation of TE due to a hyperinflammatory intracerebral immune response. The latter was caused by the reduction of the gp130-dependent neuronal production of immunosuppressive IL-27 and TGF-β (63).

Similar to T cells, the immunological function of astrocytes has to be limited in order to prevent neuronal damage in this type of chronic CNS infection. Accordingly, it has recently been shown that immunosuppressive TGF-β signaling in astrocytes is required to limit leukocyte infiltration into the CNS, to restrict cytokine and chemokine production and to prevent neuronal injury (74).

Remaining open questions

In the last two decades, substantial progress has been made in our understanding of the pathogenesis of cerebral toxoplasmosis however, many questions are still unresolved. In particular, the interactions and cross-regulation of cells of the immune system with brain-resident cell populations are incompletely defined. What is the exact identity of brain APC subsets promoting protective T cell responses in the context of LT? Is MHC I cross-presentation by (non-infected) bystander cells a major mechanism at play during chronic stage? Key to answering these open questions are the development of new mouse models allowing the selective analysis of genes within individual cell types. These models predominantly include conditional gene-deficient mice allowing Cre-mediated cell type-specific gene deletion in different cell types of the immune system, but also in brain-resident cell populations including astrocytes (64), neurons (63) and microglia (75). In addition, the development of Cre-secreting *T. gondii* allows the selective analysis of host genes in infected cells (76). In addition, we anticipate that brain-resident cells do not only regulate intracerebral leukocyte populations, but also influence each other with respect to pathogen control. Do microglia-, astrocyte or monocyte-derived anti-parasitic molecules contribute to the control of *T. gondii* in neurons? It has been demonstrated that neurons can uptake RNA-containing exosomes of non-neuronal cells (77). Uptake of exosomes containing anti-parasitic effector molecules, immunomodulatory proteins or RNA by neurons could have a strong influence on parasite control in these cells. To address these questions a combination of gene-modified parasites and hosts in combination with multi-photon microscopy will be instrumental. The latter technique will also allow us the essential real time *in vivo* monitoring of the effect of the immune response on parasite biology (37).

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Box 1

LT: latent, clinically silent phase characterized by limited immune cell infiltrates in CNS parenchyma, absence of tachyzoite replication and efficient control of cyst number. Here we will refer to this context as latent toxoplasmosis (LT).

TE: A progressive inflammatory disease presenting with bradyzoite to tachyzoite conversion, parasite replication, large leukocyte infiltrates and fatal outcome in the absence of parasiticidal treatment. We will refer to this disease as *Toxoplasma* encephalitis (TE).

Reactivated TE: LT Reactivated TE constitutes one of the most severe manifestations of toxoplasmosis in immunosuppressed patients, especially those with AIDS (Munoz et al. Immunological Reviews 2011). Reactivation upon immunosuppression occurs, when not sufficient T cell- and NK cell-derived IFN-γ is available, that leads to cyst rupture and CNS inflammation. We will refer to this situation as reactivated TE

Legends

Fig. 1. *Toxoplasma* cyst in the murine brain

Immunohistochemical detection of a *Toxoplasma* cyst in the brain of a BALB/c mouse with LT (day 30 after infection). Note that *Toxoplasma* cysts reside within neurons and are filled with hundreds of bradyzoites.

Fig. 2. Immune response to *T. gondii* in the infected brain by resident and recruited immune cells