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Immune response and immunopathology during toxoplasmosis[1]

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Abstract

Toxoplasma gondii is a protozoan parasite of medical and veterinary significance that is able to infect any warm-blooded vertebrate host. In addition to its importance to public health, several inherent features of the biology of *T. gondii* have made it an important model organism to study host-pathogen interactions. One factor is the genetic tractability of the parasite, which allows studies on the microbial factors that affect virulence and allows the development of tools that facilitate immune studies. Additionally, mice are natural hosts for *T. gondii*, and the availability of numerous reagents to study the murine immune system makes this an ideal experimental system to understand the functions of cytokines and effector mechanisms involved in immunity to intracellular microorganisms. In this article, we will review current knowledge of the innate and adaptive immune responses required for resistance to toxoplasmosis, the events that lead to the development of immunopathology, and the natural regulatory mechanisms that limit excessive inflammation during this infection.

Keywords

Toxoplasma gondii; *T. gondii*; immune response; immunopathology; pathology; infection; parasite

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that can infect any warm-blooded vertebrate, and is a pathogen of medical and veterinary significance [1]. Infection with *T. gondii* can be acquired through congenital infection [2], or through carnivory, if tissue cysts present in the chronically infected host are ingested [3,4]. Additionally, it can be acquired through the ingestion of food and water contaminated with parasites in the form of oocysts, which are shed in the feces of infected cats [5]. Following ingestion, the parasite converts to a fast replicating form known as the tachyzoite, which results in systemic dissemination of the parasite to all tissues. Under normal circumstances this systemic infection is effectively controlled by the host immune response [6,7]. The parasite then converts to a slow replicating form known as the bradyzoite, which persist in tissue cysts in the host neural and muscle tissues for the lifetime of the host [8].

The course of infection in humans can range from asymptomatic to severe, depending on the parasite strain and the immune status of the host. The majority of cases of human infection are regarded as asymptomatic and infection rates in some areas are as high as 70% [9]. In contrast, congenital infection can result in a number of birth defects, including

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hydrocephalus, chorioretinitis, intracerebral calcifications, or spontaneous abortion [10]. Toxoplasmosis can also cause severe disease in patients with primary or acquired deficiencies in T cell function, such as those present in patients with AIDS, Hyper IgM Syndrome, those receiving treatment for cancer, and transplant patients being treated with immunosuppressive drugs [11–16]. Although such instances are relatively rare, symptomatic disease in immunocompetent individuals can result from infection with highly virulent strains of *T. gondii* and can cause severe ocular disease or death [17,18]. In addition to its direct significance to public health, the genetic malleability of the parasite and its natural ability to infect laboratory animals, have made it an ideal model to study parasite genetics and host-pathogen interactions [19].

Invasion process and intracellular niche

The mechanisms by which *T. gondii* invades host cells and forms an intracellular niche have been extensively reviewed elsewhere [20], but several aspects of this process are directly relevant to immunity and pathogenesis. During invasion, three successive waves of proteins are secreted from parasite organelles, called the micronemes, dense granules, and rhoptries, into the host cell. These proteins can alter host cell function and inhibit the immune response directed towards the parasite [21]. They also serve to modify the lipid membrane surrounding the parasite, forming a specialized intracellular organelle called the parasitophorous vacuole (PV). The PV allows for the transport of essential nutrients from the host cell to the parasite, while preventing lysosomal fusion, which would lead to the killing of the parasite [22]. The sequestered nature of the parasite within the PV raises several fundamental questions regarding the mechanisms by which the parasite interacts with the immune system. For example, can host cells sense the invading parasite, and how would infected cells access parasite antigens for presentation to T cells as is required for the effective control of the parasite.

Parasite virulence

As is the case for many pathogens, the outcome of infection with *T. gondii* is highly dependent on the interplay of host and microbial factors. Genotypic studies have identified three lineages of *T. gondii* into which most strains found in North America and Western Europe can be broadly classified [23]. In mouse models, parasites belonging to the Type I lineage are highly virulent whereas the Type II and Type III lineages are considered avirulent [23,24]. These differences are also reflected in human disease, as ocular toxoplasmosis in humans is associated with Type I, but not Type II or Type III strains [17]. Given the lethality of Type I strains during murine infection, the vast majority of insights into the mechanisms by which the host immune response controls infection have been gained through studies using avirulent isolates. However, the use of reverse genetics to compare parasite strains that differ in virulence has allowed the identification of secreted *T. gondii* kinases that modify host cell function. Understanding how these parasite enzymes impact host anti-microbial mechanisms provides one approach to define the events that determine the outcome of infection [25].

Innate immune responses to *T. gondii*

Following challenge with *T. gondii*, monocytes, neutrophils and dendritic cells (DCs) are recruited to the site of infection, and all of these cell types have been implicated in resistance to this organism [26–32]. However, questions remain about their specific roles in controlling infection. One of the most critical functions of the innate immune response to *T. gondii* is the ability to sense the pathogen and produce the cytokine IL-12, which stimulates natural killer (NK) cells and T cells to produce the cytokine Interferon-gamma (IFN-γ) [33–35]. IFN-γ is the major mediator of resistance to *T. gondii* and promotes multiple intracellular

mechanisms to kill the parasite and inhibit its replication. This Th1 immune response, defined by the production of IL-12 and IFN-γ, is characteristic of infection with many intracellular pathogens, and as is the case for infection with other intracellular pathogens, mice deficient in either IL-12 or IFN-γ that are infected with *T. gondii* succumb to acute disease and demonstrate an inability to control parasite burden [34,36].

The innate production of IL-12 during toxoplasmosis requires that the parasite first be sensed by the host. Innate immune receptors called Toll Like Receptors (TLRs) appear to have a role in this process. Thus, mice deficient in the adapter molecule MyD88, which is required for downstream signaling from most TLRs, are acutely susceptible to toxoplasmosis [37]. Specific TLRs implicated in the immune response to *T. gondii* include TLRs 2, 4, 9 and 11. TLR11 responds to a profilin-like molecule conserved among protozoan parasites [38,39] whereas TLRs 2 and 4 appear to recognize glycosylphosphatidylinositols on the surface of the parasite [40]. Additionally, following oral infection with *T. gondii*, bacterial antigens translocate from the gut, and TLRs 2, 4, and 9 respond to these microbial insults, thus contributing to the development of the Th1 immune response [41]. Although deficiency in any single TLR (of those tested to date) does not result in acute susceptibility to *T. gondii*, the relative contribution of each of these TLRs is illustrated by the increased cyst burden present in infected mice deficient in one or more of these receptors [38,40]. Despite the critical importance of MyD88, other mechanisms of sensing the parasite clearly exist, as protective immunity can be induced in MyD88-deficient mice using a vaccine strain of the parasite, and IL-12 responses are not completely abolished in the absence of MyD88 [37,42].

Numerous studies have aimed to define the primary cell types responsible for production of IL-12 *in vivo* and have identified neutrophils, inflammatory monocytes, macrophages and DCs as relevant sources [27,28,37,43–45,]. The relative contribution of DCs to the production of IL-12 during toxoplasmosis has been examined using two mouse models: one in which DCs can be selectively depleted, and another in which DCs specifically lack expression of MyD88 [32,46]. In both cases, altered function or numbers of DCs resulted in lower systemic levels of IL-12 and increased susceptibility to *T. gondii*. In these models, resistance can be restored by treatment with IL-12, suggesting that DCs are an essential source of IL-12 during toxoplasmosis. Other studies have aimed to define which subsets of DCs are the most relevant sources of IL-12. Following the *in vivo* administration of soluble *T. gondii* antigens, the $CD8\alpha^+$ subset of DCs produces IL-12 [47]. More recently, mice lacking the transcription factor Batf3, which have a deficiency in $CD8\alpha^+$ DCs, have been shown to succumb to *T. gondii* associated with a severe IL-12 defect, reduced $CD8^+$ T cell responses, and high parasite burdens [48]. The finding that exogenous IL-12 restores survival of Batf3 KO mice is consistent with a model in which $CD8\alpha^+$ DCs are an essential source of IL-12.

Neutrophils are another source of IL-12 during toxoplasmosis, as they contain pre-stored IL-12 and can secrete this cytokine *in vitro* and *in vivo* in response to *T. gondii* [28,43,49]. Additionally, there are reports that neutrophil depletion results in decreased levels of IL-12 and increased parasite replication [50]. These findings are complicated by the realization that the strategy used to deplete neutrophils also affects other cell types, including inflammatory monocytes [30]. Regardless, mice deficient in the chemokine receptor CXCR2, which is essential for neutrophil recruitment to the site of infection, have higher parasite levels in the CNS, suggesting a role for neutrophils during toxoplasmosis [31]. Neutrophils are also implicated in other effector mechanisms that directly kill parasites, including phagocytosis, the release of reactive chemical species, and the formation of extracellular traps [51–54]. While phagocytosis of *T. gondii* by neutrophils has been observed *in vitro* [51,52], several groups have reported that $p47^{phox}$, an enzyme component

necessary for the oxidative burst generated by neutrophils following phagocytosis, is unnecessary for resistance to *T. gondii* [55,56]. Indeed, *in vivo* imaging studies have observed swarms of neutrophils that congregate around infected cells, but the parasites present in the neutrophils appear to be largely intact [53]. However, infection with *T. gondii* does induce increased extracellular DNA at the site of infection, which is dependent upon the presence of neutrophils, and this may be explained by the release of DNA from neutrophils to form extracellular traps [54]. *In vitro* studies suggest that the formation of these traps results in decreased parasite vitality and may contribute to the control of *T. gondii in vivo*.

Monocytes are also required for resistance during toxoplasmosis, as mice deficient in the chemokine receptor CCR2, which in necessary for monocyte recruitment to the site of infection, exhibit increased susceptibility when challenged [30,57,58]. Inflammatory monocytes produce IL-12 *in vitro* and *in vivo* when stimulated with *T. gondii*, however it is not clear whether they are a critical source of this cytokine [27, 30,45,57–59]. It has also been proposed that these populations contribute to the direct control of *T. gondii* through the generation of nitric oxide (NO), which inhibits parasite replication [60]. In support of this model, inflammatory monocytes express inducible nitric oxide synthase (iNOS), the enzyme responsible for catalyzing the production of NO, and inflammatory monocytes are able to kill and inhibit the replication of *T. gondii in vitro* [61,62,27]. Additionally, CCR2 KO mice given a low dose oral challenge of *T. gondii* succumb approximately 3–4 weeks after infection, and this is associated with decreased expression of iNOS and increased parasite burdens in the CNS [58]. Although monocytes are clearly critical for survival during toxoplasmosis, their role is not limited to production of nitric oxide, as iNOS- deficient mice survive acute challenge, while deficiencies in monocyte recruitment can lead to acute susceptibility [30,57,63]. Monocytes also produce IL-1 in response to soluble toxoplasma antigens [64], and this factor can enhance anti-toxoplasmic effector mechanisms in macrophages and astrocytes *in vitro* [65,66]. Moreover, IL-1 can synergize with IL-12 to promote production of IFN-γ from innate and adaptive sources [67,68]. It is also possible that monocytes proceed to develop into DCs that are capable of inducing adaptive immune responses [69], or macrophages that can control infection through immune GTPase-mediated mechanisms, as will be discussed later in this article.

Natural killer (NK) cells are another innate population involved in immunity to *T. gondii*, and in mice that lack T cells they provide a limited mechanism of resistance through their ability to produce IFN-γ [35,70–72]. NK cell activity peaks early during infection, and although their activity is elevated during chronic toxoplasmosis, they do not appear to be significant contributors to immunity during the chronic stage of infection [35,70–74]. Consequently, most studies have focused on the early events that control NK cell activity, leading to a model in which IL-12 produced by other innate cells (e.g. neutrophils, monocytes and DCs) promotes NK cell-mediated production of IFN-γ [33,35]. In addition to IFN-γ, NK cells produce the cytokine IL-10, the significance of which will be discussed later in this review [75]. Human and murine NK cells can also be cytotoxic for cells infected with *T. gondii* [76,77], but it has been proposed that NK cells become infected with the parasite following the lysis of infected cells, which may promote the dissemination of the parasite [78].

NK cells can also act to promote adaptive immune responses. Thus, in the absence of $CD4^+$ T cells, they can provide help to the $CD8^+$ T cell response [79]. One mechanism by which this help is accomplished is by increasing IL-12 production from DCs through interactions dependent on the molecule NKG2D [80]. Additionally, production of IFN-γ by NK cells has been implicated in the development of optimal $CD4^+$ T cell responses [81].

Adaptive Immune Responses to *T. gondii*

The importance of adaptive immune responses for resistance to *T. gondii* during human infection is demonstrated by the increased susceptibility of patients with primary or acquired defects in T cell function, and mice with deficiencies in B cells, $CD4^+$ T cells or $CD8^+$ T cells survive the acute stage of infection, but ultimately show increased susceptibility to *T. gondii* [82–84]. Understanding how these different cell populations are integrated to provide long-term resistance has been challenging, but several advances in technology have improved our ability to study adaptive immune responses to *T. gondii*. For example, the identification of the molecular epitopes of *T. gondii* recognized by $CD8^+$ T cells has allowed the measurement of antigen-specific $CD8^+$ T cell responses during infection, and provided insight into the mechanisms by which antigen is presented [85,86]. This has been complimented by the development of parasites that express model antigens such as ovalbumin, β-galactosidase, and EαRFP, as well as the use of two-photon imaging to allow visualization of immune cells following infection [19,87]. These advances are currently allowing a higher resolution analysis of the events that mediate the control of *T. gondii*, and may also provide insight into the strategies used by this parasite to persist despite the presence of an array of anti-microbial effector mechanisms.

$CD4^+$ T cell responses: Initiation and mechanisms of controlling infection

As mentioned earlier, $CD4^+$ T cells are critical for resistance during toxoplasmosis, as the emergence of severe toxoplasmosis is concomitant with the decline in T cell numbers in patients infected with HIV [88,11], and in mouse models, the lack of $CD4^+$ T cells is associated with increased susceptibility during the chronic stage of infection [83]. $CD4^+$ T cells provide several critical regulatory functions in mediating resistance to toxoplasmosis. During the early stages of infection they contribute to optimal B and $CD8^+$ T cell responses (discussed in subsequent sections of this review) [83,89], and the ability of these cells to control chronic infection may be attributed to their production of cytokines such as IFN-γ, or their expression of CD40L (also referred to as CD154), which can activate effector mechanisms in macrophages and other innate cells expressing CD40 on their surface [90–95].

The initiation of T cell responses requires that naïve $CD4^+$ or $CD8^+$ T cells encounter antigen presenting cells bearing their cognate antigen in the context of MHCII or MHCI molecules respectively, in conjunction with co-stimulatory and cytokine signals needed for T cell activation [96–99]. During toxoplasmosis, ligation of the molecules CD28 and ICOS, expressed on the surface of T cells, contributes to the co-stimulatory signals, while IL-12 provides the cytokine signal required to promote proliferation and differentiation into populations that produce IFN-γ [34,100,101].

B cells, macrophages, and DCs are all capable of presenting antigen to $CD4^+$ T cells, though DCs are generally considered the most crucial antigen presenting cell population *in vivo* [102]. Following infection with *T. gondii*, multiple populations of DCs undergo expansion and acquire an activated phenotype. Additionally, challenge of mice with parasites engineered to express the model antigen EαRFP revealed that $CD8\alpha^+$ and plasmacytoid DCs (pDCs) express complexes of MHC class II and Eα, a peptide derived from EαRFP, on their surfaces. While these studies implicate pDCs and $CD8\alpha^+$ DCs as responsible for presenting antigen to $CD4^+$ T cells during toxoplasmosis, the use of mice with deficiencies in specific DC compartments, as well as mouse models that allow for the selective depletion of DCs or DC subsets, may be useful to further define the roles of these populations in antigen presentation [48,103,104].

The mechanisms by which professional antigen presenting cells acquire parasite antigens for presentation in the context of MHCII are unclear, and there are several possible models to explain how this may be accomplished (Figure 1). Since there are multiple reports that murine DCs and monocytes infected with *T. gondii* express low levels of MHCII and co-stimulatory molecules, it has been suggested that infected cells would be poor presenters of antigen [105–107]. Thus, antigen acquisition might occur through the phagocytosis of parasites, infected cells, or parasitic debris, or through the endocytosis of antigens secreted by the parasite (Figure 1a). *In vitro* studies have demonstrated that murine DCs are able to present antigen derived from live and heat-killed parasites to $CD4^+$ T cells [108]. Because heat-killed parasites cannot invade cells, these data are consistent with a model in which antigen is acquired via the phagocytosis of parasites.

Alternatively, antigen may be acquired by antigen presenting cells through active invasion mediated by the parasite, or through abortive invasion events in which antigens are injected into cells that are not subsequently infected (Figure 1b) [109]. This model is supported by experiments conducted using human monocytes and DCs, in which cells exposed to viable parasites upregulated MHCII and co-stimulatory molecules, whereas cells exposed to heat-killed parasites did not [110,111]. Additionally, these studies found that exposure to live parasites was necessary for antigen presentation ability. These findings are not entirely consistent with the reports described earlier, although this may be attributable to differences between human and murine cells. It is also relevant to note that results obtained from *in vitro* studies may not be representative of what occurs *in vivo*. Currently, *in vivo* studies examining the mechanisms by which antigen is presented to $CD4^+$ T cells during toxoplasmosis are lacking. Other insights into the mechanisms by which antigen can be acquired during toxoplasmosis have been gained by studying antigen presentation to $CD8^+$ T cells, and will be discussed later in this article.

Humoral immunity is essential for resistance to toxoplasmosis

It has long been recognized that infection with *T. gondii* promotes antibody responses, and that these antibodies can kill the parasite [112]. Indeed, parasite-specific IgM, IgA, IgE and IgG2 antibodies have been isolated from human patients, and detection of parasite specific antibodies is an effective diagnostic tool to distinguish newly infected individuals from those in the chronic stage of infection [112–116]. The critical role of antibody in immunity to *T. gondii* is demonstrated by the phenotype of μMT mice, which are deficient in B cells. These mice develop apparently normal IFN-γ responses, but succumb to infection within 3–4 weeks following challenge, associated with high parasite burdens in the CNS [82]. This increase in susceptibility is likely due to a lack of antibodies, as the passive transfer of antibodies confers protection to B cell-deficient mice [83]. Antibodies can mediate their protective effects through a variety of mechanisms. *In vitro* studies have found that they can opsonize parasites for phagocytosis, block invasion, and also activate the classical complement pathway [51,117–121]. The *in vivo* relevance of complement activation is illustrated by studies in which treatment of mice with an antibody that binds to the complement protein C3 results in acute susceptibility to toxoplasmosis [122]. Additional studies are required to define the contribution of other antibody-mediated functions.

As mentioned previously, $CD4^+$ T cells are necessary to promote optimal B cell responses and mice deficient in or depleted of $CD4^+$ T cells display lower parasite-specific antibody titers [83,123]. Furthermore, the increased susceptibility of $CD4^+$ T cell-deficient mice can be ameliorated by the passive transfer of antibodies, indicating that the defect in antibody responses likely contributes to the failure to control parasite numbers [83]. Curiously, infection with *T. gondii* results in severe disruption of splenic architecture and the loss of distinct B cell zones [123,124]. Since B cell zones are considered the main location where $CD4^+$ T cells provide help to B cells [125], this raises the question of whether there is a

specialized microenvironment where T-B interactions occur when B cell zones are absent. Since disruption of secondary lymphoid structures is characteristic of many infections [126–131], murine models of toxoplasmosis may prove a useful system to interrogate the mechanisms by which $CD4^+$ T cells help B cell responses, and the extent to which splenic architecture contributes to such interactions.

$CD8^+$ T cell response: Initiation and control of parasite burden

Given that *T. gondii* is an intracellular pathogen, it is not surprising that $CD8^+$ T cells, which are specialized to recognize and destroy cells infected with viral, bacterial and parasitic organisms, also have a critical role in mediating resistance to this infection. $CD8^+$ T cells can control infection through the production of inflammatory cytokines such as IFN-γ, through CD40/CD40L interactions, and through the perforin-mediated cytolysis of infected host cells [84,90,91]. Indeed, mice deficient in $CD8^+$ T cells show increased susceptibility to toxoplasmosis, succumbing approximately 50 days post-infection [84]. Furthermore, the adoptive transfer of $CD8^+$ T cells from chronically infected mice, or mice vaccinated with an attenuated strain of *T. gondii*, is sufficient to confer resistance [132,133]. Additional evidence comes from *in vivo* depletion studies using chronically infected mice, in which depletion of $CD8^+$ T cells and $CD4^+$ T cells results in reactivation of the infection and severe disease, but depletion of $CD4^+$ T cells alone had limited impact [90].

As previously described, $CD8^+$ T cell responses are initiated when naïve $CD8^+$ T cells encounter their cognate antigen in the context of MHCI on the surface of antigen presenting cells, accompanied by co-stimulatory and cytokine signals. Some of the earliest studies on the $CD8^+$ T cell response identified the Surface Antigen 1 (SAG-1) protein as a target of $CD8^+$ T cells, although the specific peptide sequence of SAG-1 that the $CD8^+$ T cells recognize remains unknown [134]. More recently, technical advancements have accelerated the discovery of epitopes of *T. gondii* that are recognized by $CD8^+$ T cells. Thus, in 2008, two studies identified peptides derived from *T. gondii* that are presented in the context of the H2-L^d allele of MHCI. These include peptides from the dense granule proteins GRA4 and GRA6, and the rhoptry protein ROP7 [86,135]. Of these, the GRA4 and ROP7 epitopes are conserved across multiple strains of *T. gondii*, whereas expression of the GRA6 epitope is limited to Type II strains. Another epitope, derived from the protein Tgd_057, is presented in the context of the MHCI allele H2-K^b, and is also conserved among multiple genotypic strains [136]. The function of Tgd_057 is unclear, but despite the presence of a secretory signal, it localizes primarily to the cytosol of the parasite. It is of interest that all of these proteins, with the possible exception of Tgd_057, are secreted from the parasite. While this observation likely reflects, in part, the methods used to screen for these epitopes, it is also in agreement with studies in which parasites are engineered to express model antigens, as these studies consistently demonstrate that antigens secreted from *T. gondii* induce more robust T cell responses than antigens expressed in the cytosol [137–139]. These findings may provide insight into the mechanisms by which antigen is presented to T cells, as will be discussed later in this article.

Currently, there are several questions about the identity of the cell populations involved in antigen presentation to $CD8^+$ T cells during toxoplasmosis. DCs are known to be efficient antigen presenting cells, and are crucial for the development of $CD8^+$ T cell responses to *T. gondii*, however there is a lack of studies that clearly distinguish their role as producers of IL-12 from their role as presenters of antigen [29,32]. *In vivo* imaging studies have observed extensive interactions between DCs and antigen-specific $CD8^+$ T cells, suggesting a role for DCs in antigen presentation [124,140]. In contrast, using a bone marrow chimera approach to generate mice in which MHCI expression was limited to non-hematopoietic cells, Dzierszinski *et al.* demonstrated that challenge of these mice with *T. gondii* resulted in an apparently normal $CD8^+$ T cell response [141]. One interpretation of these data is that DCs

are not necessary for antigen presentation during toxoplasmosis. Further experimentation is therefore necessary to determine which cell type presents antigen to $CD8^+$ T cells.

As is the case for antigen presentation to $CD4^+$ T cells, there are multiple pathways by which parasite antigens may be acquired for presentation to naïve $CD8^+$ T cells (See figure). *In vitro* and *ex vivo* studies have found that infected DCs are able to present antigen, whereas cells exposed to parasites or parasite antigens that were not infected are unable to do so [108,141,142]. In contrast, the DCs observed to interact with $CD8^+$ T cells *in vivo* appear to be largely uninfected [124,140], suggesting a possible role for uninfected cells in presenting antigen to naïve $CD8^+$ T cells *in vivo*, which is consistent with numerous reports of cross-presentation in other models of infection [143].

While the cellular pathways by which phagocytosed antigens can come to be presented in the context of MHCI have been widely studied in a variety of systems [143], it is less clear how a cell infected with *T. gondii* can acquire antigen to be presented, given that the parasite resides in a specialized non-fusogenic vacuole. Several studies using reporter systems in which host cells respond to antigens derived from *T. gondii* have demonstrated that secreted antigens can enter the cytoplasm of infected cells [109,142]. These antigens would then be transported from the cytosol into the endoplasmic reticulum by the Transporter Associated with Antigen Processing (TAP) [142]. This model is consistent with studies demonstrating that secreted antigens from *T. gondii* are preferentially presented to T cells [86,135,137–139]. Alternatively, the PV can fuse with the endoplasmic reticulum, providing another mechanism by which antigens may escape sequestration and enter the protein transport pathway [108].

$CD8^+$ T cell responses to *T. gondii* are influenced by help provided by $CD4^+$ T cells [79,89]. Although depletion of $CD4^+$ T cells does not affect the magnitude of the $CD8^+$ T cell response during the early stage of $CD8^+$ T cell expansion and activation, $CD4^+$ T cells are necessary for the maintenance of $CD8^+$ T cell effector functions during the chronic stage of infection, and this help must be provided during the acute stage of infection [89]. Further insights regarding the nature of $CD4^+$ T cell help have been gained from studies using the attenuated vaccine strains of *T. gondii* ts-4 and *cpsII*, both of which require $CD4^+$ T cell help for optimal protective $CD8^+$ T cell responses [144,145]. In current models, ts-4 vaccination stimulates $CD4^+$ T cells to produce the growth factor IL-2, which provides an essential signal for $CD8^+$ T cells. Indeed, neutralization of IL-2 results in diminished $CD8^+$ T cell responses and decreased protection [144]. Other potential mechanisms by which $CD4^+$ T cells may provide help include the licensing of DCs, or direct interactions with $CD8^+$ T cells through CD40/CD40L interactions [146].

The vast majority of studies examining the $CD8^+$ T cell response have used avirulent Type II strains of *T. gondii*. Recently, it has become apparent that $CD8^+$ T cell responses are dramatically decreased following infection with the highly virulent RH strain of the parasite and there are several possible explanations for this phenotype [29]. The defective $CD8^+$ T cell response may be influenced by the activities of the parasite virulence factor ROP18 which (in addition to other functions) binds to the host protein Activating Transcription Factor 6 β (ATF6β), leading to its degradation [147]. In support of this model, ATF6β-deficient mice have a defective $CD8^+$ T cell response when infected with *T. gondii*, and ROP18-deficient parasites from an RH background induce greater production of IFN-γ from $CD8^+$ T cells, relative to WT RH parasites. Decreased $CD8^+$ T cell responses may also result from an abbreviated DC response during RH infection, relative to infection with an avirulent Type II strain [148]. As the adoptive transfer of large numbers of antigen-specific $CD8^+$ T cells is able to transiently reduce parasite burden during RH infection, it seems

likely that the decreased $CD8^+$ T cell response is a contributing factor to the virulence of the RH strain [29].

More subtle changes in $CD8^+$ T cell responses may also help to explain differences in susceptibility among mouse strains. Whereas the C57B/6 inbred mouse strain succumbs to *T. gondii* during the chronic stage of infection, BALB/c mice are relatively resistant to toxoplasmic encephalitis. This difference in susceptibility has been genetically mapped to the MHC Class I H2-L^d allele, implicating $CD8^+$ T cells as being responsible for this difference in susceptibility [149,150]. The recent identification of an immunodominant epitope from the protein GRA6, recognized by $CD8^+$ cells, that binds to the H2-L^d Allele has led to the hypothesis that recognition of this peptide is crucial for controlling *T. gondii* infection in BALB/c mice, and may account for the differences in virulence among mouse strains [86]. Because expression of this epitope is restricted to Type II strains of *T. gondii*, its relative significance could be tested by replacing the peptide with the sequence present in Type I or Type III strains. Alternatively, it may be possible to tolerize mice to this epitope through vaccination, as has been reported in other systems [151]. Regardless, these studies highlight the importance of GRA6 as a target for protective CD8+ T cells.

Effector mechanisms controlling *T. gondii* infection

As discussed in the previous section, cellular immunity mediates protection through the production of inflammatory cytokines such as IFN-γ. Other molecular signals, such as the cytokine tumor necrosis factor alpha (TNF-α) and CD40 ligation are also required for resistance during chronic toxoplasmosis [91,152–154]. This section describes how these distinct pathways are integrated to engage specific effector mechanisms required to directly control infection with *T. gondii*.

Nitric oxide inhibits replication of *T. gondii*

Since the early 1980's, it was recognized that IFN-γ can activate macrophages to kill a variety of intracellular organisms, including *T. gondii* [155], and during the late 1980's it was reported that IFN-γ is also essential *in vivo* for resistance to *T. gondii* [36]. These findings raised the fundamental question of how this cytokine promotes control of *T. gondii* and other pathogens. It was proposed that the protective effects of IFN-γ may be mediated by inducing increased synthesis of Nitric Oxide (NO) [156]. Consistent with this hypothesis, expression of inducible nitric oxide synthase (iNOS), the enzyme responsible for catalyzing the reaction that results in production of NO, is increased in macrophages by stimulation with IFN-γ, and NO inhibits replication of *T. gondii* in macrophages and other cell types [157–161]. Importantly, IFN-γ alone is not typically sufficient to activate macrophages to kill *T. gondii*, and additional signals provided by factors like TNF-α or CD40L are required for optimal iNOS expression [158,162]. *In vivo* evidence for a role of NO in controlling toxoplasmosis came from a study in which administration of the iNOS inhibitor aminoguanidine to infected mice resulted in increased parasite burdens [163]. Subsequently, iNOS-deficient mice were developed and found to display increased susceptibility to toxoplasmosis, succumbing to disease in the chronic stage of infection [63]. Although the specific mechanism by which NO inhibits replication of *T. gondii* remains to be determined, studies using intracellular bacterial pathogens have shown that NO can inhibit bacterial enzymatic activity and directly damage DNA [164], which would preferentially affect pathogen replication and account for the static effects of NO.

IFN-γ mediates protection through the p47 GTPases

The increased susceptibility of iNOS-deficient mice to toxoplasmic encephalitis clearly implicated iNOS in immunity to *T. gondii*, but also pointed toward iNOS-independent

mechanisms by which IFN-γ mediates protection during the acute phase of infection. Like iNOS, members of the p47 GTPase family (also referred to as the immune related GTPase family (IRGs)) are also upregulated in response to IFN-γ [165,166], but the importance of this family was first apparent when mice that lack the p47 GTPase IGTP (Irgm3) were infected with *T. gondii*. These mice have normal IFN-γ responses, but succumb to acute toxoplasmosis due to high parasite burdens [167]. Subsequent studies revealed other members of this family, including LRG-47 (Irgm1), IRG-47 (Irgd), IIGP1 (Irga6), and TGTP (Irgb6) to be involved in immunity to *T. gondii* as well [161,168–170]. The specific mechanisms by which individual members of the p47 GTPase family promote the clearance of *T. gondii* are the subject of ongoing studies in many laboratories [166]. There are reports that in IFN-γ activated cells p47 GTPases colocalize to the PV, which then develops a tight fitting morphology followed by a rough and disrupted appearance before being stripped away [161,170–177]. Once free in the cytosol, the parasite egresses the infected cell or becomes permeabilized and killed [161,178]. In the latter studies the host cell was observed to undergo necrosis after killing the parasite. Additionally, other studies have observed the exposed cytosolic parasite to be disposed of by xenophagy, the process by which foreign bodies within a cell are eliminated using the same cellular machinery involved in autophagy [172]. In further support of a role for autophagic machinery in immunity to *T. gondii*, the autophagy protein Atg5 has been found to be necessary for the disruption of the PV and resistance to this infection *in vivo* [174]. Additionally, CD40 ligation has been observed to induce xenophagic elimination of parasites independently of p47 GTPases, as will be discussed later in this review [93].

Given the important role of the p47 GTPases in immunity to *T. gondii*, it is not surprising that the parasite has evolved strategies to interfere with their function. At least three members of the p47 GTPase family, Irga6, Irgb6 and Irgb10, are phosphorylated by ROP18, resulting in changes in their functionality or cellular localization associated with increased virulence [170,177]. Additionally, the recruitment of GBP1, a member of the guanylate-binding protein family (GBPs), to the PV is also inhibited by the parasite-derived virulence factors GRA15, ROP16 and ROP18 [179]. As the GBP family has recently been implicated in immunity to intracellular bacteria [180], this finding may be indicative of a role for GBPs in immunity to *T. gondii*, although further research will be necessary to directly test this hypothesis.

The role of tryptophan degradation as a defense mechanism

IFN-γ can also mediate protective effects against *T. gondii* by promoting tryptophan degradation in a variety of infected cell types, including fibroblasts, macrophages, and brain cells [181–184]. Treatment of cells with IFN-γ results in the upregulation of the genes indolamine 2,3-dioxygenase 1 and 2 (IDO-1 and IDO-2), which catalyze the degradation of tryptophan [182,185]. Because *T. gondii* is a natural tryptophan auxotroph, the increased degradation of tryptophan by host cells inhibits parasite growth [186]. The *in vivo* relevance of this pathway is illustrated by the finding that long-term treatment of infected mice with inhibitors of IDO-1 and 2 results in increased susceptibility and increased parasite burdens during chronic infection [187]. Interpretation of this finding is complicated by the fact that IDO has other known immune functions such as suppression of DC and effector T cell functions, as well as promotion of regulatory T cell responses [188].

Members of the TNF family are necessary for immunity to *T. gondii*

In addition to IFN-γ, members of the TNF family such as CD40L, TNF-α and LT-α, are also required for protection during the chronic stage of infection [91,153,154]. The critical role of TNF-α is demonstrated by studies in which neutralization of this cytokine results in increased susceptibility and higher parasite burdens [189]. Additionally, mice deficient in

TNF-α (TNF-α KO) or the components of its receptor (TNFR KO) succumb to infection approximately 3–4 weeks post-challenge despite having functional IFN-γ responses [152–154]. TNF-α is produced by a number of cell populations in response to *T. gondii* or *T. gondii* antigens, including neutrophils [43,190], DCs [190], macrophages [191], microglia [192], and T cells [193]. TNF-α synergizes with IFN-γ to promote anti-parasitic mechanisms in macrophages, as well as non-hematopoietic cells [194,195]. *In vitro* studies have demonstrated that this can be mediated through the production of nitric oxide [157,158]. Additionally, TNF-α KO mice, TNFR KO mice, and mice treated with a neutralizing antibody for TNF-α display decreased iNOS expression [152,154,189]. Collectively, these data support a model in which TNF-α mediates its protection by inducing expression of iNOS. However, there are also data that suggest that susceptible TNFR KO mice infected with *T. gondii* can have appropriate levels of iNOS, suggesting that TNF-α can mediate protection through iNOS-independent mechanisms [153]. Because TNF-α KO and TNFR KO mice are capable of surviving the acute stage of infection, it is clear that TNF-α is not required for the IGTP-mediated elimination of the parasite [152–154]. This notion is also supported by *in vitro* studies, in which macrophages show no defect in their ability to kill parasites in the absence of TNF-α signaling [196]. However, interpretation of these results is complicated by the finding that TNF-α plays a more prominent role in activating macrophages when concentrations of IFN-γ are limiting [153]. Thus, the chronic susceptibility of mice deficient in TNF-α signaling may result from changes in the expression of IFN-γ during the course of infection rather than a deficiency in any one specific effector mechanism that is absolutely dependent upon TNF-α.

Another component of the TNF family involved in immunity to *T. gondii* is CD40L, which is expressed on T cells and binds to CD40 expressed on macrophages and other cell populations [197]. The importance of CD40/CD40L interactions to promote immunity to *T. gondii* is evidenced by the increased susceptibility of patients with Hyper-IgM syndrome, a disease characterized by defective CD40L expression [12–14]. During human toxoplasmosis, CD40/CD40L interactions are necessary to promote optimal production of IFN-γ and class switched antibody [12,198]. In contrast, these interactions are not critical for production of IFN-γ in the murine model, yet mice deficient in CD40L display increased susceptibility during chronic infection [91]. While CD40L can act synergistically with IFN-γ to inhibit parasite replication, there is also evidence that CD40L can act independently of IFN-γ [91–93,95]. One IFN-γ independent mechanism by which CD40L controls infection is through the induction of xenophagic killing of the parasite, which has been shown to be independent of the p47 GTPase family, but dependent upon the autophagic molecule Beclin-1 [93–95]. Beclin-1-heterozygous mice also demonstrate increased susceptibility to *T. gondii* infection, indicating that CD40-mediated xenophagy may be a unique and critical mechanism for controlling chronic toxoplasmosis.

Lymphotoxin alpha (LT-α) is another member of the TNF family essential for immunity to *T. gondii*. Like TNF and TNFR KO mice, LT-α KO mice succumb to this infection within the first 4 weeks, associated with a high parasite burden [154]. These mice display functional but delayed IFN-γ responses and antibody titers, and decreased expression of iNOS. These defects may conceivably result from a critical role for LT-α in signaling to directly promote effector functions, or they may be a secondary consequence of the defective splenic architecture observed in LT-α KO mice [199].

Thus, cytokines and the effector mechanisms they induce are able to control toxoplasmosis, allowing the parasite and the host to co-exist. Parasite virulence factors or immunodeficiency can disrupt this equilibrium, leading to severe disease or the death of the host. However, the immune effector mechanisms that control parasite burden can also bear a fitness cost upon the host, as will de described in the following section.

Severe immunopathology and the mechanisms that prevent it

As is true for many infections, maintaining immune homeostasis during toxoplasmosis requires not only the ability to limit the replication of the pathogen, but also the ability to control the host immune response. In WT mice this is illustrated by severe infection-induced inflammation in the gut and central nervous system that is mediated by $CD4^+$ T cells. This section will review the factors that contribute to these pathologic events and the mechanisms by which they are controlled.

Intestinal Ileitis following infection with *T. gondii*

Immunopathology can occur in the ileum following oral infection with *T. gondii* in mice and other species, and this ileitis has been proposed as a model to understand the basis for immune-mediated gastrointestinal disease in humans [200,201]. The infection-induced ileitis is characterized by the development of severe necrosis and inflammatory foci, and is dependent upon the host's sex and genetic background [200,202]. That this process is immune mediated is demonstrated by studies in which C57B/6 mice lacking $CD4^+$ T cells or mice depleted of $CD4^+$ T cells fail to develop this phenotype [200]. Development of the ileitis is a complex process involving numerous cell types, including intraepithelial lymphocytes, natural killer T cells, and NK cells [203–206]. Factors that promote T cell responses, such as CD40/CD40L interactions and the cytokines IL-12 and IL-23 also contribute to ileitis development [207–209]. Other cytokines, such as IFN-γ, TNF-α, IL-18, IL-22, and the macrophage migration inhibitory factor (MIF) have also been implicated in mediating pathology [208–211]. The Th2 cytokines IL-4 and IL-5 have also been implicated in ileitis development, although another report found IL-4-deficient mice to be more susceptible to oral challenge [212–214].

As work on this model has progressed, it has become clear that the commensal bacteria present in the gut contribute to the development of this infection-induced ileitis [215]. Recent findings have led to a model in which oral challenge with *T. gondii* results in a dramatic increase in the quantity of Gram-negative bacteria in the gut flora and bacterial translocation to subepithelial tissues [215] where TLR4 senses these bacteria and amplifies the local inflammation [216]. Additionally, mice deficient in TLR11, which binds to profilin expressed by the parasite, do not develop ileitis, suggesting that the innate response to *T. gondii* also contributes to this process [41]. While these findings implicate both parasitic and bacterial antigens in stimulating the pathologic immune response, it remains to be determined for which antigens the $CD4^+$ T cells that mediate ileitis are specific.

$CD4^+$ T cell mediated immunopathology during Toxoplasmic encephalitis

Another example of severe pathology occurs in mouse models of chronic toxoplasmic encephalitis. Although $CD4^+$ T cells are essential for long-term resistance to *T. gondii*, they also can induce severe pathology in the central nervous system. Thus, in susceptible mice large numbers of $CD4^+$ T cells are present in the brain during the chronic encephalitis, and depletion of $CD4^+$ T cells can ameliorate pathology without affecting parasite burden [217]. Similarly, CD28 KO mice infected with *T. gondii* have normal parasite burdens but exhibit enhanced resistance to TE that correlates with decreased numbers of $CD4^+$ T cells in the brain [218]. This work contrasts with studies in which depletion of $CD4^+$ T cells was sufficient to reactivate disease [219]. These seemingly conflicting results may be partially explained by differences in depletion efficiency, as complete depletion of $CD4^+$ T cells in the central nervous system can be difficult to obtain, and is not necessarily reflected by the depletion efficiency in other tissue sites [220]. Thus, while $CD4^+$ T cells are required for control of infection, partial inhibition of $CD4^+$ T cell responses may be beneficial to the host in the context of chronic toxoplasmic encephalitis.

IL-10 inhibits CD4$^+$ T cell mediated immunopathology

Since 1996, the use of various knockout mice has led to the identification of factors critical for limiting the development of immune pathology during toxoplasmosis. These studies have provided a novel insight into the nature of host-pathogen interactions, which is perhaps best illustrated by studies in which mice deficient in the cytokine IL-10 (IL-10 KO) were challenged with *T. gondii* [221]. IL-10 is produced by a number of cell types, including macrophages, NK cells, T cells and B cells, and functions by inhibiting the activation of accessory cells and adaptive immune responses [222]. The central role for IL-10 in limiting inflammation was confirmed by the finding that IL-10 KO mice develop spontaneous colitis [223]. However, IL-10 is also a potent antagonist of the ability of macrophages to kill intracellular bacteria and parasites, such as *T. gondii*, and infection with a number of pathogens, including *T. gondii*, increases expression of IL-10 [189,224–227]. These findings led to the idea that pathogens induce IL-10 production as a means to evade the immune response [227]. However, challenge of IL-10 KO mice with *T. gondii* revealed that these mice display normal parasite burdens, but develop severe liver damage, increased production of pro-inflammatory cytokines, and succumb to a CD4$^+$ T cell mediated hyper-inflammatory response [221]. These results provide one of the first examples of an infection in which the host must control its own immune response and tolerate pathogen persistence in order to survive.

While IL-10 is clearly critical for survival during the acute stage of infection, studies analyzing the role of IL-10 during toxoplasmic encephalitis have yielded more ambiguous results. There is general agreement that IL-10 expression is upregulated in the brain during chronic toxoplasmosis, and macrophages and CD4$^+$ T cells represent a local source of IL-10 in the central nervous system [220,228]. One study observed that neutralization of IL-10 during chronic toxoplasmosis was not lethal to infected mice, and resulted in decreased parasite burden [228]. In contrast, another study reported that blocking of the IL-10R resulted in decreased survival of chronically infected mice [229]. Similarly, in a system that allowed IL-10 KO mice to survive acute infection, the chronically infected IL-10 KO mice displayed normal parasite burden but also developed severe immunopathology mediated by CD4$^+$ T cells associated with increased production of pro-inflammatory cytokines [220]. Together these results suggest that while IL-10 may partially inhibit effector mechanisms that could otherwise reduce parasite burden, it is crucial in the acute and chronic stage of infection to prevent severe immunopathology.

Attempts to identify the cellular sources of IL-10 during toxoplasmosis have revealed that there are multiple contributors, including macrophages [228], NK cells [75], CD4$^+$ T cells [228,229] and CD8$^+$ T cells [228]. Innate sources of IL-10 are significant during toxoplasmosis, as the loss of IL-10 expression from SCID mice can dramatically extend their survival [230], and NK cells are regarded as a major innate source of IL-10 during the acute stage of infection [75]. Nevertheless, the use of a cre-flox system to selectively eliminate IL-10 production from CD4$^+$ and CD8$^+$ T cells revealed that these mice still develop severe immunopathology upon infection [231], indicating that non-T cell sources of IL-10 were not sufficient to limit pathology. Subsequent work has demonstrated that depletion of CD4$^+$ T cells dramatically decreases expression of IL-10, and that the CD4$^+$ T cells that produce IL-10 express the transcription factor T-bet and low levels of CD25, suggesting that they are effector T cells as opposed to regulatory T cells [229].

IL-27 inhibits CD4$^+$ T cell mediated immunopathology

IL-27 is another cytokine critical for regulating CD4$^+$ T cell responses during toxoplasmosis. Initial reports concluded that the IL-27 receptor was important in promoting Th1 immune responses [232], however when mice deficient in WSX1, a component of the

IL-27 receptor, were infected with *T. gondii*, these mice were found to exhibit enhanced Th1 responses and dramatically increased susceptibility to this challenge [233]. Similar to the IL-10 KO mice, infected WSX1 KO mice have normal parasite burdens but develop severe liver and lung pathology, and increased numbers of activated $CD4^+$ and $CD8^+$ T cells. Furthermore, depletion of $CD4^+$ T cells prevents the infection-induced pathology. Although initial studies indicated that the suppressive effects of IL-27 were independent of IL-10 [233], subsequent work has demonstrated that IL-27 promotes IL-10 expression [234]. However, this property of IL-27 does not fully explain its suppressive activities. IL-27 can also act directly on $CD4^+$ T cells to inhibit production of IL-2, a growth and survival factor for T cells, and neutralization of IL-2 results in enhanced survival of WSX1-KO mice [233]. Another recent study found that deficiency in EBI3, a second component of the IL-27 receptor, correlated with decreased expression of the inhibitory molecule PD-L1 on $CD4^+$ T cells during toxoplasmosis, providing another potential mechanism by which IL-27 may mediate its protective effects [235].

Although WSX1 KO mice normally do not survive to chronic infection, treatment with anti-toxoplasma drugs or immune blockade can prevent acute susceptibility, allowing the role of IL-27 during chronic infection to be examined [236]. Under these circumstances, WSX1 KO mice show no defect in their ability to control parasite burden but do develop severe immunopathology in the central nervous system, which correlated with decreased production of IL-10 and increased production of IL-17 [234,236]. These findings led to the recognition that IL-27 can directly inhibit production of IL-17 from Th17 cells [236]. These results collectively support a model in which IL-27 controls immunopathology during toxoplasmosis by inhibiting multiple facets of T cell activation, and the principles established using *T. gondii* have been shown to be relevant to the immunosuppressive effects of IL-27 in a variety of systems, including infections with other intracellular parasites [237,238], helminth infection [239], bacterial infection [240, 241], and numerous autoimmune models [242–247].

The role of Ahr and Lipoxin in controlling immunopathology

Another factor that contributes to the control of chronic toxoplasma infection is Lipoxin A4 (LXA4), a product of the reaction catalyzed by the enzyme 5-lipoxygenase (5LO). Mice deficient in 5LO succumb to toxoplasmosis approximately one month post infection, having reduced parasite burdens relative to WT controls [248]. 5LO- deficiency is associated with increased infiltration of inflammatory cells into the brain, and elevated production of the cytokines IL-12, IFN-γ, and TNF-α. Expression of LXA4 is induced by infection with *T. gondii* and administration of an LXA4 analogue can rescue 5LO-deficient mice, implicating this molecule as a negative regulator of inflammation. One known mechanism by which LXA4 can inhibit immune responses is by serving as a ligand for the aryl hydrocarbon receptor (Ahr) [249]. Ahr is an intracellular signaling molecule that translocates to the nucleus upon binding to its ligand [250]. Ligation of Ahr by LXA4 inhibits the production of IL-12 from DCs in a manner dependent upon the signaling molecule Suppresser of Cytokine Signaling 2 (SOCS2), which is also required for survival during toxoplasmosis [251,252]. Consistent with this mechanism as a means to control the immune response during toxoplasmic encephalitis, mice deficient in Ahr have reduced parasite burdens, but succumb to chronic infection with *T. gondii* [253].

Other factors involved in controlling immune pathology

TLR11 is another factor necessary to control the immune response during acute toxoplasmosis [254]. In the absence of TLR11, mice develop fat necrosis and pancreatic inflammation, which does not correlate with increased parasite burden in the pancreas. This inflammation is mediated by IL-12, IFN-γ and NK cells, but is independent of T

lymphocytes. The cytokines IL-18 and IL-1β are also partially responsible for the development of pancreatic inflammation, as neutralization of either of these cytokines decreases inflammation. These results raise the question as to what anti-inflammatory mechanisms may be initiated by TLR11 ligation. Given the prominent role of IL-12 in eliciting production of IL-10 from NK cells [75], it may be that decreased IL-12 levels in the absence of TLR11 result in insufficient amounts of IL-10 to prevent pancreatic pathology, although additional experiments are required to test this model.

Another pathway involved in protection of mice against severe immunopathology during acute toxoplasmosis involves the cleavage of fibrinogen to produce fibrin, as part of the cascade responsible for blood clotting [255]. Fibrin levels are increased upon infection with *T. gondii* in a TNF-α dependent manner, while IFN-γ negatively regulates these events [256]. Mice lacking fibrin exhibit normal control of parasite burden and intact immune responses, but succumb to infection within the first 15 days, associated with an IFN-γ dependent liver pathology characterized by necrosis, hemorrhaging, and diffuse inflammatory infiltrates [257]. Thus, fibrin prevents hemorrhaging and severe pathology during toxoplasmosis.

Concluding remarks and future perspectives

Since the discovery that *T. gondii* infects humans 75 years ago, there have been many advancements in understanding the mechanisms by which this parasite establishes persistent infection and how the host's immune system can control it. Additionally, the study of immunity to *T. gondii* has advanced our understanding of basic immunology, host-pathogen interactions, and provided a model system to study other inflammatory conditions. Despite these advances, there are many fundamental questions that remain unanswered, as highlighted throughout this review. The identification of parasite virulence factors shows great promise in providing insights into the pathogenesis of this infection. These studies have led to the identification of a role for ATF6β in immunity to *T. gondii*, and highlighted the potential role of GBPs. The mechanisms by which other parasite factors, such as ROP5, mediate virulence remain unknown [258], but determining their mechanisms of action may elicit the identification of novel components of the host immune response.

There are also many open questions about the mechanisms by which antigen is presented to T cells following infection with *T. gondii*. Recently, protective $CD8^+$ T cell responses were found to be induced in mice vaccinated with a replication-deficient strain of *T. gondii* [133,145,259]. Understanding the fate of these live attenuated parasites, and the mechanisms by which host cells acquire and present antigens from them may provide insights for designing efficacious vaccines for humans that elicit T cell mediated memory– a long-standing goal for vaccine design that has remained elusive [260].

The advent of two-photon imaging technology has had a profound impact on the study of immunology by enabling researchers to visualize the interactions of immune populations as they occur *in vivo* or *in situ*. Most recently, this technology was utilized to model the migratory patterns of $CD8^+$ T cells in the brains of mice chronically infected with *T. gondii* [261]. This study found that $CD8^+$ T cells use a migration pattern that is conserved among other predatory animals such as sharks, which maximizes their searching efficiency. This approach provides a foundation for other studies examining the migration patterns of other cell types in other tissue locations. An improved understanding of the migration patterns of effector cells and their interactions with infected cells may provide insight into how these cells can efficiently eliminate parasites while minimizing damage to the host, or how parasites manage to evade destruction. A recurring theme throughout this review has been the ability of $CD4^+$ T cells to mediate severe immunopathology through the production of

inflammatory cytokines. However, relatively little is known about the downstream effector mechanisms involved in this process. Nitric oxide has been implicated in the pathology mediated in the gut following infection, and could potentially contribute to the pathology that occurs in the absence of IL-10 or IL-27. Further insight may by provided by imaging the $CD4^+$ T cells in the context of immunopathology. Such studies have the potential to greatly advance our understanding of host-pathogen interactions and the development of immunopathology.

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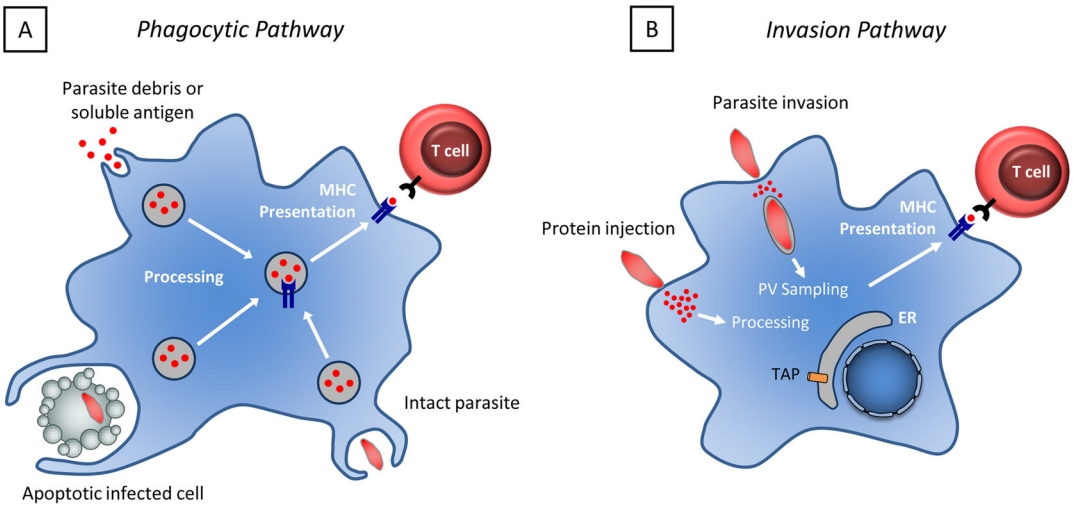

Figure 1. Potential antigen presentation pathways

A. Antigen may be acquired through the phagocytosis of infected cells, intact parasites, parasite antigens, or through the endocytosis of parasite debris. **B.** Antigen may also be acquired by infected cells through the release of soluble antigens from the parasite, or through the sampling of antigens from the parasitophorous vacuole, which may be mediated by fusion of the parasitopherous vacuole with the endoplasmic reticulum. Antigens may also be injected into the host cell through abortive invasion events.

Table

Cytokines necessary for survival during toxoplasmosis

Cytokines	Sources	Phenotype of knockout	Functions
• IL-12	• Dendritic cells [32, 46, 48] • Neutrophils [43,45,49] • Inflammatory monocytes [27, 45] • Macrophages [27,45,221]	• Succumb within 10 days of infection [34, 262] • Inability to control parasite burden [34, 262]	• Promotes T cell proliferation and differentiation [34, 101] • Promotes Natural Killer cell responses [33, 35] • Promotes IFN-γ production [33, 34] • Promotes IL-10 expression from natural killer cells [75]
• IFN-γ	• Natural killer cells [70–72, 35] • $CD4^+$ T cells [90] • $CD8^+$ T cells [90]	• Succumb within 10 days of infection [36] • Inability to control parasite burden [36]	• Promotes iNOS expression [156] • Promotes p47 GTPase-mediated killing of *T. gondii* [166,167] • Promotes Tryptophan degradation [181–184]
• TNF-α	• Neutrophils [43, 190] • Dendritic cells [190] • Macrophages [191] • Microglia [192] • T cells [193]	• Succumb ~3–4 weeks post-infection [152–154] • Inability to control parasite burden [152– 154]	• Promotes macrophage activation [194] • Promotes control of parasite in non-hematopoietic cells [195] • Promotes iNOS expression [152,154,158,189]
• IL-6	• Monocytes [263] • Astroglia [264] • Stromal cells [265] • Retinal pigment epithelial cells [266]	• Increased susceptibility 2–4 weeks post-infection [267] • Increased parasite burden [267, 268]	• Necessary for optimal neutrophil responses [267] • Necessary for optimal IFN-γ responses [267, 268]
• LT- α	• Lymphocytes [269]	• Succumb 2–4 weeks post- infection [154]	• Necessary for normal secondary lymphoid architecture [199] • Necessary for optimal antibody and IFN-γ

Cytokines	Sources	Phenotype of knockout	Functions
			responses early during infection [154] • Necessary for optimal expression of iNOS [152]
• IL-10	• Natural killer cells [75] • Macrophages [228] • $CD4^+$ T cells [229] • $CD8^+$ T cells [228]	• Succumb 1–2 weeks post- infection [221] • Severe immunopathology [221]	• Inhibits CD4+ T cell-mediated pathology [221]
• IL-27	• Antigen Presenting Cells [270]	• Succumb within 15 days post-infection [233] • Severe immunopathology [233]	• Inhibits IL-17 production [236] • Inhibits IL-2 production [233] • Promotes IL-10 production [234] • Promotes PD-L1 expression [235]
• CD40L (Surface protein)	• Expressed on T cells [271]	• Succumb 30–80 days post- infection [91] • Inability to control parasite burden [91]	• Promotes Th1 responses in humans [198] • Promotes iNOS expression [162] • Promotes xenophagic killing of *T. gondii* [95]