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Review

# CD8$^+$ T Cell Responses to *Toxoplasma gondii*: Lessons from a Successful Parasite

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***Toxoplasma gondii* infection in mice provides an excellent model for the study of CD8$^+$ T cell responses. Natural and engineered *T. gondii* antigens have led the way to understanding the factors regulating antigen presentation from vacuolar pathogens. *T. gondii* infection of resistant and sensitive mouse strains provides unique models to study both effective CD8$^+$ T cell function and protection in a well-controlled infection attributed to a novel T cell population, and T cell exhaustion in a progressing chronic infection. Additionally, the long-term persistence of the parasite in the brain provides a unique model of neurotropic infection used to study CD8$^+$ T cell entry, retention, and function in the brain. Here we discuss recent advances in each of these areas.**

## Highlights

Investigation into how antigens from the vacuolar pathogen *T. gondii* access the MHC-I antigen presentation reveals distinct pathways, depending on the mode of antigen secretion and intracellular trafficking.

*T. gondii* infection in mice provides a unique contrast of MHC-linked susceptibility versus protection against infection.

Studies in a susceptible mouse strain reveal roles for CD4$^+$ T cells and cytokines in preventing CD8$^+$ T cell dysfunction.

Studies in a resistant mouse strain reveal the cellular mechanisms that maintain continuous production of functional CD8$^+$ T cell effector cells.

Factors that regulate T cell entry, retention, and function in the brain during infection have been elucidated using the *T. gondii* model.

## CD8$^+$ T Cell Responses to *T. gondii*

The protozoan intracellular parasite, *Toxoplasma gondii*, is a highly successful pathogen. It is found world-wide in a variety of birds and mammals, including an estimated 20–50% of humans. Its success is largely achieved by the parasite's ability to establish a stable balance with the host immune response, thus allowing the parasite to rapidly spread through the body and establish persistent infection, while also maintaining the viability of the host. A key part of this balance involves CD8$^+$ T cells, the primary cell type that controls the parasite in the mammalian adaptive immune response.

Experimental *T. gondii* infection of mice (a natural host) provides an outstanding model for understanding CD8$^+$ T cell responses. Propagating the parasite in the laboratory is relatively safe and easy, and a large variety of genetically engineered parasite strains are available. The parasite spreads systemically, allowing for the study of CD8$^+$ T cell responses in a variety of lymphoid and nonlymphoid tissues, including the brain, the primary site of long-term persistence. Finally, the CD8$^+$ T cell response differs dramatically in different inbred mouse strains, leading to differential control of the parasite during chronic infection, and providing an opportunity to explore the factors that determine CD8$^+$ T cell efficacy.

In this review, we summarize recent progress in using the mouse–*T. gondii* infection model to uncover various aspects of CD8$^+$ T cell responses, including antigen presentation and effector T cell differentiation.

## How CD8$^+$ T Cells Detect Vacuolar Pathogens

In many viral and intracellular bacterial infections, pathogen-derived antigens are present in the host cell cytosol and can be processed and presented to CD8$^+$ T cells via the classical **major histocompatibility complex class I (MHC-I)** (see Glossary) presentation pathway. However, vacuolar pathogens such as *T. gondii* pose particular challenges for MHC-I presentation, since their antigens do not have access to the host cytosol. Nevertheless, *T. gondii* infection elicits a robust and protective CD8$^+$ T cell response. That fact, together with a collection of several natural antigens that have been identified (Table 1), and the ability to generate parasites expressing engineered antigens (Table 2), makes *T. gondii* a valuable model for the study of CD8$^+$ T cell responses to vacuolar pathogens.

Although in some settings inert, phagocytosed antigens can be **cross-presented** by certain **antigen-presenting cells (APCs)** [1], presentation of *T. gondii* antigens requires interactions of live parasites with APCs [2,3]. Moreover, presentation of *T. gondii* antigens is strongly affected by their mode of secretion from the parasite into host cells, and their location and membrane topology within the host cell (Table 1). For example, constitutive secretion via organelles termed **dense granules** favors

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| Antigenic peptide | Source protein | MHC restriction | Subcellular location | T cell response | Refs |
|---|---|---|---|---|---|
| HPGSVNEFDF (HF10) | GRA6 | H-2L$^{d}$ | Constitutively secreted by dense granules in PV; localizes to PV membrane; C terminal epitope may extend into cytosol | Immunodominant response; responsible for protection in BALB/c mice | [9,11,12,54] |
| SPMNGGYYM (SM9) | GRA4 | H-2L$^{d}$ | Constitutively secreted by dense granules into PV lumen; localizes to **intravacuolar network (IVN)** | Subdominant response; not protective | [10,18] |
| YAVANYFFL (YAL9) | ROP5 | H-2D$^{b}$ | Secreted by rhoptries into host cell during invasion | Subdominant response; not protective | [19] |
| IPAAAGRFF (IF9) | ROP7 | H-2L$^{d}$ | Secreted by rhoptries into host cell during invasion | Subdominant response; not protective | [10,18] |
| SVLAFRRL (SVL8) | Tgd057 | H-2K$^{b}$ | Secreted by dense granules in PV lumen | High frequency of Tgd057-specific T cells post vaccination; adoptive transfer of CTLs protective against lethal parasite challenge | [11,41,71] |

**Table 1. Natural CD8$^{+}$ T Cell Epitopes in *Toxoplasma gondii***

antigen presentation, whereas retention of the antigen within the parasite prevents presentation [2,4,5]. In addition, soluble antigens that are secreted into the **parasitophorous vacuole (PV)** lumen appear to enter the MHC-I presentation pathway via a Sec22b-dependent endoplasmic reticulum (ER) to Golgi intermediate compartment (ERGIC) pathway [1,6,7], and are processed in a proteasome/transporter associated with antigen processing (TAP)-dependent manner [2,8] (Figure 1). In contrast, the immunodominant GRA6 antigen, which is also secreted via dense granules, does not require Sec22b [9]. After secretion, the GRA6 protein associates with the PV membrane (PVM) with its C terminal epitope protruding into the host cytosol, and this orientation favors efficient presentation [9–11] (Figure 1). Presentation of the HF10-GRA6 epitope has been shown to require components of the classical MHC-I pathway such as the proteasome, TAP, and ER aminopeptidase associated with antigen processing (ERAAP) [12]; however, it is unknown how the epitope is released from the PVM to access this pathway. It has been hypothesized that an unidentified protease may cleave the C terminal epitope which protrudes into the host cell cytosol. Interestingly, some host proteins involved in cross-presentation, such as Rab22a and Sec61, associate directly with the PV [6,13], suggesting that there may be direct access of parasite antigens to the cross-presentation pathway. On the other hand, for some antigens, access to the cytosol may be accomplished through damage to the PVM by host defense mechanisms such as **immunity-related GTPases (IRGs)** [14,15].

In addition to constitutive secretion via dense granules, *T. gondii* also possesses a distinct secretory pathway via organelles termed **rhoptries**. Rhoptries directly discharge their contents into host cells during the invasion process, and during abortive invasion events [16,17]. This model of secretion might be expected to favor MHC-I presentation, since the antigens have direct access to the host cytosol and the classic MHC-I presentation machinery (Figure 1). However, rhoptry-targeted antigens

## Glossary

**Antigen-presenting cell (APC)**: a cell that displays pathogen-derived peptides bound to MHC for T cell recognition.
**B lymphocyte-induced maturation protein-1 (Blimp-1)**: a transcriptional repressor that regulates T cell effector differentiation, expressed in exhausted T cells.
**Blood–brain barrier**: a layer of endothelial cells that separates the CNS from the blood; it restricts migration of pathogens, and it becomes leaky during inflammation.
**Bradyzoites**: slow-growing stage of the parasite contained in tissue cysts.
**Checkpoint blockade**: antibody-mediated neutralization of inhibitory receptors, mainly PD-1; it can boost T cell responses by reversing the effects of inhibitory receptors.
**Cross-presentation**: a process whereby exogenous antigens are taken up by an APC and presented via MHC-I.
**CXCR3**: a receptor for the chemokines CXCL9 and CXCL10; it is expressed on activated CD8$^{+}$ T cells, and it controls effector CD8$^{+}$ T cell differentiation and migration.
**Cysts**: structures containing bradyzoites that form in the brain of infected hosts during chronic infection.
**Dense granules**: constitutive secretory organelles; they release contents into the PV lumen in invaded cells.
**Exhaustion**: a process observed in chronic infections whereby T cells lose their effector capabilities over time due to persistent exposure to antigen or inflammation.
**Immunity-related GTPases (IRGs)**: interferon-inducible GTPases that play a role in resistance against intracellular pathogens; in *T. gondii* infection, IRGs accumulate at the PV and destroy the vacuole, releasing parasites into the host cell cytosol.
**Interferon-γ (IFNγ)**: a pleiotropic proinflammatory cytokine critical for *T. gondii* control.
**Interleukin-12 (IL-12)**: an early proinflammatory cytokine produced by APCs that promotes CD4$^{+}$ Th1 and CD8$^{+}$ T cell responses.

| Antigen | Description | Effect on antigen presentation and T cell response | Refs | Conclusion |
|---|---|---|---|---|
| SAG1ΔGPI-OVA | Model antigen OVA secreted by dense granules | OVA secreted into the PV results in efficient presentation; low $CD8^+$ T cell activation with nonsecreted OVA | [2,5] | Secreted antigen > nonsecreted antigen |
| Cytoplasmic β-galactosid-ase | Cytoplasmic expression of β-galactosidase under the tachyzoite-specific promoter, SAG1 | No β-gal-specific $CD8^+$ T cell response | [4] | |
| Secreted β-galactosid-ase | β-galactosidase secreted into the PV lumen | Production of long-lasting dominant response against $L^d$ restricted β-gal epitope | [4] | |
| GRA6-YAL9 | YAL9 peptide targeted to dense granules instead of rhoptries | Increased presentation, higher $CD8^+$ T cell response | [19] | Dense granule targeting > rhoptry targeting |
| GRA6-ROP5 | ROP5 protein targeted to dense granules instead of rhoptries | Increased presentation, 10- to 20-fold higher $CD8^+$ T cell response (more than GRA6-YAL9); better parasite control | [19] | |
| ROP5-HF10 | HF10 peptide targeted to rhoptries instead of dense granules | Decreased presentation, 3- to 4-fold lower $CD8^+$ T cell response | [19] | |
| GRA6II-L, GRA6II-HA | GRA6 protein extended by a leucine or HA tag | HF10 presentation is lost; no T cell response; weaker protection | [10] | C terminal epitope position is important |
| GRA6II-SM9Cter | SM9 peptide at C terminus of GRA6 protein | Enhanced presentation; stronger $CD8^+$ T cell response; stronger parasite control | [10] | |
| TgRH GRA5-HF10 | HF10 epitope at C terminus of GRA5 protein: HF10 inverted to face the PV lumen | Reduced presentation *in vitro* | [9] | Host cell cytosolic epitope > PV lumenal epitope |
| SAG1-GRA6-HF10 | SAG1 N terminal domain with GRA6-HF10: converts to soluble protein | Reduced presentation | [11] | PV membrane localization > soluble within PV lumen |
| TgRH GRA6-HF10 gra2KO | GRA2 KO eliminates the IVN; GRA6 preferentially localizes to PV membrane | Enhanced presentation and $CD8^+$ T cell response | [11] | |

**Table 2. Engineered CD8 T Cell Antigens**

*(Continued on next page)*

**Intravacuolar network (IVN)**: a network of membrane tubules and vesicles generated by the parasites within the vacuolar space.
**Killer cell lectin-like receptor subfamily G member 1 (KLRG1)**: a surface receptor expressed by effector $CD8^+$ T cells.
**Major histocompatibility complex (MHC) molecules**: cell surface proteins that bind and present antigens to T cells; MHC class I presents antigens to $CD8^+$ T cells.
**Ovalbumin (OVA)**: a protein used as a model antigen for T cell studies.
**Parasitophorous vacuole (PV)**: a structure produced by apicomplexan parasites after invasion of a host cell; it protects the parasite from host cell phagolysosomes.
**Programmed cell death protein 1 (PD-1)**: an inhibitory receptor that suppresses T cell responses to avoid overactivation of the immune system; it is a key surface marker for exhausted T cells.
**Rhoptries**: secretory organelles at the apical pole of the parasite, secreted upon invasion of host cells or during abortive invasion events.
**Tachyzoites**: a rapidly dividing stage of the parasite.
**Tissue resident memory T cells ($T_{RM}$)**: a distinct lineage of cells that confer protection directly in relevant tissues rather than within the lymphatic system and do not recirculate; they are identified by CD69 and CD103 surface expression.
**Toxoplasmic encephalitis (TE)**: a severe and often lethal outcome of *T. gondii* infection characterized by inflammation in the CNS.

| Antigen | Description | Effect on antigen presentation and T cell response | Refs | Conclusion |
|---|---|---|---|---|
| Tg.pGRA6/GRA6-OVA | OVA-derived SIINFEKL epitope at the C terminus of the GRA6 protein, under the GRA6 promoter | Antigen processed more efficiently than vacuolar SIINFEKL epitope from parental strain; lower parasite load in the brain; reduced brain inflammation | [68] | Dense granule targeting > soluble vacuolar localization |
| SAG1/GRA6-OVA | Same protein construct as above, but restricted to tachyzoites by SAG1 promoter | OVA-specific $CD8^+$ T cell response not significantly affected; effective parasite control in the CNS | [68] | Tachyzoite restricted antigen presentation is sufficient to confer protection |

**Table 2. *Continued***

are relatively weak antigens [18], and antigenicity can be improved by retargeting a rhoptry epitope for dense granule secretion [19] (Table 2). This may reflect the transient nature of rhoptry secretion, and failure to accumulate sufficient parasite antigen in the cytosol.

## A Model for Effector $CD8^+$ T Cell Responses

Most of our knowledge about $CD8^+$ T cell responses to persistent infections comes from studies with lymphocytic choriomeningitis virus (LCMV), in which chronic infection and persistently high antigen load leads to functional impairment of T cells, or T cell **exhaustion** [20–22]. HIV, which is responsible for one of the most medically important chronic viral infections, also results in T cell exhaustion [23–25] but is difficult to study due to lack of an appropriate mouse model. Well-controlled viral infections can be complicated by factors such as periods of viral latency or the phenomena of epitope switching and 'memory inflation' observed in mouse cytomegalovirus (MCMV) infection [26,27]. Therefore, our understanding of the mechanisms that maintain effective control during infection with persistent pathogens is limited and could benefit from additional mouse models. Studies focusing on T cell responses during *T. gondii* infection have provided insights into T cell function and differentiation.

## Features of the $CD8^+$ Response to *T. gondii*

$CD8^+$ T cells are critical for effective control of many intracellular pathogens, including *T. gondii* [28]. Early work utilizing depletion or knockout studies demonstrated that $CD8^+$ T cells are the most protective adaptive immune cell type during infection [29–31]. **Interferon-γ (IFNγ)** has been identified as a key mediator of protection [29,32,33], and $CD8^+$ T cells, in addition to $CD4^+$ T cells, serve as important cytokine producers. $CD8^+$ T cells can also mediate lysis of infected cells, and recently direct lysis of parasites through granulysin secretion in the acute stage has been demonstrated as well [34].

The observation that BALB/c ($H\text{-}2^d$) mice are better at controlling *T. gondii* infection compared with C57BL/6 mice ($H\text{-}2^b$) led to this trait being mapped to the MHC-I $L^d$ gene in BALB/c mice [35]. We and our colleagues have shown that this is due to the induction of an immunodominant and protective $CD8^+$ T cell response directed against a peptide from the parasite protein GRA6 [12]. BALB/c mice are able to establish effective long-term control of the parasite, while C57BL/6 mice, which lack $L^d$ and therefore cannot present the GRA6 peptide, develop progressing disease. These two contrasting settings provide a unique opportunity to study the differentiation, exhaustion, and maintenance of $CD8^+$ T cell responses to infection in the context of a well-controlled infection versus a poorly controlled progressing infection.

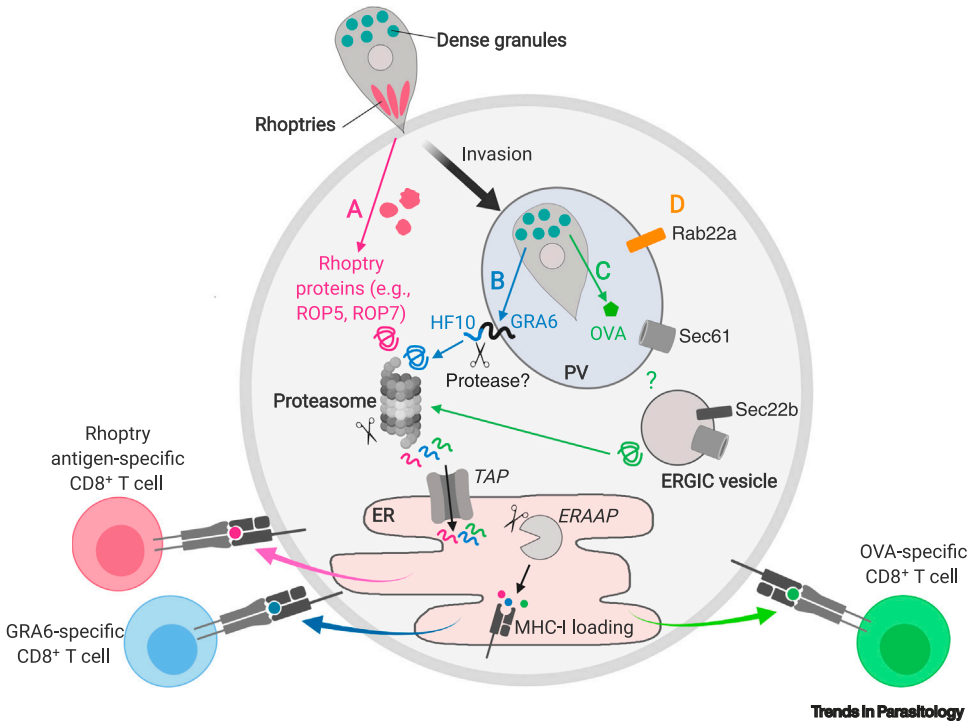


**Figure 1. Modes of MHC-I Presentation of Vacuolar Antigens.**

Distinct pathways of MHC-I antigen processing and presentation are shown for different *T. gondii* antigens. (A) During host cell invasion or abortive invasion events, parasites secrete the contents of their rhoptries into the host cell cytosol, allowing rhoptry proteins to access the classical MHC-I presentation pathway. In the classical MHC-I pathway, antigens are cleaved by the proteasome and transported to the endoplasmic reticulum (ER) for further processing and loading onto MHC-I. MHC-I–peptide complexes are transported to the surface of the antigen-presenting cells (APCs) to be presented to $CD8^+$ T cells. (B,C) After invasion of the host cell, the parasite generates a parasitophorous vacuole (PV), and dense granules are constitutively secreted into the PV lumen. The dense granule antigen GRA6 (B) associates with the parasitophorous vacuole membrane (PVM), with the C terminal antigenic peptide protruding into the host cell cytosol. Processing of this peptide requires components of the classical MHC-I pathway. It has been hypothesized that a protease may cleave the C terminal epitope. Soluble dense granule proteins such as the model antigen ovalbumin (OVA) (C) secreted from transgenic parasites localize within the PV lumen. The antigen may be trafficked to the host cytosol following fusion of ER-Golgi intermediate compartment (ERGIC) vesicles with the PV, mediated by the Sec22b SNARE protein; however, this part of the pathway is not fully understood. (D) Additional proteins involved in cross-presentation such as Rab22a GTPase, which may play a role in recycling MHC-I molecules, accumulate on the PV in infected dendritic cells (DCs). Figure created with BioRender. Abbreviation: ERAAP, ER aminopeptidase associated with antigen processing.

## $CD8^+$ T Cell Differentiation during Acute Infection

During an immune response, naive pathogen-specific $CD8^+$ T cells differentiate into functionally heterogeneous populations characterized by distinct memory potential and effector functions [36]. This differentiation is regulated by various signals, including T cell receptor signaling, co-stimulatory signals, and cytokine signals received from APCs or other bystander cells. In particular, inflammatory cytokines such as **interleukin-12 (IL-12)** and type-I interferons have been shown to function as a 'signal 3', directly signaling activated $CD8^+$ T cells to promote proliferation and effector functions *in vitro* and *in vivo* [37–39].

The specific cytokines that drive early effector differentiation can vary between models, and studies examining $CD8^+$ T cell fate during acute *T. gondii* infection in C57BL/6 mice have identified a critical

role for IL-12 in this system. IL-12 knockout mice fail to generate large numbers of effector $CD8^+$ T cells and show reduced expression of **killer cell lectin-like receptor subfamily G member 1 (KLRG1)** [40,41], a widely used marker of effector differentiation. Coinfection of mice with *T. gondii* and an intestinal helminth can promote the type-II cytokine IL-4, which, together with IL-10, suppresses IL-12 responses and dampens effector $CD8^+$ differentiation [42]. IL-12 impacts the different stages of effector T cell differentiation both by signaling directly to the T cell or through activating other cell types to make IFNγ [43] (Figure 2, Key Figure).

As $CD8^+$ T cells differentiate during acute infection, their localization within lymphoid tissues changes. Shah *et al.* tracked the location of T cell populations in the spleen as they progressed through various stages of differentiation after vaccination with an attenuated parasite strain [43]. As cells matured from naïve to terminally differentiated effectors, they moved from the white pulp through the marginal zone into the red pulp. This migration into the red pulp appears to be regulated by the chemokine receptor **CXCR3** and its ligands CXCL9 and CXCL10, similar to the role of CXCR3 on $CD8^+$ T cells in other models [44,45]. This relationship between fate and location suggests that there may be environmental signals promoting distinct fates in these different regions.

## $CD8^+$ T Cells in Progressing Chronic Infection

Although C57BL/6 mice are able to mount effective $CD8^+$ T cell responses during acute infection, they are ultimately unable to control the parasite in chronic stages of infection, resulting in mouse mortality due to **toxoplasmic encephalitis (TE)**. In this setting, $CD8^+$ T cells upregulate the inhibitory receptor **programmed cell death protein 1 (PD-1)**, a marker of T cell exhaustion typically upregulated in chronic viral infections [46]. These $CD8^+$ T cells also lose effector functions, and become more susceptible to apoptosis [47]. *In vivo* blockade of the receptor for PD-1, PD-L1, referred to as **checkpoint blockade**, rescues $PD\text{-}1^+$ $CD8^+$ T cells, leading to improved $CD8^+$ T cell responses and prevention of TE-associated mortality [45]. However, the infection model may impact the efficacy of this treatment, as neutralization of inhibitory receptors failed to reverse $CD8^+$ T cell exhaustion and rescue infected mice after challenge with hypervirulent strains [48].

Recently, a progenitor-like $CD8^+$ T cell population that responds to checkpoint blockade in certain models of chronic infection and tumors has been identified and characterized [49–51]. These cells share some common features across models, the most prominent being expression of *Tcf7*/TCF1, a transcription factor associated with self-renewal capacity. It will be interesting to determine whether a similar progenitor-like $CD8^+$ T cell population is present in mice chronically infected by *T. gondii*, and whether its presence or absence correlates with the response of mice to checkpoint blockade.

$CD4^+$ T cell help plays a role in preventing or reversing $CD8^+$ T cell exhaustion during infection with *T. gondii* and other chronic viral pathogens. Expression of the transcription factor **B lymphocyte-induced maturation protein-1 (Blimp-1)** in $CD4^+$ T cells correlates with $CD4^+$ T cell exhaustion during *T. gondii* infection (Figure 2), and deletion of Blimp-1 restores function of these $CD4^+$ T cells [52]. Boosting $CD4^+$ T cell function by deletion of Blimp-1 or adoptive transfer of functional $CD4^+$ T cells into chronically infected mice can also restore $CD8^+$ T cell function after exhaustion [52]. IL-21 is a key cytokine produced by follicular helper $CD4^+$ T cells (Tfh) that has an important role in proper B cell activation. Interestingly, IL-21 also appears to be critical in maintaining $CD8^+$ T cell functionality during chronic *T. gondii* infection. Exhausted $CD4^+$ T cells lose IL-21 production but regain production after Blimp-1 ablation [52,53]. In addition, IL-21 knockout mice have impaired $CD8^+$ responses and reduced ability to control the parasite compared with wild-type mice [53].

## $CD8^+$ T Cells in a Well-Controlled Chronic Infection

Utilizing the unique model of parasite control in $H\text{-}2^d$-expressing mice allows for investigations of the factors regulating $CD8^+$ T cells in a well-controlled chronic infection and provides an interesting contrast to the $H\text{-}2^b$ model of susceptibility. $H\text{-}2^d$-linked protection is mediated by an immunodominant $CD8^+$ T cell response against the parasite protein GRA6 [12]. Investigations into the processing and presentation of the GRA6 epitope have advanced our understanding of MHC-I presentation (see

above), and this unique response has begun to provide key insights into novel features of $CD8^+$ T cell function as well. Unlike T cells in most chronic infections, GRA6-specific $CD8^+$ T cells do not upregulate PD-1 or other inhibitory receptors and retain cytotoxic capacity and cytokine production throughout chronic infection [54]. Investigating the exact mechanisms behind this may provide novel therapeutic targets to prevent or reverse T cell exhaustion.

The protective capacity of $CD8^+$ T cell responses against GRA6 have also been demonstrated by other groups. Sanecka *et al.* showed that GRA6-specific transnuclear $CD8^+$ T cells significantly reduced parasite burden during the acute phase of infection [55], while Sa *et al.* provided evidence that GRA6-specific $CD8^+$ T cells play a role in **cyst** removal during chronic infection [56].

The GRA6 response also develops a unique differentiation profile. GRA6-specific T cells maintain a balance between three functionally distinct populations defined by surface expression of CXCR3 and KLRG1 [54]. A long-lived memory population ($T_{MEM}$) gives rise to a highly proliferative intermediate population ($T_{INT}$) that allows the generation of a large number of terminally differentiated effectors ($T_{EFF}$). Modeling studies demonstrated the importance of this novel $T_{INT}$ population in rapidly producing new $T_{EFF}$ cells without depleting the $T_{MEM}$ population, and subdominant responses lacking this $T_{INT}$ population were not maintained well throughout the chronic stages of infection.

Certain similarities exist between the wave of effector T cell differentiation that occurs following *T. gondii* vaccination and the continuous production of effector T cells that occurs during well controlled *T. gondii* infection (Figure 2). In both settings, CXCR3 is expressed early but is subsequently lost as cells progress to terminal differentiation. KLRG1 expression is regulated inversely, with initial upregulation being observed during the intermediate stages of differentiation and high expression continuing through terminal differentiation. Both models also feature proliferation specifically within a $CXCR3^+$ $KLRG1^+$ population [43,54]. In light of these similarities it will be important to investigate the location of each GRA6-specific population within the spleen during chronic infection and the role of inflammatory cytokines like IL-12 and IFNγ.

## $CD8^+$ T Cell Responses in the Brain

During infection, *T. gondii* crosses the **blood–brain barrier** and establishes brain cysts that allow the parasite to persist into the chronic phase. Control of the parasite necessitates an ongoing immune response in the brain, providing a unique opportunity to study $CD8^+$ T cell entry, retention, and function in the brain.

### T Cell Entry into the Brain

T cell entry into the brain is a multistep process mediated by interactions with endothelial cells and dependent on an array of adhesion molecules and chemokine receptors [57]. *T. gondii* infection induces endothelial cells to express increased amounts of integrins and selectins like platelet endothelial cell adhesion molecule (PECAM-1), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion protein 1 (VCAM-1). These integrins appear to be critical for T cell entry, as antibody blocking of VLA-4, the ligand for VCAM-1, prevents entry [58]. Infection also increases the expression of the inflammatory chemokines CXCL9 and CXCL10 in the brain [59], which function as ligands for the chemokine receptor CXCR3. CXCR3 plays a critical role in T cell entry in the brain in other viral and parasitic infection models [60–62]. Antibody blockade of CXCL9 [63] or CXCL10 [59] during infection dramatically reduces the number of brain-infiltrating $CD8^+$ T cells, supporting a vital role of this axis during *T. gondii* infection as well. Together, these studies indicate that inflammation appears to be a key element regulating T cell infiltration into the brain. Anti-PDL1 treatment also results in increases in $CD3^+$ and $CD8^+$ cells in the brains of *T. gondii*-infected mice [64]. While the exact mechanisms remain unclear, it is possible that increased inflammation following checkpoint blockade mediates this increase in T cell infiltration in the brain.

## Key Figure

### CD8$^+$ T Cell Fate during *Toxoplasma gondii* Infection

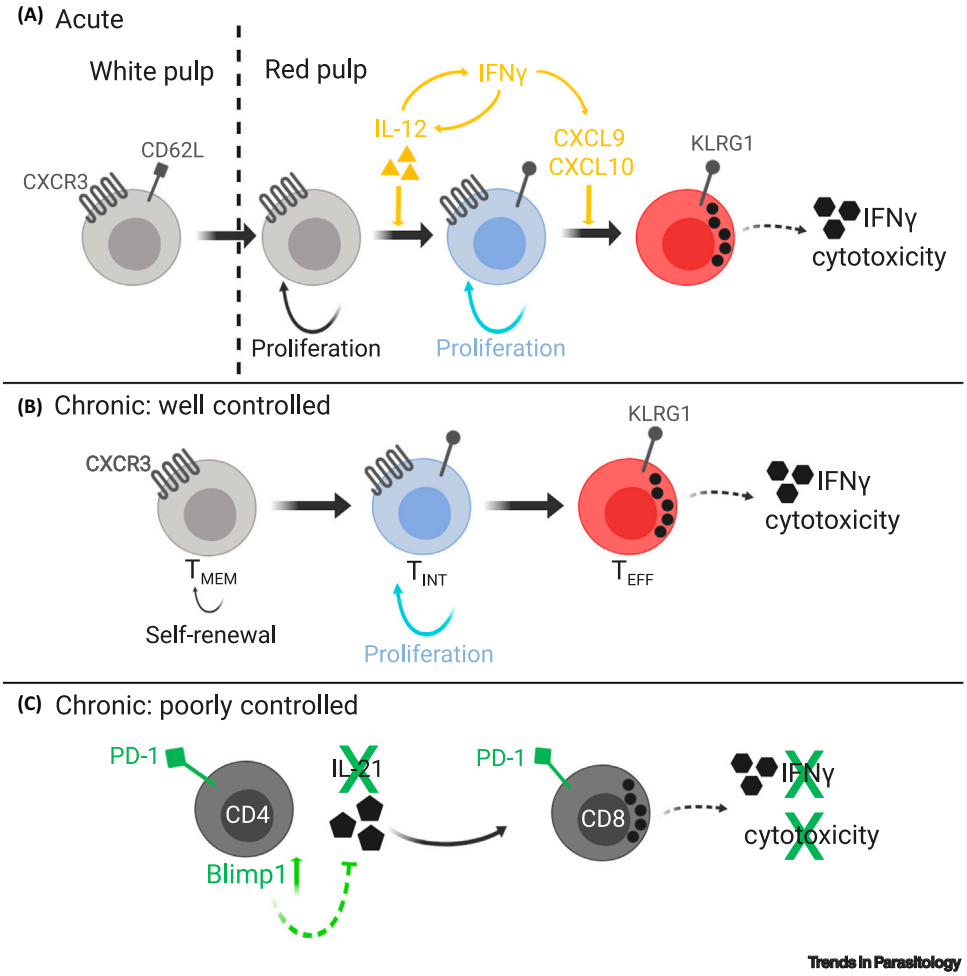


**Figure 2.** Features of CD8$^+$ T cell differentiation and function are shown for the different settings of *T. gondii* responses in mice. (A) During an acute response or after vaccination, CD8$^+$ T cell differentiation is linked to their migration and exposure to environmental signals. As naive cells are activated and differentiate into effector cells, they migrate from the white pulp of the spleen to the red pulp in a CXCR3:CXCL9/10-dependent manner. This migration results in exposure to a network of key cytokines like interleukin (IL)-12 and interferon (IFN)γ (yellow) that drive changes in CXCR3 and killer cell lectin-like receptor subfamily G member 1 (KLRG1) expression (gray) and promote effector differentiation [43]. (B) CD8$^+$ T cells during well-controlled chronic infection give rise to three functionally distinct subsets of antigen-specific T cells. The differentiation of these populations shares many similarities to acute infection, including the expression pattern of surface receptors CXCR3 and KLRG1 on different subsets (gray) and a proliferative intermediate population (blue) that generates terminally differentiated effectors (red). However, unlike the vaccine setting, in which a single wave of T cell effector differentiation occurs, during well-controlled chronic infection, effector T cells are continuously replenished from memory and intermediate precursor populations. (C) During infection, CD4$^+$ T cells provide help to CD8$^+$ T cells in the form of key cytokines like IL-21. In the setting of poorly controlled progressing infection (green), CD4$^+$ T cells become exhausted and lose their ability to produce these cytokines, resulting in subsequent CD8$^+$ T cell exhaustion. Figure created with BioRender. Abbreviations: Blimp 1, B lymphocyte-induced maturation protein-1; PD-1, programmed cell death protein 1; $T_{MEM}$, long-lived memory T cells; $T_{INT}$, highly proliferative intermediate T cells; $T_{EFF}$, terminally differentiated effector T cells.

## T Cell Retention in the Brain

While inflammatory signals are sufficient to mediate the initial entry of antigen-experienced T cells into the brain, only T cells with the appropriate antigen specificity are efficiently retained in the brain. **Ovalbumin (OVA)**-specific $CD8^+$ T cells activated *in vitro*, and then transferred into mice infected with *T. gondii*, were observed in the brain, despite the absence of the OVA epitope in this model [31]. While these OVA-specific T cells were capable of entering the brain, their numbers rapidly declined in this setting. T cells' affinity for their antigen may also regulate their retention. Two T cell clones with different affinities for the same epitope were shown to enter the brain in similar numbers, but the lower affinity clone exhibited more rapid contraction in the brain 5 weeks postinfection [65].

## T Cell-Mediated Protection in the Brain

While antigen recognition appears to promote T cell retention and effector function in the brain, the nature of the brain cells that present parasite antigens to T cells remains incompletely understood. Static imaging of brain sections revealed that parasite-specific T cells interacted with granuloma-like aggregates of $CD11b^+$ cells harboring parasite material [31]. In contrast, no interaction was observed between T cells and intact cysts [31], which were likely contained within neurons [66,67]. However, recent evidence suggests that neurons can present antigen directly to T cells, and that this presentation is critical for parasite control in the brain [68]. $L^d$-expressing mice with a neuron-specific $L^d$ knockout had dramatically increased numbers of brain cysts and increased amounts of parasite DNA despite high numbers of antigen-specific $CD8^+$ T cells. Restricting the expression of the immunodominant antigen specifically to the **tachyzoite** stage did not significantly reduce the $CD8^+$ T cell response, nor reduce their ability to protect from encephalitis (Table 2). Together with the previous imaging data, this suggests that tachyzoite recognition within neurons may result in critical T cell:neuron interactions, while **bradyzoites** within cysts remain relatively invisible to T cells.

## Resident Memory T Cells

**Tissue resident memory T cells ($T_{RM}$)** are a distinct lineage of cells that confer protection directly in the relevant tissues rather than within the lymphatic system. Traditionally, tissue residence has been defined by an inability of these cells to recirculate in the mouse as shown by parabiosis experiments. $CD8^+$ T cells that remain in the brain during *T. gondii* infection appear to adopt a $T_{RM}$ phenotype, evidenced by surface expression of CD69 and CD103 [54,65]. Supporting their status as bona fide $T_{RM}$ cells, $CD8^+$ T cells in the brains of *T. gondii*-infected mice also have a transcriptional profile resembling a $T_{RM}$ signature [69], though parabiosis experiments have not demonstrated their tissue residence status using the classic definition. *T. gondii* infection may provide a unique opportunity to study these cells, and future work investigating the development and protective capacity of these cells will be of great interest.

## Concluding Remarks

Studies of *T. gondii* infection in mice have led to seminal discoveries in the field of $CD8^+$ T cell biology and our understanding of how $CD8^+$ T cells detect and respond to intracellular pathogens. The *T. gondii*–mouse infection model will be instrumental in addressing additional questions in the field (see Outstanding Questions).

The *T. gondii* parasite provides us with a unique model to investigate the requirements for presentation of vacuolar antigens, which cannot be studied with a viral model in which antigens are translated in the cytosol. As we learn more about how *T. gondii* antigens are processed and presented to $CD8^+$ T cells, it is becoming clear that different antigens use distinct pathways based on their location and trafficking within host cells. Future studies to address the detailed mechanisms of these pathways will provide additional insight into the general problem of how exogenous and vacuolar antigens are cross-presented via MHC-I. Moreover, *T. gondii* shows considerable promise as a vaccine vector [70], and understanding the factors that lead to efficient processing and presentation of parasite antigens may also help to realize the full potential of this approach.

Much of our understanding of how $CD8^+$ T cells respond to chronic infections is based on a limited number of mouse infection models, particularly the widely studied LCMV clone 13 model of chronic infection. The addition of the *T. gondii* infection model had broadened our perspective on this topic. *T. gondii* infection has contributed to our understanding of $CD8^+$ T cell responses to infection in the central nervous system (CNS), as neurotropic viral mouse models are limited. Additionally, the difference in control of *T. gondii* infection between two strains of inbred mice provides the unique opportunity to compare $CD8^+$ T cell function in a progressing disease setting versus a well-controlled chronic infection. Studies have identified a novel T cell population fundamental for control in the resistant setting, as well as cellular mechanisms leading to disease in susceptible mice.

One example demonstrating the potential of this system can be found in Salvioni *et al.*, where the authors engineered a parasite strain expressing the OVA epitope in a manner similar to the immunodominant GRA6 epitope [68] (Table 2). This system allowed the authors to recapitulate H-$2^d$-mediated protection in the typically susceptible H-$2^b$ setting, highlighting the critical importance of antigen processing in generating parasite control. Similar efforts to take lessons from the H-$2^d$-restricted protective setting and manipulate the H-$2^b$-restricted susceptible setting to improve protective capacity may lead to exciting advancements in our understanding of $CD8^+$ T cell function.

**Outstanding Questions**

How does antigen trafficking within distinct host cell compartments influence effective MHC-I presentation?

How do T cell location and different cytokine environments impact differentiation during chronic infection?

What population of cells is responsive to checkpoint blockade? Do they resemble the responding cells that have been identified in other infection models?

Why are GRA6-specific $CD8^+$ T cells able to mount such a robust and persistent response? Do T cell-intrinsic or MHC-intrinsic factors play a significant role?

Do $CD69^+$ $CD103^+$ cells in the brain recirculate or are they true resident memory cells? What leads to development of $T_{RM}$ cells in the brain? Do they play a vital protective role?

How do brain cysts remain immunologically silent? How do we induce immune recognition and promote parasite clearance?

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