Trends in Parasitology

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Review

Toxoplasma and Dendritic Cells: An Intimate Relationship That Deserves Further Scrutiny

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***Toxoplasma gondii* (*Tg*), an obligate intracellular parasite of the phylum Apicomplexa, infects a wide range of animals, including humans. A hallmark of *Tg* infection is the subversion of host responses, which is thought to favor parasite persistence and propagation to new hosts. Recently, a variety of parasite-secreted modulatory effectors have been uncovered in fibroblasts and macrophages, but the specific interplay between *Tg* and dendritic cells (DCs) is just beginning to emerge. In this review, we summarize the current knowledge on *Tg*–DC interactions, including innate recognition, cytokine production, and antigen presentation, and discuss open questions regarding how *Tg*-secreted effectors may shape DC functions to perturb innate and adaptive immunity.**

Toxoplasma Infection Induces Robust Th1 and Cytotoxic Responses

Toxoplasma gondii (*Tg*) is a widespread parasite, infecting a large range of warm-blooded animals, including humans who are considered to be accidental hosts in the *Tg* life cycle. For optimal transmission, parasites need to preserve their host and strike a delicate balance so that efficient immune responses control the parasite burden, while restricting immunopathology. Possibly reflecting the diversity of its natural hosts, multiple genotypes of *Tg* exist worldwide. Yet most parasites in North America and Europe fall within one of three clonal lineages: type I, II, or III, which are also the most studied in laboratory mice [1]. In immune-competent humans, a generally mildly symptomatic acute phase of tachyzoite dissemination is followed by the development of bradyzoite-containing cysts in the brain, leading to lifelong persisting infection. Latent *Tg* infection of the brain is now considered as a possibly underestimated cause of behavioral alterations and mental disorders in humans [2,3]. Moreover, in immunosuppressed individuals, parasite conversion to highly replicative tachyzoites can induce brain tissue damage and fatal acute neuroinflammation. Congenital infections can also be life-threatening for the developing fetus, causing severe neurological pathologies at birth, or milder, ocular toxoplasmosis later in life. When considering laboratory mice, type II and type III parasites typically exhibit a relatively low virulence during the acute phase, while type I strains kill their host prematurely due to hyperinflammation and uncontrolled parasite dissemination, and thus fail to establish latent infections. However, this phenotype only applies in laboratory mice, and type I strains can lead to persistent infection in wild mice and possibly other hosts, due to genetic variations in host resistance loci [4,5].

As an obligatory intracellular parasite, *Tg* has the ability to invade all nucleated cells and to reside within a **parasitophorous vacuole (PV)** (see Glossary). To modulate cell-intrinsic defenses, the parasite releases effectors into the host through apicomplexan-specific secretory organelles called **rhoptries (ROP)** and **dense granules (GRA)**. These effectors operate in concert to manipulate host cell responses involved in cytokine and chemokine secretion, cell-cycle progression, host survival, and metabolic homeostasis [6,7]. Strikingly, while a growing number of effectors dampening cell-autonomous immunity are being characterized, very few effectors promoting parasite latency and preservation of the host have been identified.

During natural oral *Tg* infection, dendritic cells (DCs) (Box 1) are one of the first cell types that encounter parasites in the intestine [8]. DCs are key players in the local activation of cell-mediated immunity against *Tg* by producing interleukin (IL)-12 that activates interferon (IFN)-γ production by T cells and natural killer (NK) cells [9]. T cells play a major role in controlling acute dissemination and keeping chronic cerebral infection under control [9]. While the mechanisms by which *Tg* modulates host responses have been extensively investigated in fibroblasts, monocytes, and macrophages

Highlights

Tg innate recognition by murine DCs involves TLR11 and TLR12 signaling, resulting in IL-12 secretion.

Innate sensing and cytokine secretion triggered by *Tg* infection requires live parasite phagocytosis in human DCs.

Tg modulates the antigen-presentation pathways of infected DCs.

Tg enhances the migratory ability of DCs.

The different DC subsets likely display distinct responses to *Tg* infection in terms of cytokine responses, antigen presentation, and parasite clearance.

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[6,7], how DC functions are modulated by the parasite remains ill-defined. The consequences of these interactions most likely impact on immunomodulatory functions of DCs and on T cell activation, and are therefore important for the outcome of infection. Since our current knowledge on these questions mainly derives from the study of mouse models, this review concentrates on mouse studies. One section is also dedicated to emerging findings on the specific responses of human DCs, which substantially differ (e.g., in parasite sensing).

Innate Recognition of *Tg* by DCs

The *Tg* Profilin-TLR11/12-IL-12 Axis: A Major Pathway in DC Responses in Mice

Macrophages and DCs detect and respond to microbial products by **pattern-recognition receptors (PRRs)**, such as Toll-like receptors (TLRs). PRR signaling influences multiple biological processes, including antigen presentation, cell survival, cytokine production, and antimicrobial responses. During *Tg* infection, the molecular processes enabling *Tg* sensing by infected DCs are not fully uncovered.

Box 1. DC Functions and Subsets

DCs represent an efficient patrolling system sampling tissues in search of danger signals, and a first line of defense against invading pathogens. At steady-state, DCs reside in immature or semimature states in the periphery, where they constantly take up and process self-antigens to maintain self-tolerance. In response to infection, DCs undergo a program of maturation involving the upregulation of MHC and co-stimulatory molecules at the cell surface, the CCR7-dependent migration to T cell-rich zones of secondary lymphoid organs, and the release of cytokines promoting the differentiation of naïve T cells into effector/memory cells, as well as the local activation of other immune cells [103]. Depending on the nature of the microbial compounds they sense, DCs produce distinct cytokines and shape the differentiation of different types of effector T cells, adapting T cell polarization to the specific threat. Moreover, DCs bridge innate and adaptive immunity by presenting processed antigenic peptides on MHC molecules to prime naïve antigen-specific T cells. DCs exist as functionally distinct subsets, which can be distinguished based on their tissue distribution, cell surface markers, and transcriptional programs (Table I).

DC subsets	Monocyte DC	cDC1	cDC2	pDC
Type	Migratory	Lymphoid resident (CD8α^+) Migratory (CD103$^+$)	Lymphoid resident DC	Lymphoid resident DC
Mouse markers	CD11b$^+$ Ly6C$^{hi/lo}$ CD64$^+$	CD11c$^+$ MHC-II$^+$ XCR1$^+$ CLEC9A$^+$ FLT3$^+$ CD11b$^-$ CD8α^+ or CD103$^+$	CD11c$^+$ MHC-II$^+$ CD11b$^+$ CD24$^+$ CD172a$^+$ CD8α^-	CD11c^{int} MHC-II^{int} B220$^+$ PDCA1$^+$ SiglecH$^+$ Ly6C$^+$
Human markers	CD11b$^+$, CD14$^+$, CD206$^+$ CD209$^+$	CD11c^{int} MHC-II$^+$ XCR1$^+$ CLEC9A$^+$FLT3$^+$ BDCA3$^+$ CD11b^{-}	CD11c$^+$ MHC-II$^+$ CD11b$^+$ CD172a$^+$ FLT3$^+$ BDCA1$^+$	CD11c$^-$ MHC-II^{int} BDCA2$^+$ BDCA4$^+$ CD123$^+$ CD304$^+$
Functions	TNF, iNOS, and ROI production; Presentation on MHC-II; CD4 T cell response	Against intracellular pathogens; IL-12 production; TLR3 induced; Cross-presentation on MHC-I; CD8 T cell response	Against extracellular pathogens IL-12, TNFα production Presentation on MHC-II CD4 T cell response	IFN I/III, IL-12, TNF-α, IL6 production TLR7, TLR9 induced CD4 and CD8 T cell response

Table I. DC Subsets[a]

[a] Abbreviations: iNOS, inducible nitric oxide synthase; ROI, reactive oxygen intermediates.

Glossary

Active invasion: *Toxoplasma gondii* invades host cells by secreting effectors (rhoptry and microneme proteins) that enable parasite adhesion to the host plasma membrane and penetration into the forming vacuole.

Cross-presentation: refers to the presentation by MHC-I molecules of peptides derived from an extracellular source (from endocytosed proteins or microorganisms internalized through phagocytosis or after active invasion).

Dense granule (GRA): specific apicomplexan secretory organelle containing proteins involved in the maturation of the parasitophorous vacuole, the formation of the intravacuolar network, and the modulation of host cell responses when secreted beyond the vacuole limiting membrane.

Endolysosome: intracellular organelle resulting from the fusion of a lysosome with an endocytic vesicle.

Endoplasmic reticulum (ER): a network of membranous tubules within the cytoplasm continuous with the nuclear membrane involved in protein and lipid synthesis and cellular homeostasis.

Exosome: extracellular vesicle originated from the endocytic compartment involved in cell-to-cell communication.

Immunity-related GTPase (IRG) and **guanylate-binding proteins (GBPs)**: GTPases induced upon interferon gamma stimulation, which accumulate at the parasite vacuole limiting membrane and trigger its degradation.

Inflammasome: intracellular multiprotein complex that detects microorganisms and other stressors, which activates IL-1β and IL-18 production. It also induces a form of cell death called pyroptosis.

Intravacuolar network (IVN): a network of membrane tubules and vesicles, present in the vacuolar space of the parasitophorous vacuole, that interconnect the parasites and also connect the parasites to the vacuole-limiting membrane.

Parasitophorous vacuole (PV): an intracellular vacuole in which the parasites replicate, protected from the host defense mechanisms.

Pioneer studies reported that, upon *Tg* systemic infection, the induction of IL-12 production by macrophages, DCs, and neutrophils depends on the signaling molecule MyD88, suggesting a TLR-mediated microbial detection. Similar to MyD88$^{-/-}$ animals, mice in which MyD88 deletion is restricted to CD11c^{+} DCs fail to control type II *Tg* systemic infection and exhibit a strong decrease in IFN-γ production [10,11], demonstrating the major role of DCs in inducing a protective T helper cell (Th)1 immunity. In contrast to macrophages, IL-12 production in DCs downstream of MyD88 was found to be independent from the nuclear factor (NF)-κB pathway but to rely on the transcription factor interferon regulatory factor 8 (IRF8) [12] (Figure 1A, Key Figure). TLR11 and TLR12, two endolysosomal TLRs, which are able to form heterodimers and are stimulated in an UNC93B1-dependent manner, were then identified as the major players in innate sensing of *Tg* by DCs, via the recognition of *Tg* profilin (*Tg*PRF) (Figure 1A) [13]. Mice deficient in these sensing molecules display a drastic defect in IL-12 production by splenic DCs. Consequently, IFN-γ production is severely impaired, and mice become highly susceptible to infection [13–17]. There seems to be a functional specificity between both TLRs, since TLR12, but not TLR11, is involved in IL-12 production by plasmacytoid DCs (pDCs) (Box 1). Similar to DC-MyD88$^{-/-}$ mice, TLR12$^{-/-}$ mice display a more severe susceptibility to infection compared with TLR11$^{-/-}$ mice, which is due to a defect in the early activation of IFN-γ production by NK cells and impaired parasite clearance in the peritoneum [16]. Of note, TLR11/TLR12-mediated recognition of *Tg*PRF only operates in mice and not in humans, in which the ortholog TLR11/12 genes are not functional.

Sensing *Tg* beyond TLR11 and TLR12

TLR2 and TLR4 represent major mammalian surface TLRs that recognize lipoproteins and liposaccharides derived from bacteria, viruses, fungi, and parasites. They can also signal from endosomes after internalization of ligand/receptor complexes. In contrast to macrophages [18], TLR2 and TLR4 were proposed to not operate in murine DCs in the sensing of *Tg* **pathogen-associated molecular patterns (PAMPs)** based on the observation that IL-12 production is not altered in splenic DCs of TLR2$^{-/-}$ and TLR4$^{-/-}$ mice injected with soluble tachyzoïte antigens (STAgs) [19]. However, the responses of splenic DCs isolated from infected mice or of splenic DCs infected *ex vivo* with live parasites have not been explored in this study. Along this line, parasite surface adhesins *Tg*MIC1/MIC4 were recently reported to stimulate IL-12 secretion in a TLR2-dependent manner in bone-marrow-derived DCs (BMDCs) [20]. Alterations in the production of additional TLR2-stimulated cytokines, notably type I interferons (IFN-I) [21], or in the regulation of other DCs functions (e.g., antigen presentation [22]) were not examined in TLR2$^{-/-}$ infected BMDCs. Thus, further exploration of TLR2 signaling after *Tg* infection may reveal novel regulatory mechanisms of DC responses. Most importantly, while TLR2$^{-/-}$, TLR4$^{-/-}$ and TLR9$^{-/-}$ mice infected systemically with *Tg* demonstrate limited susceptibility and modest defects in IL-12 production, severe mortality was demonstrated in the same animals receiving the parasite orally [18,23,24]. Notably, DC maturation and migration to draining mesenteric lymph nodes (MLNs) were altered in TLR9$^{-/-}$ mice together with decreased CD4^{+} T cell responses [23]. TLR2, TLR4, and TLR9 were also demonstrated to be essential for the development of efficient IFN-γ responses by T cells in the small intestine after oral infection [23,24]. In addition, parasite-induced damage of the intestinal mucosa is decreased in TLR4$^{-/-}$ mice and in mice treated with broad-spectrum antibiotics, suggesting that mucosal immune responses to *Tg* also rely on the indirect stimulation of DCs by normal gut microflora [25]. Hence, the route of infection likely impacts on the mechanisms of *Tg* innate sensing, which may also differ after oral infection between the different DC subsets present in the intestinal mucosa.

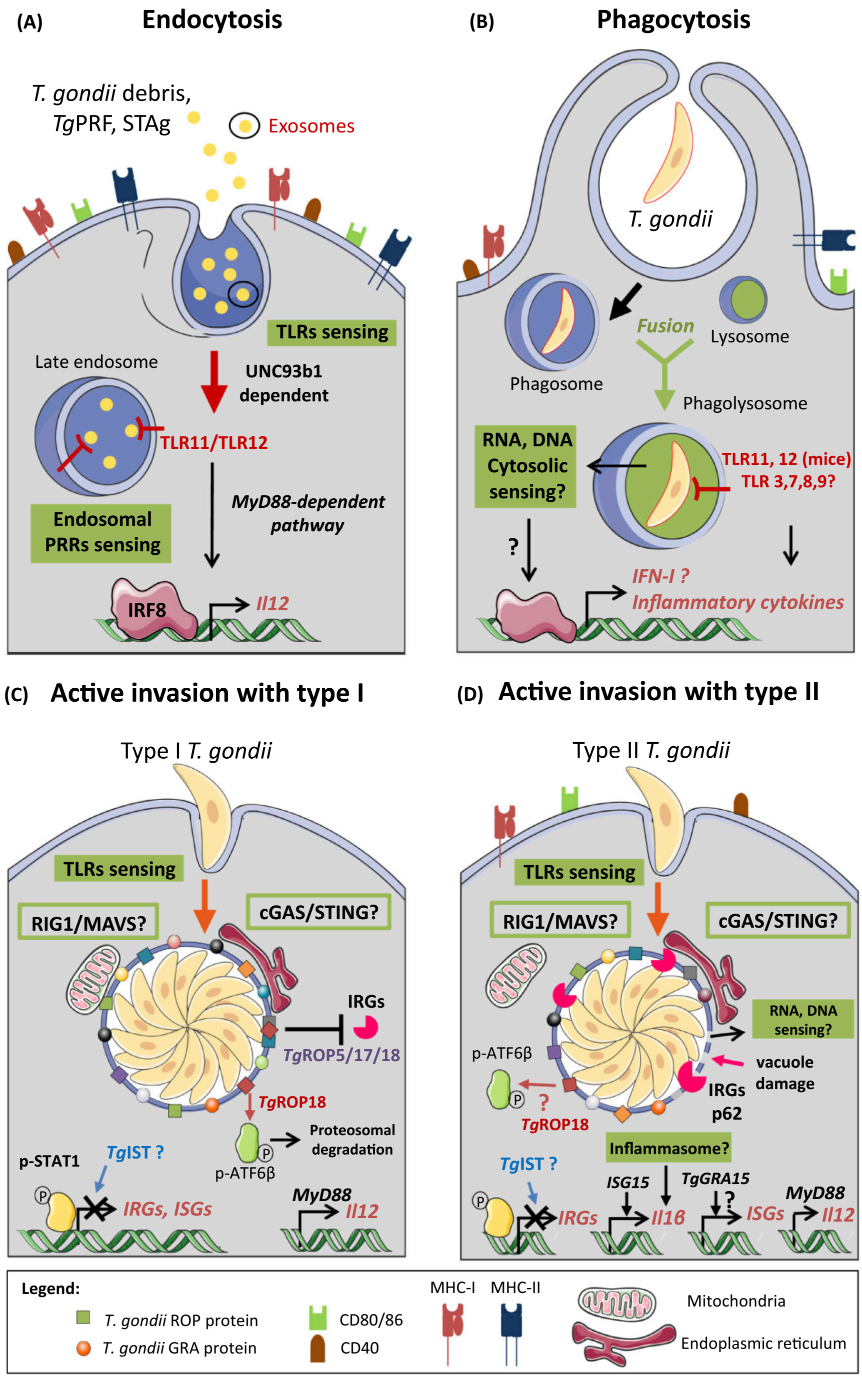
Tg Innate Sensing in Human DCs

Deciphering the respective roles of TLRs in the sensing of *Tg* PAMPs is of particular relevance for human DCs, which lack functional TLR11 and TLR12 genes. In contrast to murine DCs, human monocyte-derived DCs (MDDCs) fail to respond to STAgs as well as heat-killed (HK) or fixed parasites [26,27]. The TLRs involved in *Tg* sensing in human DCs have not yet been identified; however, studies carried out in human blood-derived monocytes, which are major producers of IL-12 upon infection, revealed that parasite invasion is not essential to trigger cytokine responses. Furthermore, phagocytic uptake of live parasites and the acidification of the resulting phagosome enhance cytokine production

Pathogen-associated molecular patterns (PAMPs): small molecular motifs found in microbial molecules recognized by pattern-recognition receptors.
Pattern-recognition receptors (PRRs): host sensors that detect PAMPs and trigger signaling pathways involved in innate immune responses.
Phagocytosis: a mechanism by which phagocytes engulf and digest large particles and microorganisms.
Rhoptry (ROP): a specific apicomplexan secretory organelle containing proteins involved in parasite invasion, PV formation, and modulation of host cell responses.
Unfolded protein response (UPR): ER stress response induced by an accumulation of misfolded proteins in the ER, changes in lipid homeostasis, TLR stimulation, and oxidative stresses. It is characterized by the activation of three ER sensors: ATF6, PERK, and IRE1α, which induce transcriptional programs aiming to restore cell homeostasis but can lead to the induction of cell apoptosis.

Key Figure

Innate Sensing and Modulation of Signaling Pathways in *Tg*-infected Dendritic Cells (DCs)



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compared with **active invasion** (Figure 1B) [27]. When parasite **phagocytosis** (but not adhesion) is inhibited, cytokine responses are abrogated, indicating that surface TLRs are not involved in *Tg* sensing. In addition, as phagocytosis of HK but not irradiated parasites fails to stimulate cytokine production, efficient innate recognition of *Tg* may rely on the detection of heat-labile compounds, possibly parasite RNA [27]. Thus, based on what is known for phagosomes containing inert or microbial antigenic material, phagocytosis of live parasites in human monocytes and DCs may trigger the stimulation of endosomal TLRs following phago-lysosomal fusion, or of cytosolic PRRs after phagosomal membrane disruption [28]. Importantly, as opposed to the mouse context in which conventional cDC1s dominate the IL-12 response, the human cDC1 are nonresponsive to *Tg* infection, and IL-12 and TNF-α production following *Tg* stimulation is restricted to the cDC2 subset [27].

How Do PRRs Sense Parasites Residing in Intracellular Vacuoles?

Even in murine DCs, which have been more extensively studied, the molecular processes of PAMP sensing from vacuolar parasites remain a conundrum. Using trans-well experiments, Pifer *et al.* demonstrated that contacts between murine DCs and DC-depleted splenocytes infected by tachyzoites are not required for TLR11-mediated IL-12 production [13]. This suggests that *Tg*PRF, a cytoskeleton protein localized in the cytosol of parasites, is released into the medium and further internalized by DCs into **endolysosomes**, where TLR11/TLR12 are localized [13] (Figure 1A). Whether *Tg*PRF detection by distant DCs relies on the presence of the protein in apoptotic bodies, necrotic debris, or **exosomes** remains an open question. In line with this idea, two independent studies recently demonstrated an active role for exosomes released by type I and type II tachyzoites in stimulating IL-10, IL-12, and TNF-α production by macrophages *in vitro*. When injected into mice, these exosomes were able to induce specific anti-*Tg* humoral responses and CD8$^+$ T cell-mediated immunity, suggesting that *Tg* exosome internalization by DCs contributes to MHC-I and MHC-II antigen presentation [29,30]. Furthermore, in correlation with the detection of *Tg*PRF by immune cells 'at a distance' from infected cells, bystander splenic DCs, which do not contain phagocytosed or invaded parasites, induce high amounts of IL-12 [31]. Yet, one may envision that, while TLR11/12 stimulation by

Figure 1. (A) Internalization of parasite debris, soluble tachyzoite antigen (STAg), *Tg* profilin (*Tg*PRF), or *Tg* exosomes stimulate interferon regulatory factor 8 (IRF8)-mediated production of interleukin (IL)-12 via a Toll-like receptor (TLR)11/12- and MyD88-dependent pathway in mice. (B) Phagocytosis of live or killed parasites results in TLR activation after phagolysosomal fusion. TLR11 and TLR12 play a major role in triggering a cytokine response in mice. However, other endosomal TLRs, such as TLR 3/7/8/9, may be also stimulated, notably in human DCs, which lack functional TLR11 and TLR12 genes. Parasite degradation and partial disruption of the phagosomal membrane may lead to the release of RNA/DNA from the parasite sensed by cytosolic pattern-recognition receptors (PRRs) described to induce type I interferon (IFN) production. (C) Innate sensing of type I parasites that have actively invaded DCs relies on MyD88-dependent activation of TLRs, and has yet to be fully uncovered. Possible additional mechanisms may involve the RIG-1 and STING sensors localized at the host mitochondria and endoplasmic reticulum, respectively, two compartments tightly associated with the parasitophorous vacuole (PV). IFN-γ stimulation triggers the phosphorylation of STAT1, but the transcriptional activation of interferon-stimulated gene (ISG), notably immunity-related GTPase (IRG), is impaired in type I-infected DCs, likely by a *Tg*IST-mediated process. The *Tg* ROP5/17/18 effectors also block IRG-induced vacuole damage upon IFN-γ stimulation, while *Tg* ROP18 triggers ATF6β phosphorylation and degradation, resulting in decreased CD8$^+$ T cell activation. Also, MHC-I, MHC-II, and co-stimulatory molecule surface expression are down regulated by type I parasites. (D) The innate sensing of type II parasites also relies on MyD88-dependent activation of TLRs. Distinct sensing mechanisms compared with type I parasites may be induced, correlating with partial vacuole disruption. Upon IFN-γ stimulation, PV damage and antigen escape to the cytosol is mediated by autophagy proteins (ATG and p62) and IRG/ guanylate-binding protein (GBP) recruitment at the PV membrane. Other ISG genes may be also induced in a *Tg*GRA15II- and nuclear factor (NF)-κB-dependent manner. Possible leakage of parasite RNA/DNA upon PV disruption may activate cytosolic PRRs. In addition, ISG15 induces IL-1β production in infected conventional (c)DC1. A putative activation of the inflammasome has not been investigated. Type II infection itself also triggers the phosphorylation of STAT1 but *Tg*IST effector likely reduces STAT1-mediated IRG/GBP expression. *Tg*IST is also likely involved in the downregulation of MHC-II molecule surface expression upon IFN-γ stimulation. Abbreviations: cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; RIG1, retinoic acid inducible gene 1 protein; MAVS, mitochondrial antiviral signaling protein.

*Tg*PRF is critical to trigger IL-12 secretion, active invasion by parasites may activate distinct endosomal TLRs or cytosolic PRRs (Figure 1C,D). Cytosolic PRR stimulation would imply a process of *Tg* PAMP export from the PV to the host cytosol, in particular nucleic acids. Although speculative, it is logical to consider that this process may be facilitated by vacuolar membrane breakdown following IFN-γ and STAT1-induced **immunity-related GTPase (IRG)** and **guanylate-binding protein (GBP)** recruitment at the PV membrane (PVM) [6,32]. Supporting this hypothesis, polymorphic *Tg* ROP18, ROP5, and ROP17 proteins are essential secreted factors localized at the PVM that disrupt IRG/GBP-mediated parasite restriction, albeit in a strain-specific manner (Figure 1C). Type II strains possess an inactive ROP5 allele, which hampers blockade of PV disruption in IFN-γ-activated macrophages (Figure 1D). It is likely that similar mechanisms exist in DCs. In support of this hypothesis, infection of IFN-γ-primed BMDCs by type II, but not by type I, parasites induces the recruitment of ubiquitin and the P62 sequestosome (SQSTM1) at the PVM, via a process dependent on the IRG-regulatory proteins Irgm1/Irgm3 and the autophagy-related proteins (ATGs) ATG3, ATG5, ATG7, and ATG16L1 (Box 2). Accumulation of p62 on parasite-containing vacuoles occurs after partial damage by IFN-γ-inducible IRG and GBP and contribute to the cytosolic release of luminal antigens that activate CD8$^+$ T cell responses [33]. In addition, in BMDCs, the infection itself is sufficient to trigger STAT1 phosphorylation and nuclear translocation in a strain-independent manner, and these responses are accentuated by the addition of IFN-γ [34] (Figure 1C,D). However, parasites impede STAT1 activity by inducing the formation of aberrant transcriptional complexes at the promoter of IFN-stimulated genes (ISGs), such as *Irf1* and *Igtp* [34] (Figure 1C). In macrophages, this inhibitory process is mediated by the *Tg*-secreted effector IST (inhibitor of STAT1 transcriptional activity) in both type I and type II strain-infected cells [35–37]. Most likely, similar *Tg*IST-mediated inhibitory mechanisms take place in infected DCs; however, this remains to be formally established. As previously mentioned, upon IFN-γ stimulation of BMDCs, type II parasites, but not type I parasites, induce Irgm1/Irgm3 recruitment at the PVM, which is not sufficient to induce parasite clearance but triggers partial PV disruption [33]. Although this difference is likely linked to the inactive ROP5-II allele, it is worth mentioning that the distinct *Tg* strains can differentially modulate specific aspects of IFN-γ signaling through the polymorphic effector proteins ROP16 and GRA15. In particular, GRA15 from type II strains was reported to upregulate a certain set of ISGs in human fibroblasts in a STAT1-independent but NF-κB-dependent manner [38,39].

Therefore, strain-specific induced PV damage may promote *Tg* PAMP export to the host cytosol, resulting in strain-specific innate sensing mechanisms and activation of corresponding downstream

Box 2. *Tg* Modulation of Autophagy in DCs

Canonical autophagy is activated in *Tg*-infected cells upon CD40 signaling. This pathway is dependent on ULK1, accompanied by LC3 accumulation onto the PV and it involves lysosomal elimination of the parasite [104,105]. Autophagic targeting of *Tg* occurs in macrophages and endothelial cells where it plays an important role in parasite restriction, in particular regarding access to the neural tissue [106]. As a survival counter-strategy, *Tg* activates signaling cascades that antagonize host autophagy through at least two distinct mechanisms, involving the activation of Akt and the inhibition of PKR. In addition, some autophagy-related proteins are required for the IRG/GBP-mediated restriction of *Tg* in the context of IFN-γ-stimulated macrophages and fibroblasts. However, these processes are distinct from bona fide autophagy, which typically involves fusion with and degradation in lysosomes [104,105].

Autophagy regulates several DC functions, including antigen presentation, cytokine production, and migration [107]. In *Tg*-infected DCs, the recruitment of autophagy protein at the PVM regulates parasite antigen escape to the host cytosol and subsequent T cell activation [33,86,87], albeit in a process that is likely not dependent on canonical autophagy [86,87]. Moreover, it is currently unknown to what extent autophagy contributes to *Tg* parasite restriction in DCs and if autophagy avoidance pathways are mobilized by the parasite in this cell type. Interestingly, host autophagy may be hijacked by the parasite for the acquisition of nutrients to sustain its growth [108]. Knowing that the PV in *Tg*-infected DCs intersects with various host organelles [46,68,69,73], it would be interesting to investigate how the autophagosome–PV interplay regulates both the parasite and DC metabolisms.

pathways. Of particular interest is the activation of the **inflammasome**. NLRP3 activation and IL-1β production in human monocytes and bone marrow-derived macrophages were reported to be specifically triggered by type II strains and dependent on the secretion of *Tg*GRA15 into host cells [40,41]. Whether the inflammasome is activated in *Tg*-infected DCs remains to be addressed.

Finally, although not yet explored in *Tg*-infected immune cells, other mechanisms of microbial sensing may control the immune response outcome. The mitochondrial antiviral-signaling protein (MAVS), stimulated downstream of the nucleic acid cytosolic sensor RIG-I (retinoic acid-inducible gene I) as well as STING (stimulator of interferon genes), downstream of the cyclic GMP-AMP synthase (cGAS) sensor, are localized at the mitochondrial membrane and the **endoplasmic reticulum (ER)**, respectively [42] (Figure 1C,D). Interestingly, these two compartments tightly associate with the PVM, therefore are likely to be in direct contact with *Tg* PAMPs. Of note, only type I and type III strains, but not type II strains, were reported to trigger the recruitment of mitochondria at the PVM in human fibroblasts [43]. Additionally, a cell achieves frequent contacts between the ER and late endosomes/lysosomes, where endosomal TLRs reside [44,45], and the PV has been recently shown to intersect with various endosomal compartments [46]. Hence, mechanisms distinct from PVM damage may promote the delivery of PAMPs from vacuolar parasites into intracellular compartments implicated in microbial innate sensing.

DC Cytokine Secretion: Beyond IL-12

Owing to the crucial role of IL-12 in the induction of the cytotoxic and Th1 responses, most studies have focused on the mechanisms by which splenic DCs secrete this cytokine. Nonetheless, the distinct DC subsets operating during infection may secrete additional cytokines contributing to the regulation of various immune responses (Box 1). This aspect is illustrated by a recent study showing that ISG15 production, an IFN-I-stimulated proinflammatory molecule, enhances the recruitment of cDC1 specifically producing IL-1β at the site of infection during type II parasite systemic infection [47] (Figure 1D). Along this line, IFN-I plays an important role in parasite infections [48,49]. In *Tg*-infected mice, pDCs, which expand after oral infection with type II parasites and play a key role in $CD4^+$ T cell priming, were shown to secrete high levels of IFN-α [16,50]. In contrast, IFN-β is mainly produced by inflammatory monocytes in the MLN and requires parasite phagocytosis [51]. Of note, human DCs actively phagocytose parasites and might therefore trigger IFN-I secretion upon *Tg* infection. In addition, in the mouse, IFN-I serum levels gradually increase during systemic *Tg* infection, and the cytokine can be detected in the brain and spleen of infected mice. $Ifnar^{-/-}$ (interferon alpha and beta receptor 1) mice orally infected with *Tg*, have increased parasite loads compared with wild-type mice, correlated with slightly decreased survival [51,52]. Importantly, IFN-I are prominent regulators of cDC1 turnover *in vivo* through a dose-dependent modulation of multiple processes, including DC apoptosis, proliferation, migration [53], and maturation [54] as well as their **cross-presentation** efficacy [55]. Hence, the role of IFN-I (via an autocrine effect or produced by surrounding cells) in the modulation of DC functions would deserve further exploration.

Moreover, the immune system associated with the intestinal mucosa comprises distinct lymphoid cells, including antigen-experienced $CD8^+$ and $CD4^+$ T cells and various subsets of innate lymphoid cells (ILCs), which reside in the epithelium and the underlying lamina propria (LP). DCs are central players in determining tolerance versus immunity during inflammation and infection. Among the DC subsets in the intestinal LP and MLN, $CD103^+$ DCs play an important role in maintaining intestinal homeostasis and inducing regulatory or effector T cells (Box 1). Two recent studies highlighted the role of IL-22 in gut protection against inflammation-triggered tissue necrosis during *Tg* type II infection [56,57]. DCs being potent regulators of T cell and ILC functions, secretion of cytokines by $CD103^+$ DCs and other DC subsets in the intestinal mucosa may contribute to the establishment of protective $CD4^+$ T cell and ILC responses against immunopathology. These responses may be manipulated by the parasite as suggested by the downregulation of retinoic acid production previously observed in mucosal DCs [8].

To conclude, while in mice, *Tg*PRF detection by TLR11/12 is critical in triggering the secretion of IL-12 and the IFN-γ response for early parasite clearance, active invasion of DCs and parasite phagocytosis in human cells likely stimulate additional innate sensing mechanisms, which have not yet been fully unraveled (Figure 1). These distinct mechanisms of *Tg* detection may not only regulate DC secretion of various chemokines and cytokines, but also modulate other DC functions, including antigen presentation and T cell priming [58–60]. In addition, it is possible that *Tg* secretes yet to be identified, strain-specific factors that target PRR-induced signaling pathways, impacting on the outcome of inflammatory responses. Finally, based on the diversity of DC phenotypes and functions, it appears essential to explore in more depth the mechanisms of *Tg* innate sensing in the different tissue-resident DC subtypes, notably after oral infection.

Modulation of *Tg* Antigen Presentation and T Cell Responses

T cell activation relies on three signals: (i) signal 1 elicited by MHC/peptide complex engagement of the TCR; (ii) signal 2 triggered when co-stimulatory receptors (e.g., CD28) bind their ligands (e.g., CD80, CD86) on antigen-presenting cells; (iii) and signal 3 integrating the cytokine environment to complete T cell differentiation. Thus, antigen availability, expression of co-stimulatory molecules and cytokine release are critical factors regulating T cell activation and memory formation that can be targeted by *Tg* to promote parasite dissemination and/or persistence.

Mechanisms of MHC-I Presentation by *Tg* and Modulation Thereof

Through the process of cross-presentation, MHC-I molecules can present antigenic peptides derived from extracellular sources, including those from microorganisms that reside in a phagosome or a vacuole, like *Tg*. The pathways by which *Tg* antigens access the MHC-I pathway have been partially unraveled [61]. Active secretion of parasite antigens into the infected host cell is a major requirement for efficient MHC-I presentation [62–65]. Moreover, emerging evidence shows that luminal and membrane-bound antigens follow processing mechanisms that exhibit similarities as well as distinctions. The analogies include the implication of the transporter associated with antigen processing (TAP) and the proteasome, suggesting that, in both cases, antigenic precursors exit the vacuole and reach the host cytosol before loading onto MHC-I molecules [66,67]. For luminal antigens, access to the DC cytosol is facilitated by fusion of vesicles of the host ER-Golgi intermediate compartment (ERGIC) onto the vacuole [68] through a mechanism that depends on the soluble NSF attachment protein receptor (SNARE) protein Sec22b [69]. This process promotes the recruitment of components of the ER-associated degradation machinery onto the PVM, then allowing retrotranslocation of vacuolar antigens to the host cytosol. Intriguingly, Sec22b-mediated ER–PV interactions are not involved in the presentation of the membrane-bound protective GRA6 antigen [70]. Instead, optimal processing of GRA6 requires the epitope to be located at the C terminus of GRA6 and to protrude into the host cytosol [70,71]. Of note, the protective membrane-bound GRA6 antigen associates with a membranous, tubulovesicular **intravacuolar network (IVN)** generated in the vacuolar space by parasite effectors. In the absence of this network, GRA6 redistributes to the PVM and is presented more efficiently by MHC-I, leading to enhanced CD8$^+$ T cell responses [72]. Thus, by trapping membrane-bound antigens, the network plays a negative modulatory function on recognition of *Tg*-infected cells by CD8$^+$ T cells (Figure 2).

Another way by which the parasite may potentially affect DC antigen presentation capabilities is by redirecting organelles towards the PV. In *Tg*-infected fibroblasts, a variety of Rab-containing organelles, including Golgi and endocytic compartments, are sequestered in the PV, allowing lipid acquisition by the parasite [46]. In DCs as well, the PV intersects with endocytic recycling compartments, resulting in accumulation of the Rab22a GTPase onto the PVM. In this case, this process seems to benefit the host as it promotes *Tg* MHC-I presentation [73]. Yet at this stage, besides effectors that generate the IVN [46], the effectors driving the PV–host organelle connections remain ill-defined. Furthermore, the exact implications of these new infection-related trafficking pathways on DC functions and parasite fitness remain to be studied.

Additional indirect mechanisms may contribute to the modulation of MHC-I presentation by the parasite. Of particular interest is the role of the ER-associated **unfolded protein response (UPR)** induced by viruses, bacteria, and parasite infections [74]. UPR induction in DCs has recently been implicated in the modulation of MHC-I antigen presentation in normal and pathological conditions [75–77]. Interestingly, type I *Tg*ROP18 was reported to directly phosphorylate the host UPR sensor ATF6β, resulting in its degradation by the proteasome [78] (Figure 1C). DCs isolated from ATF6β knockout (KO) mice exhibit a reduced ability to restimulate primed CD8$^+$ T cells, but not CD4$^+$ T cells, suggesting a yet unexplored function of ATF6β in MHC-I antigen presentation (Figure 2). However, since an enhanced IFN-γ production by CD4$^+$ T cells in mice infected with *Tg*ROP18 KO parasites was also observed, it is likely that additional mechanisms beyond the targeting of ATF6β-mediated cross-presentation dampen T cell activation [78]. Therefore, whether *Tg*ROP18 directly impacts on MHC-I presentation requires further investigation. Finally, it remains to be studied whether MHC-I presentation is modulated during the chronic phase of the infection by persistent parasites present in tissues, such as the muscles, the retina, and the central nervous system. Indeed, we have recently shown that MHC-I presentation of tachyzoite-derived antigens by neurons is pivotal for CD8$^+$ T cell-mediated parasite control in the brain [79]. However, it remains to be established if and how effectors secreted by bradyzoites interfere with MHC-I presentation, both in the target neuronal cells and in the surrounding DCs.

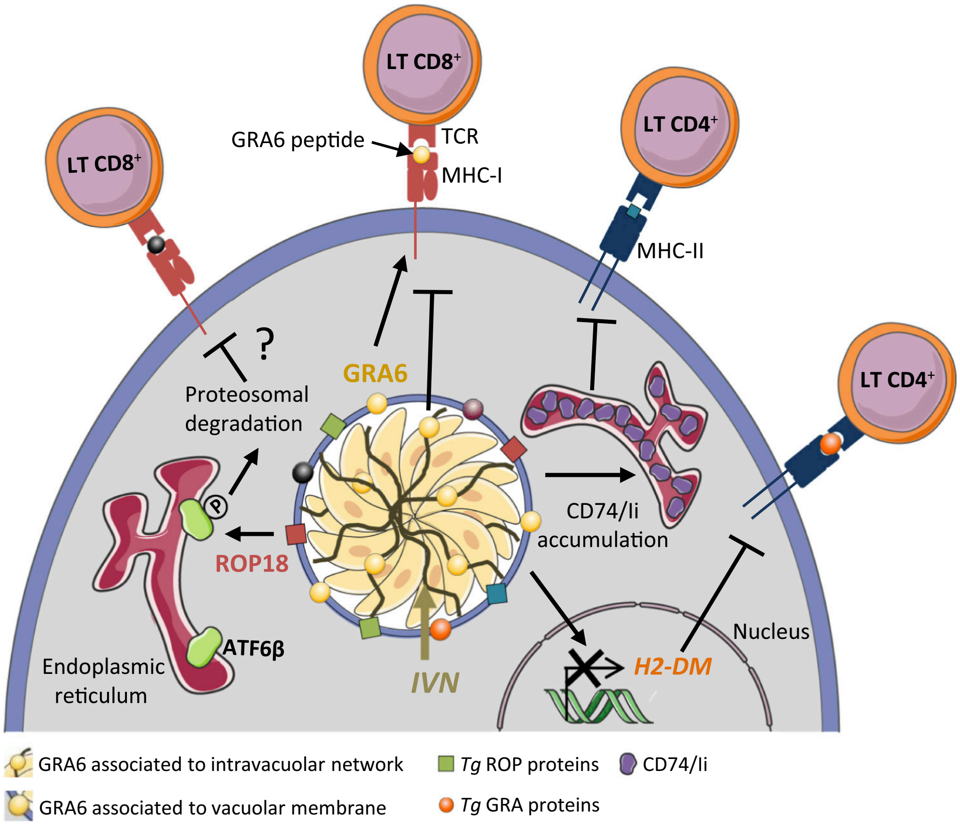

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Figure 2. Modulation of Antigen Presentation by *Tg* Effectors.
The intravacuolar tubulovesicular network (IVN) reduces GRA6 localization at the vacuole limiting membrane, therefore impeding access of the C-terminal fragment, that contains the antigenic peptide, into the host cytosol. This ultimately reduces presentation of the GRA6-derived peptide by MHC-I molecules on the cell surface. *Tg*ROP18 downregulates CD8 T cell activation by a yet unknown mechanism that involves *Tg*ROP18-mediated phosphorylation and degradation of the endoplasmic reticulum (ER) sensor ATF6β. *Tg* infection represses H2-DM expression and induces CD74/Ii accumulation in the ER, thereby impairing MHC-II antigen presentation and CD4 T cell activation.

Modulation of MHC-II Presentation by *Tg*

The manipulation of the MHC-II antigen-presentation pathway by *Tg* tachyzoites has been reported both at steady-state and following IFN-γ stimulation (Box 3).

At steady-state, *Tg* promotes the accumulation of CD74/Ii in the ER of infected cells and reduces the transcription of H2-DM, a chaperone essential for MHC-II loading. These combined effects result in the inhibition of MHC-II presentation of parasite-derived antigens [80]. Accumulation of CD74 is triggered by type I and type II strains, requires invasion by live parasites (but not replication), and is observed in macrophages and cDC1 isolated from the lymph nodes (LNs) of infected mice. In fact, *Tg* infection is known to impede IFN-γ-mediated upregulation of MHC-II in multiple cell types [81,82]. This is due to the ability of *Tg* to disturb chromatin remodeling at the class II transactivator CIITA locus [35] through the blockade of STAT1 activity (Figure 2) [83]. The parasite effector(s) responsible for this effect were reported to be rhoptry and/or dense granule-derived proteins [80]. *Tg*IST, which represses STAT1-dependent promoters, is thus a very likely candidate for the dysregulation of MHC-II molecule expression [36,37].

By preventing the upregulation of MHC-II on the surface of infected DCs, *Tg* alters the activation of $CD4^+$ T cells *in vivo* [84] and impedes parasite control. In agreement, $CD74^{-/-}$-$H2\text{-}DM^{-/-}$ KO mice fail to control infection by type II strains, correlating with high cerebral cyst burden, showing the important role of MHC-II antigen presentation during chronic toxoplasmosis [80]. Furthermore, immunization with the *Tg* AS15 peptide, a natural $CD4^+$ T cell antigen (Box 3), lowered cyst burden and parasite load in the brain of infected mice [85].

Moreover, DCs deficient in the autophagy protein ATG5, but not ATG7, were reported to induce diminished $CD4^+$ T cell production of IL-2 and IFN-γ after type II strain infection, suggesting that noncanonical autophagy may stimulate delivery of antigens to the MHC-II presentation pathway (Box 2) [86,87]. In general, in contrast to macrophages, the role of autophagy in the modulation of DC responses upon *Tg* infection remains mostly unexplored (Box 2).

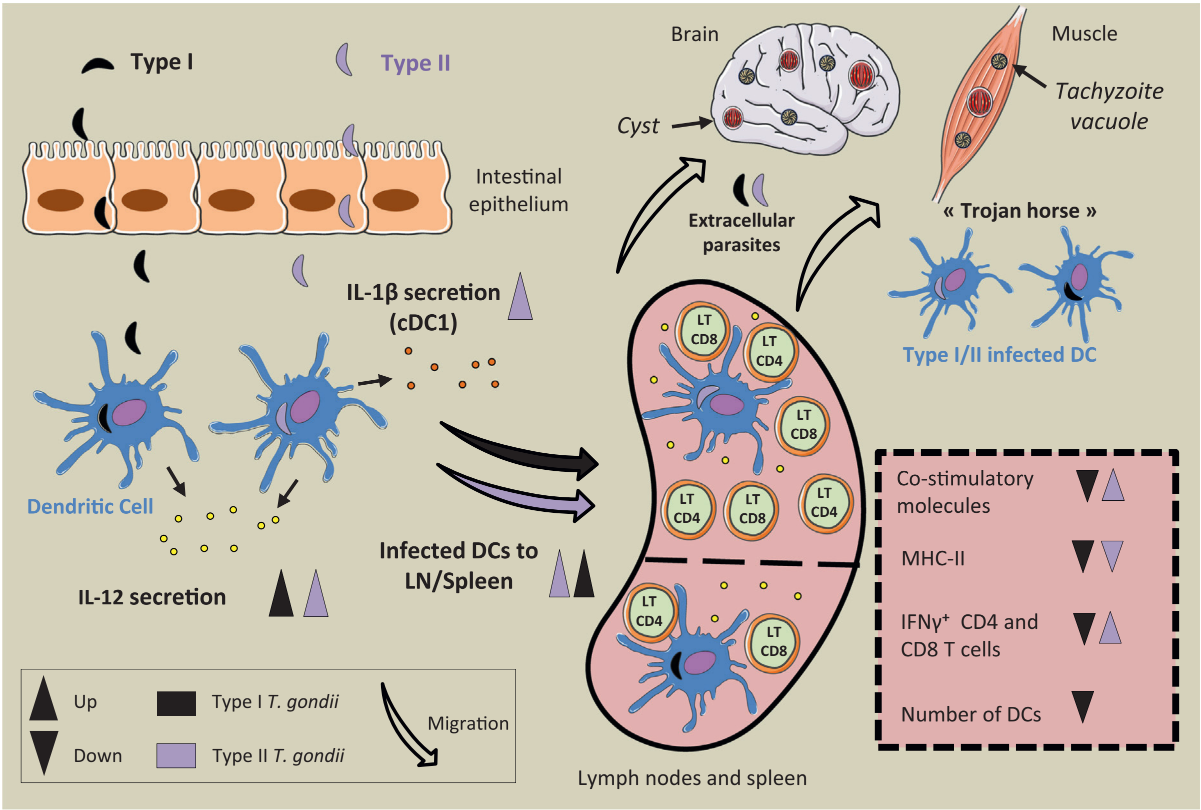
Modulation of DC Maturation by *Tg*

Contrasting results were obtained *in vitro* on the modulation of co-stimulatory molecule expression following *Tg* infection. Infection by type I parasites leads to the activation of human DCs by increasing the expression of CD40, CD80, CD86, and HLA-DR [26,88]. In contrast, immature murine BMDCs are activated after exposure to HK parasites or STAg (increased CD40, CD80, CD86, and MHC-II expression) but not after invasion by type I parasites, which also render them resistant to subsequent activation by TLR ligands [84] (Figure 3). Importantly, Tait *et al.* reported that infection by type I parasites is correlated with a decrease in the number of DCs in peritoneal exudate cells (PECs) and LNs, compared with type II infection [89]. DCs recruited at the site of infection display a reduced expression of CD80 and MHC-I compared with DCs analyzed during type II infection. As expected, the

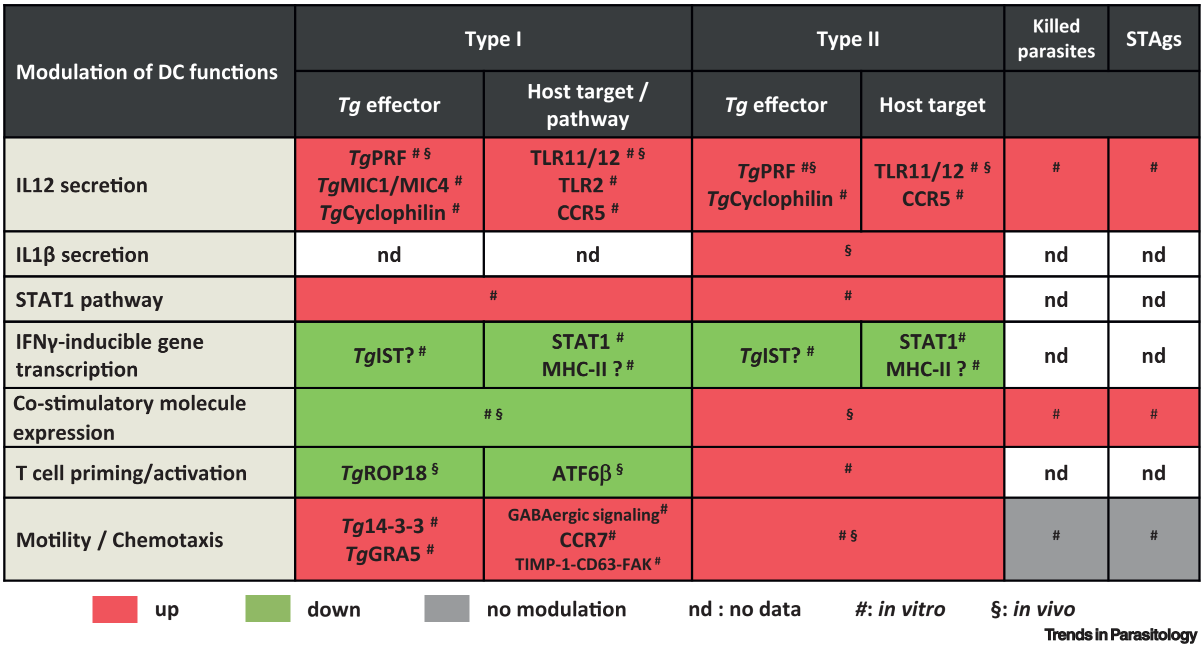
Box 3. Mechanisms of MHC-II Presentation by *Tg*

Two natural $CD4^+$ T cell antigens from *Tg* have been described so far: (i) the AS15 peptide derives from the TgCD4Ag28m protein [85], predicted to be a secreted protein, and (ii) the second one derives from *Tg*PRF [109]. Based on the fact that the *Tg* CD4Ag28m-derived AS15 peptide is robustly presented even when DCs are fed with heat-killed (HK) parasites, it is likely that both secreted and nonsecreted parasite antigens have access to the MHC-II antigen degradative compartments where peptide loading on MHC-II molecules occurs [85,110]. The mechanisms promoting antigen entry into the degradative compartments in the case of replicating parasites residing in a vacuole are still elusive. DCs deficient in the autophagy protein ATG5, but not ATG7, induce diminished $CD4^+$ T cell production of IL-2 and IFN-γ after type II strain infection [86,87]. This defect is not accompanied by changes in MHC-II and co-stimulatory molecule expression or impaired production of IL-12, IL-1β, or TNF-α [86], suggesting that it may be linked to a reduced delivery of antigens to the MHC-II presentation pathway.

(A)



(B)



Modulation of DC functions	Type I		Type II		Killed parasites	STAgs
	Tg effector	Host target / pathway	*Tg* effector	Host target		
IL12 secretion	🟥 *Tg*PRF # § *Tg*MIC1/MIC4 # *Tg*Cyclophilin #	🟥 TLR11/12 # § TLR2 # CCR5 #	🟥 *Tg*PRF #§ *Tg*Cyclophilin #	🟥 TLR11/12 # § CCR5 #	🟥 #	🟥 #
IL1β secretion	nd	nd	🟥 §		nd	nd
STAT1 pathway	🟥 #		🟥 #		nd	nd
IFNγ-inducible gene transcription	🟩 *Tg*IST? #	🟩 STAT1 # MHC-II ? #	🟩 *Tg*IST? #	🟩 STAT1# MHC-II ? #	nd	nd
Co-stimulatory molecule expression	🟩 # §		🟥 §		🟥 #	🟥 #
T cell priming/activation	🟩 *Tg*ROP18 §	🟩 ATF6β §	🟥 #		nd	nd
Motility / Chemotaxis	🟥 *Tg*14-3-3 # *Tg*GRA5 #	🟥 GABAergic signaling# CCR7# TIMP-1-CD63-FAK #	🟥 # §		▨ #	▨ #

🟥 up 🟩 down ▨ no modulation nd : no data #: *in vitro* §: *in vivo*

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(Figure legend at the bottom of the next page.)

diminished number of activated DCs altered the generation of specific CD8$^+$ T cell responses. In addition, IL-12 treatment during type I infection only modestly increased the number of antigen-specific CD8$^+$ T cells, reinforcing the hypothesis that type I parasites restrict CD8$^+$ T cell priming. The observed DC reduction upon type I infection may be a result of parasite-triggered cell death or impaired recruitment in response to altered chemokine environment. Overall, this may well explain the higher virulence of type I strains in comparison with type II strains and suggest that type II parasite virulence may be efficiently controlled by murine DCs. Supporting this hypothesis, systemic infection of mice by type II parasites resulted in increased expression of CD40, CD80, CD86, and MHC-II markers and elevated IL-12 production by both CD8α^- and CD8α^+ splenic DCs [90] (Figure 3). In addition, CD40–CD154 interactions were found to be essential to maintain splenic DC activation during infection [90]. In agreement, CD40 is upregulated in cDC1 following STAg injection in mice [91]. Therefore, strain-dependent modulation of DC maturation appears critical to shape the generation of *Tg* antigen-specific T cells and determine the outcome of infection in mice.

Nonetheless, the precise mechanisms by which the parasite interferes with key DC functions, such as antigen presentation, DC maturation, and survival, need to be elucidated. Also, an improved characterization of *Tg* antigen-presentation pathways in human DCs, as well as in distinct tissue-resident DC subtypes, is an important goal for future studies.

Tg Modulation of DC Migratory Functions

Pioneer experiments suggested that DCs and monocytes participate to the transport of the parasite from the primary site of infection and are used as 'Trojan horses' to enhance rapid parasite dissemination towards distant organs [92]. This phenomenon likely complements the ability of the parasites to exploit cerebral endothelial cells as a replication niche and a direct portal of entry into the brain parenchyma [93].

Supporting the 'Trojan horse' hypothesis, type I strain-infected murine BMDCs display enhanced ability to disseminate *in vivo* compared with free tachyzoites [94]. Phagocytosis of HK parasites or incubation with *Tg* secretory fraction do not enhance migration of DCs *in vitro* [94,95], suggesting a modulation of host signaling by secreted effectors. In addition, DCs infected by type II parasites, but not bystander cells, migrate significantly longer distances compared with type I-infected DCs in a 3D matrix [96]. Similarly, an enrichment of migratory type II-infected DCs compared with type I strain-infected DCs was monitored in spleen and MLN 16 h after inoculation of preinfected BMDCs [95]. Importantly, infection of monocytes, NK, B and T cells failed to induce an enhanced migratory phenotype [97], indicating a DC-specific modulation of host responses by the parasite via regulatory mechanisms that have been partially uncovered and recently expertly reviewed [98]. It is proposed that a transition from mesenchymal to the ameboid migration mode enables high-speed locomotion of the parasite through interstitial tissues and across biological barriers, which, in conjunction with enhanced chemotaxis, promotes *Tg* dissemination (Box 4).

Nonetheless, parasite effectors that stimulate the migratory ability of DCs need to be characterized. *Tg*GRA5, secreted and inserted at the PVM during parasite intracellular replication, was identified as a factor stimulating migration of human DCs *in vitro* [99]. The authors further pinpointed a peptide derived from the type I-*Tg*GRA5 hydrophilic N-terminal region, which, after internalization by micropinocytosis, triggers the expression of CCR7 and enhances DC directional migration via a

Figure 3. Modulation of Dendritic Cell (DC) Functions during *Tg* Infection.

(A) Scheme summarizing the modulation of DC functions in mice infected by the *Tg* type I and type II strains during the different steps of the infection from parasite entry into the intestine, to their migration in secondary lymphoid organs. *Tg* parasites can also disseminate to distant organs such as the brain and muscles, possibly by using DCs as shuttle leucocytes or as extracellular parasites in the blood. For simplification, modulations of human DC functions by the infection were not included in this figure. (B) Table summarizing the functions of murine DCs that are upregulated (red) or downregulated (green), compared with unchallenged DCs, by live *Tg* type I and type II strain infection, killed parasites, or soluble tachyzoïte antigens (STAgs). When identified, the parasite effectors and targeted host pathways were indicated, as well as whether these findings were obtained *in vitro* in the context of differentiated or isolated murine DCs (#) or *in vivo* in infected mice (§). Abbreviations: LN, lymph node; *Tg*PRF, *Tg* profilin.

Box 4. Modulation of DC Migratory Functions by *T. gondii*

In vitro, BMDCs and human MDDCs secrete GABA after infection with type I, II, or III strains [111]. GABA secretion activates, via an autocrine effect, functional GABA$_A$ receptors, which induce the *in vitro* transmigration and chemotaxis of type II infected murine DCs and promote parasite dissemination after their adoptive transfer in mice [111,112]. The signaling molecule calcium also plays a central role for this migratory activation as signal transduction following GABAergic activation is mediated via the L-type voltage-dependent calcium channel (L-VDCC) subtype Ca$_v$1.3. More recently, the Barragan laboratory further identified a TIMP-1-CD63-ITGB1-FAK signaling axis hijacked by *Tg* to drive high-speed ameboid migration [113]. Moreover, infected DC-enhanced migration is preceded by morphological changes. Human and murine DCs infected by type II parasites display cytoskeletal actin remodeling, exhibit a process of podosome dissolution and CD18 and CD11c integrin redistribution, thereby adopting an integrin-independent ameboid-like motility [96,114]. These morphological changes require parasite invasion of the DCs but are independent of GABAergic signaling, in agreement with the hypermotility phenotype being independent of chemotactic cues [114]. In particular, the initial hypermotility phenotype of infected DCs does not depend on CCR7 or CCR5, but infected DCs upregulate CCR7 to potentiate their chemotactic migration [111,114].

JNK-dependent signaling [99]. However, how this peptide acts at the PVM during infection by live parasites remains to be determined. In addition to *Tg*GRA5, *Tg*14-3-3, a protein located in the vacuolar space, was shown to induce *in vitro* DC hypermotility and enhanced chemotaxis via an unknown mechanism that may involve sequestration of host 14-3-3 at the PVM [100] (Figure 3). Thus, it is likely that a synergistic effect of several parasite effectors contributes to the modulation of distinct signaling pathways and transcriptional responses altering DC migration. The identification and subsequent depletion of these effectors will provide a useful means to assess the importance of parasite-driven activation of DC migration in *Tg* virulence and the establishment of latent infections in mice. It is noteworthy that, after intraperitoneal type I parasite injection, *Tg* was reported to preferentially target pDCs in the peritoneum and to enhance their migration towards the marginal zone and T cell areas of the spleen. This process was found to be partially dependent on CCR2 [101]. Therefore, further *in vivo* investigations are required to clearly identify which DC subset(s) are targeted by the parasite at the site of infection after oral infection [8], and whether a differential impact on DC migratory functions is observed in an *in vivo* context between type I and type II parasites.

Concluding Remarks

DCs critically bridge innate and adaptive immunity through their capacity to sense microbial compounds and drive antigen-specific T cell activation. DCs perform this task by patrolling tissues in search of danger signals, by antigen presentation, and by secreting immunomodulatory cytokines that shape innate and adaptive immune responses. Hence, it is assumed that any manipulation of these key activities by the parasite may directly impact on parasite dissemination or persistence. A growing body of evidence suggests that the parasite targets each of these processes (summarized in Figure 3). In laboratory mice, type I strain infection results in uncontrolled parasite growth, rapid dissemination, and detrimental tissue inflammation that eventually kills the host. In contrast, type II parasite burden and inflammation are kept under control, allowing latent infections to establish in organs distant from the gut, the primary site of parasite entry. While a growing list of parasite effectors modulating host responses in macrophages and monocytes has been characterized, only a few were identified as possibly manipulating DC functions. It is likely that effectors previously identified in other cell types may hijack similar host pathways in DCs, notably *Tg*GRA24 [102], which targets the p38 MAPK signaling, and *Tg*IST, which impairs STAT1-mediated ISG transcription [37]. However, it is also possible that certain *Tg* effectors exert specific functions in DCs (see Outstanding Questions). Importantly, the genetic polymorphisms of some effectors delineate the hypervirulence of type I strain versus the latency of avirulent type II strains in mice, with a major impact of type I parasite-mediated modulation of cell-autonomous immunity. However, the ability of the different DC subsets to kill or restrict parasite growth remains elusive. This may have major consequences on the stimulation of distinct *Tg* innate sensing mechanisms. Whether *Tg* manipulates PRR-mediated detection of *Tg* PAMPs, and thereby the downstream pathways controlled by PRR stimulation, needs to be explored.

Outstanding Questions

How do surface or endocytic pattern-recognition receptors sense parasites residing in an intracellular vacuole?

Do previously identified parasite-secreted effectors modulate DC responses similarly to other cell types?

Which parasite effectors regulate DC migratory functions?

Are there parasite effectors that hijack the specialized endocytic pathway of DCs to sustain their intracellular life?

Are there type II strain-specific effectors involved in promoting parasite latency and host maintenance?

Are there tissue-specific responses of DCs to *T. gondii* infection?

DCs exhibit specialized trafficking pathways (e.g., dedicated for cross-presentation); it is thus possible that DC-specific *Tg* effectors hijack such pathways to support their intracellular lifestyle. Up to now, no type II-specific effector that manipulates host-cell responses to eventually promote parasite latency and host maintenance has been identified. Hence, identifying the key DC subsets operating in the gut of orally infected mice, or the brain and muscular tissues during parasite latency, and the characterization of their responses upon virulent versus latent infection are important goals for future studies. Based on the specificity of DC functions compared with macrophages, in-depth exploration of DC–*Toxoplasma* interactions is needed to open novel therapeutic avenues that would reduce inflammation-induced pathology and boost antigen-specific T cell responses, which could ultimately ameliorate the clinical outcome of cerebral chronic toxoplasmosis.

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