



Toxoplasma and Dendritic Cells

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***Toxoplasma* and dendritic cells: an intimate relationship that deserves further scrutiny**

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Abstract

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.

26 ***Toxoplasma* infection induces robust Th1 and cytotoxic responses**

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

46 As an obligatory intracellular parasite, *Tg* has the ability to invade all nucleated cells and to reside
47 within a **parasitophorous vacuole (PV)**, see Glossary). To modulate cell-intrinsic defenses, the
48 parasite releases effectors into the host through apicomplexan-specific secretory organelles called
49 **rhoptries (ROP)** and **dense granules (GRA)**. These effectors operate in concert to manipulate host
50 cell responses involved in cytokine and chemokine secretion, cell cycle progression, host survival

51 and metabolic homeostasis [6,7]. Strikingly, while a growing number of effectors dampening cell-
52 autonomous immunity are being characterized, very few effectors promoting parasite latency and
53 preservation of the host have been identified.

54 During natural oral *Tg* infection, dendritic cells (DCs) (Box 1) are one of the first cell types that
55 encounter parasites in the intestine [8]. DCs are key players in the local activation of cell-mediated
56 immunity against *Tg* by producing IL-12 that activates IFN- γ production by T cells and Natural
57 Killer (NK) cells [9]. T cells play a major role in controlling acute dissemination and keeping
58 chronic cerebral infection under control [9]. While the mechanisms by which *Tg* modulates host
59 responses have been extensively investigated in fibroblasts, monocytes and macrophages [6,7], how
60 DC functions are modulated by the parasite remains ill-defined. The consequences of these
61 interactions most likely impact on immuno-modulatory functions of DCs and on T cell activation,
62 and are therefore important for the outcome of infection. Since our current knowledge on these
63 questions mainly derives from the study of mouse models, this review concentrates on mouse
64 studies. One section is also dedicated to emerging findings on the specific responses of human DCs,
65 which substantially differ (e.g. parasite sensing).

66

67 **Innate recognition of *Tg* by DCs**

68 *The *Tg* Profilin-TLR11/12-IL-12 axis: a major pathway in DC responses in mice*

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

74 Pioneer studies reported that upon *Tg* systemic infection, the induction of IL-12 production by
75 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 Th1 immunity. In contrast to macrophages, IL-12 production in DCs downstream of MyD88 was found to be independent from the NFκB pathway but to rely on the transcription factor IRF8 [12] (Figure 1a). 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 to 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.

92

93 *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 tachyzoite antigens (STAg) [19]. However, the responses of splenic DCs isolated from infected mice or of splenic DCs infected *ex*

101 *vivo* with live parasites have not been explored in this study. Along this line, parasite surface
102 adhesins *Tg*MIC1/MIC4 were recently reported to stimulate IL-12 secretion in a TLR2-dependent
103 manner in bone marrow-derived DCs (BMDCs) [20] (Figure 3b). Alterations in the production of
104 additional TLR2-stimulated cytokines, notably type I interferons (IFN-I) [21], or in the regulation of
105 other DCs functions (e.g. antigen presentation [22]) were not examined in TLR2^{-/-} infected BMDCs.
106 Thus, further exploration of TLR2 signaling after *Tg* infection may reveal novel regulatory
107 mechanisms of DCs responses. Most importantly, while TLR2^{-/-}, TLR4^{-/-} and TLR9^{-/-} mice infected
108 systemically with *Tg* demonstrate limited susceptibility and modest defects in IL-12 production,
109 severe mortality was demonstrated in the same animals receiving the parasite orally [18,23,24].
110 Notably, DC maturation and migration to draining mesenteric lymph nodes (MLN) were altered in
111 TLR9^{-/-} mice together with decreased CD4⁺ T cell responses [23]. TLR2, TLR4 and TLR9 were also
112 demonstrated to be essential for the development of efficient IFN- γ responses by T cells in the small
113 intestine after oral infection [23,24]. In addition, parasite-induced damage of the intestinal mucosa is
114 decreased in TLR4^{-/-} mice and in mice treated with broad-spectrum antibiotics, suggesting that
115 mucosal immune responses to *Tg* also rely on the indirect stimulation of DCs by normal gut
116 microflora [25]. Hence, the route of infection likely impacts on the mechanisms of *Tg* innate sensing,
117 which may also differ after oral infection between the different DC subsets present in the intestinal
118 mucosa.

119

120 *Tg* innate sensing in human DCs

121 Deciphering the respective roles of TLRs in the sensing of *Tg* PAMPs is of particular relevance for
122 human DCs, which lack functional TLR11 and TLR12 genes. In contrast to murine DCs, human
123 monocyte-derived DCs fail to respond to STAGs as well as heat-killed (HK) or fixed parasites
124 [26,27]. The TLRs involved in *Tg* sensing in human DCs are not yet identified, however studies led
125 in human blood-derived monocytes, which are major producers of IL-12 upon infection, revealed

126 that parasite invasion is not essential to trigger cytokine responses. In line, phagocytic uptake of live
127 parasites and the acidification of the resulting phagosome enhance cytokine production compared to
128 **active invasion** (Figure 1b) [27]. When parasite **phagocytosis** (but not adhesion) is inhibited,
129 cytokine responses are abrogated, indicating that surface TLRs are not involved in *Tg* sensing. In
130 addition, as phagocytosis of HK but not irradiated parasites fails to stimulate cytokine production,
131 efficient innate recognition of *Tg* may rely on the detection of heat-labile compounds, possibly
132 parasite RNA [27]. Thus, based on what is known for phagosomes containing inert or microbial
133 antigenic material, phagocytosis of live parasites in human monocytes and DCs may trigger the
134 stimulation of endosomal TLRs following phago-lysosomal fusion, or of cytosolic PRRs after
135 phagosomal membrane disruption [28]. Importantly, as opposed to the mouse context in which the
136 cDC1 dominate the IL-12 response, the human cDC1 are non-responsive to *Tg* infection, and IL-12
137 and TNF α production following *Tg* stimulation is restricted to the cDC2 subset [27].

138
139 *How do PRRs sense parasites residing in intracellular vacuoles?*

140 Even in murine DCs, which have been more extensively studied, the molecular processes of PAMP
141 sensing from vacuolar parasites, remain a conundrum. Using trans-well experiments, Pifer et al.
142 demonstrated that contacts between murine DCs and DCs-depleted splenocytes infected by
143 tachyzoites are not required for TLR11-mediated IL-12 production [13]. This suggests that *Tg*PRF, a
144 cytoskeleton protein localized in the cytosol of parasites, is released into the medium and further
145 internalized by DCs into **endolysosomes**, where TLR11/TLR12 are localized [13] (Figure 1a).
146 Whether *Tg*PRF detection by distant DCs relies on the presence of the protein in apoptotic bodies,
147 necrotic debris, or **exosomes** remains an open question. In line with this idea, two independent
148 studies recently demonstrated an active role of exosomes released by type I and type II tachyzoites in
149 stimulating IL-10, IL-12 and TNF- α production by macrophages *in vitro*. When injected into mice,
150 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 distance” of 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 *Tg*PRF is critical to trigger IL-12 secretion, active invasion by parasites may activate distinct endosomal TLRs or cytosolic PRRs (Figure 1c and 1d). 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 proteins (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 (ATG) 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 and 1d). 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 up-regulate a certain set of ISGs in human fibroblasts in a STAT1-independent but NF- κ B-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 pathways. Of particular interest is the activation of the **inflammasome**. NLRP3 activation and IL-1 β 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 *TgGRA15* 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 cGAS sensor, are localized at the mitochondrial membrane and the **endoplasmic reticulum (ER)**, respectively [42] (Figure 1c and 1d). Interestingly, these two compartments tightly associate with the PVM, therefore are likely 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,

201 where endosomal TLRs reside [44,45] and the PV has been recently shown to intersect with various
202 endosomal compartments [46]. Hence, mechanisms distinct from PVM damage may promote the
203 delivery of PAMPs from vacuolar parasites into intracellular compartments implicated in microbial
204 innate sensing.

205

206 *DC cytokine secretion: beyond IL-12*

207 Owing to the crucial role of IL-12 in the induction of the cytotoxic and Th1 responses, most studies
208 have focused on the mechanisms by which splenic DCs secrete this cytokine. Nonetheless, the
209 distinct DC subsets operating during infection may secrete additional cytokines contributing to the
210 regulation of various immune responses (Box 1). This aspect is illustrated by a recent study showing
211 that ISG15 production, a IFN-I stimulated pro-inflammatory molecule, enhances the recruitment of
212 cDC1 specifically producing IL-1 β at the site of infection during type II parasite systemic infection
213 [47] (Figure 1d). Along this line, IFN-I play an important role in parasite infections [48,49]. In *Tg*-
214 infected mice, pDCs, which expand after oral infection with type II parasites and play a key role in
215 CD4⁺ T cell priming, were shown to secrete high levels of IFN- α [16,50]. In contrast, IFN- β is
216 mainly produced by inflammatory monocytes in the MLN and requires parasite phagocytosis [51].
217 Of note, human DCs actively phagocytose parasites and might therefore trigger IFN-I secretion upon
218 *Tg* infection. In addition, in the mouse, IFN-I serum levels gradually increase during systemic *Tg*
219 infection and the cytokine can be detected in the brain and spleen of infected mice. *Ifnar*^{-/-}
220 (Interferon alpha and beta receptor 1) mice orally infected with *Tg*, have increased parasite loads
221 compared to wild-type mice, correlated with slightly decreased survival [51,52]. Importantly, IFN-I
222 are prominent regulators of cDC1 turnover *in vivo* through a dose-dependent modulation of multiple
223 processes including DC apoptosis, proliferation, migration [53], and maturation [54] as well as their
224 **cross-presentation** efficacy [55]. Hence, the role of IFN-I (via an autocrine effect or produced by
225 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 down-regulation of retinoic acid production previously observed in mucosal DCs [8].

To conclude, while in mice, *Tg*PRF detection by TLR11/12 is critical to trigger the secretion of IL-12 and the IFN- γ response for early parasite clearance, active invasion of DCs by parasites and parasite phagocytosis in human cells likely stimulate additional innate sensing mechanisms, which are not 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.

248

249 **Modulation of *Tg* antigen presentation and T cell responses**

250

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

257 258 *Mechanisms of MHC-I presentation by Tg and modulation thereof*

259 Through the process of cross-presentation, MHC-I molecules can present antigenic peptides derived
260 from extracellular sources, including from microorganisms that reside in a phagosome or a vacuole
261 like *Tg*. The pathways by which *Tg* antigens access the MHC-I pathway have been partially
262 unraveled [61]. Active secretion of parasite antigens into the infected host cell is a major requirement
263 for efficient MHC-I presentation [62–65]. Moreover, emerging evidence shows that luminal and
264 membrane-bound antigens follow processing mechanisms that exhibit similarities as well as
265 distinctions. The analogies include the implication of the TAP transporter and the proteasome
266 suggesting that in both cases, antigenic precursors exit the vacuole and reach the host cytosol before
267 loading onto MHC-I molecules [66,67]. For luminal antigens, access to the DC cytosol is facilitated
268 by fusion of vesicles of the host ERGIC onto the vacuole [68] through a mechanism that depends on
269 the soluble NSF attachment protein receptor (SNARE) protein Sec22b [69]. This process promotes
270 the recruitment of components of the ER-Associated Degradation machinery onto the PVM, then
271 allowing retro-translocation of vacuolar antigens to the host cytosol. Intriguingly, Sec22b-mediated
272 ER-PV interactions are not involved in the presentation of the membrane-bound protective GRA6
273 antigen [70]. Instead, optimal processing of GRA6 requires the epitope to be located at the C-
274 terminus of GRA6 and to protrude into the host cytosol [70,71]. Of note, the protective membrane-
275 bound GRA6 antigen associates with a membranous, tubulo-vesicular **intravacuolar network (IVN)**

276 generated in the vacuolar space by parasite effectors. In the absence of this network, GRA6
277 redistributes to the PVM and is presented more efficiently by MHC-I, leading to enhanced CD8⁺ T
278 cell responses [72]. Thus, by trapping membrane-bound antigens, the network plays a negative
279 modulatory function on recognition of *Tg*-infected cells by CD8⁺ T cells (Figure 2).

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

289 Additional indirect mechanisms may contribute to the modulation of MHC-I presentation by the
290 parasite. Of particular interest is the role of the ER-associated **unfolded protein response (UPR)**
291 induced by viruses, bacteria and parasite infections [74]. UPR induction in DCs has recently been
292 implicated in the modulation of MHC-I antigen presentation in normal and pathological conditions
293 [75–77]. Interestingly, type I *Tg*ROP18 was reported to directly phosphorylate the host UPR sensor
294 ATF6 β , resulting in its degradation by the proteasome [78] (Figure 1c). DCs isolated from ATF6 β
295 KO mice exhibit a reduced ability to re-stimulate primed CD8⁺ T cells, but not CD4⁺ T cells,
296 suggesting a yet unexplored function of ATF6 β in MHC-I antigen presentation (Figure 2). However,
297 since an enhanced IFN- γ production by CD4⁺ T cells in mice infected with *Tg*ROP18 KO parasites
298 was also observed, it is likely that additional mechanisms beyond the targeting of ATF6 β -mediated
299 **cross-presentation** dampen T cell activation [78]. Therefore, whether *Tg*ROP18 directly impacts on
300 MHC-I presentation requires further investigation. Finally, it remains to be studied whether MHC-I

301 presentation is modulated during the chronic phase of the infection by persistent parasites present in
302 tissues, such as the muscles, the retina and the central nervous system. Indeed, we have recently
303 shown that MHC-I presentation of tachyzoite-derived antigens by neurons is pivotal for CD8⁺ T cell-
304 mediated parasite control in the brain [79]. However, it remains to be established if and how
305 effectors secreted by bradyzoites interfere with MHC-I presentation, both in the target neuronal cells
306 as well as in the surrounding DCs.

307

308 *Modulation of MHC-II presentation by Tg*

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

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

322 By preventing the upregulation of MHC-II on the surface of infected DCs, *Tg* alters the activation of
323 CD4⁺ T cells *in vivo* [84] and impedes parasite control. In agreement, CD74^{-/-}-H2-DM^{-/-} KO mice
324 fail to control infection by type II strains, correlating with high cerebral cyst burden, showing the
325 important role of MHC-II antigen presentation during chronic toxoplasmosis [80]. In line,

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 non canonical 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).

333

334 *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 the PEC and LN, compared to type II infection [89]. DCs recruited at the site of infection display a reduced expression of CD80 and MHC-I compared to DCs analyzed during type II infection. As expected, the 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 to 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 CD8 α ⁻ and CD8 α ⁺ 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 to 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 line, DCs infected by type II parasites, but not bystander cells, migrate significantly longer distances compared to type I-infected DCs in a 3D matrix [96]. Similarly, an enrichment of migratory type II-infected DCs compared to type I strain-infected DCs was monitored in spleen and MLN 16 hours after inoculation of pre-infected BMDCs [95]. Importantly, infection of monocytes, NK, B and T cells fail 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 amoeboid 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 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 contribute 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 mean to assess the importance of parasite-driven activation of DC migration in *Tg* virulence and the establishment of latent infections in mice. Noteworthy, 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.

400

401 **Concluding remarks**

402 DCs critically bridge innate and adaptive immunity through their capacity to sense microbial
403 compounds and drive antigen-specific T cell activation. DCs perform this task by patrolling tissues
404 in search of danger signals, by antigen presentation, and by secreting immuno-modulatory cytokines
405 that shape innate and adaptive immune responses. Hence, it is assumed that any manipulation of
406 these key activities by the parasite may directly impact on parasite dissemination or persistence. A
407 growing body of evidence suggests that the parasite targets each of these processes (summarized in
408 Figure 3). In laboratory mice, type I strain infection results in uncontrolled parasite growth, rapid
409 dissemination and detrimental tissue inflammation that eventually kills the host. In contrast, type II
410 parasite burden and inflammation are kept under control, allowing latent infections to establish in
411 organs distant from the gut, the primary site of parasite entry. While a growing list of parasite
412 effectors modulating host responses in macrophages and monocytes has been characterized, only a
413 few were identified as possibly manipulating DC functions. It is likely that effectors previously
414 identified in other cell types may hijack similar host pathways in DCs, notably *TgGRA24* [102],
415 which targets the p38 MAPK signaling and *TgIST*, which impairs STAT1-mediated ISG
416 transcription [37]. However, it is also possible that certain *Tg* effectors exert specific functions in
417 DCs (see Outstanding Questions). Importantly, the genetic polymorphisms of some effectors
418 delineates the hyper-virulence of type I strain *versus* the latency of avirulent type II strains in mice,
419 with a major impact of type I parasite-mediated modulation of cell-autonomous immunity. However,
420 the ability of the different DC subsets to kill or restrict parasite growth remains elusive. This may
421 have major consequences on the stimulation of distinct *Tg* innate sensing mechanisms. Whether *Tg*
422 manipulates PRR-mediated detection of *Tg* PAMPs and thereby the downstream pathways controlled
423 by PRR stimulation, needs to be explored.

424 DCs exhibit specialized trafficking pathways (e.g. dedicated for cross-presentation), it is thus
425 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 to 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.

434

References

- 1 Sibley, L.D. *et al.* (2009) Genetic diversity of *Toxoplasma gondii* in animals and humans. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364, 2749–2761
- 2 Fuglewicz, A. *et al.* (2017) Relationship between toxoplasmosis and schizophrenia: A review. *Adv. Clin. Exp. Med.* 26, 1033–1038
- 3 Bayani, M. *et al.* (2019) *Toxoplasma gondii* infection and risk of Parkinson and Alzheimer diseases: A systematic review and meta-analysis on observational studies. *Acta Trop.* 196, 165–171
- 4 Hassan, M.A. *et al.* (2019) Clonal and atypical *Toxoplasma* strain differences in virulence vary with mouse sub-species. *Int. J. Parasitol.* 49, 63–70
- 5 Murillo-León, M. *et al.* (2019) Molecular mechanism for the control of virulent *Toxoplasma gondii* infections in wild-derived mice. *Nat. Commun.* 10,
- 6 Hakimi, M.-A. *et al.* (2017) *Toxoplasma* Effectors Targeting Host Signaling and Transcription. *Clin. Microbiol. Rev.* 30, 615–645

449 7 Lima, T.S. and Lodoen, M.B. (2019) Mechanisms of Human Innate Immune Evasion by
450 *Toxoplasma gondii*. *Front. Cell. Infect. Microbiol.* 9,

451 8 Cohen, S.B. and Denkers, E.Y. (2015) Impact of *Toxoplasma gondii* on Dendritic Cell Subset
452 Function in the Intestinal Mucosa. *J. Immunol. Baltim. Md 1950* 195, 2754–2762

453 9 Dupont, C.D. *et al.* (2012) Immune response and immunopathology during toxoplasmosis.
454 *Semin. Immunopathol.* 34, 793–813

455 10 Liu, C.-H. *et al.* (2006) Cutting edge: dendritic cells are essential for in vivo IL-12 production
456 and development of resistance against *Toxoplasma gondii* infection in mice. *J. Immunol.*
457 *Baltim. Md 1950* 177, 31–35

458 11 Hou, B. *et al.* (2011) Critical coordination of innate immune defense against *Toxoplasma*
459 *gondii* by dendritic cells responding via their Toll-like receptors. *Proc. Natl. Acad. Sci. U. S. A.*
460 108, 278–283

461 12 Raetz, M. *et al.* (2013) Cooperation of TLR12 and TLR11 in the IRF8-dependent IL-12
462 response to *Toxoplasma gondii* profilin. *J. Immunol. Baltim. Md 1950* 191, 4818–4827

463 13 Pifer, R. *et al.* (2011) UNC93B1 is essential for TLR11 activation and IL-12-dependent host
464 resistance to *Toxoplasma gondii*. *J. Biol. Chem.* 286, 3307–3314

465 14 Yarovinsky, F. *et al.* (2005) TLR11 activation of dendritic cells by a protozoan profilin-like
466 protein. *Science* 308, 1626–1629

467 15 Plattner, F. *et al.* (2008) *Toxoplasma* profilin is essential for host cell invasion and TLR11-
468 dependent induction of an interleukin-12 response. *Cell Host Microbe* 3, 77–87

469 16 Koblansky, A.A. *et al.* (2013) Recognition of profilin by Toll-like receptor 12 is critical for host
470 resistance to *Toxoplasma gondii*. *Immunity* 38, 119–130

471 17 Melo, M.B. *et al.* (2010) UNC93B1 mediates host resistance to infection with *Toxoplasma*
472 *gondii*. *PLoS Pathog.* 6, e1001071

- 473 18 Debierre-Grockiego, F. *et al.* (2007) Activation of TLR2 and TLR4 by
474 glycosylphosphatidylinositols derived from *Toxoplasma gondii*. *J. Immunol. Baltim. Md 1950*
475 179, 1129–1137
- 476 19 Scanga, C.A. *et al.* (2002) Cutting edge: MyD88 is required for resistance to *Toxoplasma*
477 *gondii* infection and regulates parasite-induced IL-12 production by dendritic cells. *J. Immunol.*
478 *Baltim. Md 1950* 168, 5997–6001
- 479 20 Sardinha-Silva, A. *et al.* (2017) *Toxoplasma gondii* microneme proteins 1 and 4 bind to Toll-
480 like receptors 2 and 4 N-glycans triggering innate immune response. *bioRxiv* DOI:
481 10.1101/187690
- 482 21 Dietrich, N. *et al.* (2010) Murine toll-like receptor 2 activation induces type I interferon
483 responses from endolysosomal compartments. *PloS One* 5, e10250
- 484 22 Corridoni, D. *et al.* (2019) NOD2 and TLR2 Signal via TBK1 and PI31 to Direct Cross-
485 Presentation and CD8 T Cell Responses. *Front. Immunol.* 10, 958
- 486 23 Minns, L.A. *et al.* (2006) TLR9 is required for the gut-associated lymphoid tissue response
487 following oral infection of *Toxoplasma gondii*. *J. Immunol. Baltim. Md 1950* 176, 7589–7597
- 488 24 Benson, A. *et al.* (2009) Gut commensal bacteria direct a protective immune response against
489 *Toxoplasma gondii*. *Cell Host Microbe* 6, 187–196
- 490 25 Heimesaat, M.M. *et al.* (2007) Exacerbation of murine ileitis by Toll-like receptor 4 mediated
491 sensing of lipopolysaccharide from commensal *Escherichia coli*. *Gut* 56, 941–948
- 492 26 Subauste, C.S. and Wessendarp, M. (2000) Human dendritic cells discriminate between viable
493 and killed *Toxoplasma gondii* tachyzoites: dendritic cell activation after infection with viable
494 parasites results in CD28 and CD40 ligand signaling that controls IL-12-dependent and -
495 independent T cell production of IFN-gamma. *J. Immunol. Baltim. Md 1950* 165, 1498–1505

- 496 27 Tosh, K.W. *et al.* (2016) The IL-12 Response of Primary Human Dendritic Cells and
497 Monocytes to *Toxoplasma gondii* Is Stimulated by Phagocytosis of Live Parasites Rather Than
498 Host Cell Invasion. *J. Immunol. Baltim. Md 1950* 196, 345–356
- 499 28 Pauwels, A.-M. *et al.* (2017) Patterns, Receptors, and Signals: Regulation of Phagosome
500 Maturation. *Trends Immunol.* 38, 407–422
- 501 29 Silva, V.O. *et al.* (2018) Extracellular vesicles isolated from *Toxoplasma gondii* induce host
502 immune response. *Parasite Immunol.* 40, e12571
- 503 30 Li, Y. *et al.* (2018) Characterization of exosomes derived from *Toxoplasma gondii* and their
504 functions in modulating immune responses. *Int. J. Nanomedicine* 13, 467–477
- 505 31 Christian, D.A. *et al.* (2014) Use of transgenic parasites and host reporters to dissect events that
506 promote interleukin-12 production during toxoplasmosis. *Infect. Immun.* 82, 4056–4067
- 507 32 Saeij, J.P. and Frickel, E.-M. (2017) Exposing *Toxoplasma gondii* hiding inside the vacuole: a
508 role for GBPs, autophagy and host cell death. *Curr. Opin. Microbiol.* 40, 72–80
- 509 33 Lee, Y. *et al.* (2015) p62 Plays a Specific Role in Interferon- γ -Induced Presentation of a
510 *Toxoplasma* Vacuolar Antigen. *Cell Rep.* 13, 223–233
- 511 34 Schneider, A.G. *et al.* (2013) *Toxoplasma gondii* triggers phosphorylation and nuclear
512 translocation of dendritic cell STAT1 while simultaneously blocking IFN γ -induced STAT1
513 transcriptional activity. *PloS One* 8, e60215
- 514 35 Lang, C. *et al.* (2012) Impaired chromatin remodelling at STAT1-regulated promoters leads to
515 global unresponsiveness of *Toxoplasma gondii*-infected macrophages to IFN- γ . *PLoS Pathog.*
516 8, e1002483
- 517 36 Olias, P. *et al.* (2016) *Toxoplasma* Effector Recruits the Mi-2/NuRD Complex to Repress
518 STAT1 Transcription and Block IFN- γ -Dependent Gene Expression. *Cell Host Microbe* 20,
519 72–82

520 37 Gay, G. *et al.* (2016) *Toxoplasma gondii* TgIST co-opts host chromatin repressors dampening
521 STAT1-dependent gene regulation and IFN- γ -mediated host defenses. *J. Exp. Med.* 213, 1779–
522 1798

523 38 Rosowski, E.E. and Saeij, J.P.J. (2012) *Toxoplasma gondii* clonal strains all inhibit STAT1
524 transcriptional activity but polymorphic effectors differentially modulate IFN γ induced gene
525 expression and STAT1 phosphorylation. *PloS One* 7, e51448

526 39 Rosowski, E.E. *et al.* (2011) Strain-specific activation of the NF-kappaB pathway by GRA15, a
527 novel *Toxoplasma gondii* dense granule protein. *J. Exp. Med.* 208, 195–212

528 40 Gov, L. *et al.* (2013) Human innate immunity to *Toxoplasma gondii* is mediated by host
529 caspase-1 and ASC and parasite GRA15. *mBio* 4,

530 41 Gorfu, G. *et al.* (2014) Dual role for inflammasome sensors NLRP1 and NLRP3 in murine
531 resistance to *Toxoplasma gondii*. *mBio* 5,

532 42 Schlee, M. and Hartmann, G. (2016) Discriminating self from non-self in nucleic acid sensing.
533 *Nat. Rev. Immunol.* 16, 566–580

534 43 Pernas, L. *et al.* (2014) *Toxoplasma* effector MAF1 mediates recruitment of host mitochondria
535 and impacts the host response. *PLoS Biol.* 12, e1001845

536 44 Wijdeven, R.H. *et al.* (2015) ER contact sites direct late endosome transport. *BioEssays News*
537 *Rev. Mol. Cell. Dev. Biol.* 37, 1298–1302

538 45 Cabukusta, B. and Neefjes, J. (2018) Mechanisms of lysosomal positioning and movement.
539 *Traffic Cph. Den.* 19, 761–769

540 46 Romano, J.D. *et al.* (2017) The parasite *Toxoplasma* sequesters diverse Rab host vesicles
541 within an intravacuolar network. *J. Cell Biol.* 216, 4235–4254

542 47 Napolitano, A. *et al.* (2018) Cysteine-Reactive Free ISG15 Generates IL-1 β -Producing CD8 α +
543 Dendritic Cells at the Site of Infection. *J. Immunol. Baltim. Md 1950* 201, 604–614

544 48 Beiting, D.P. (2014) Protozoan parasites and type I interferons: a cold case reopened. *Trends*
545 *Parasitol.* 30, 491–498

546 49 Silva-Barrios, S. and Stäger, S. (2017) Protozoan Parasites and Type I IFNs. *Front. Immunol.* 8,
547 50 Pepper, M. *et al.* (2008) Plasmacytoid dendritic cells are activated by *Toxoplasma gondii* to
548 present antigen and produce cytokines. *J. Immunol. Baltim. Md 1950* 180, 6229–6236

549 51 Han, S.-J. *et al.* (2014) Internalization and TLR-dependent type I interferon production by
550 monocytes in response to *Toxoplasma gondii*. *Immunol. Cell Biol.* 92, 872–881

551 52 Orellana, M.A. *et al.* (1991) Role of beta interferon in resistance to *Toxoplasma gondii*
552 infection. *Infect. Immun.* 59, 3287–3290

553 53 Mattei, F. *et al.* (2009) Type I IFN regulate DC turnover in vivo. *Eur. J. Immunol.* 39, 1807–
554 1818

555 54 Pantel, A. *et al.* (2014) Direct type I IFN but not MDA5/TLR3 activation of dendritic cells is
556 required for maturation and metabolic shift to glycolysis after poly IC stimulation. *PLoS Biol.*
557 12, e1001759

558 55 Schiavoni, G. *et al.* (2013) Type I Interferons as Stimulators of DC-Mediated Cross-Priming:
559 Impact on Anti-Tumor Response. *Front. Immunol.* 4, 483

560 56 Couturier-Maillard, A. *et al.* (2018) Interleukin-22-deficiency and microbiota contribute to the
561 exacerbation of *Toxoplasma gondii*-induced intestinal inflammation. *Mucosal Immunol.* 11,
562 1181–1190

563 57 Ryffel, B. *et al.* (2019) Blockade of IL-33R/ST2 Signaling Attenuates *Toxoplasma gondii*
564 Ileitis Depending on IL-22 Expression. *Front. Immunol.* 10, 702

565 58 Blander, J.M. (2018) Regulation of the Cell Biology of Antigen Cross-Presentation. *Annu. Rev.*
566 *Immunol.* 36, 717–753

567 59 Iwasaki, A. and Medzhitov, R. (2010) Regulation of adaptive immunity by the innate immune
568 system. *Science* 327, 291–295

569 60 Nair-Gupta, P. *et al.* (2014) TLR signals induce phagosomal MHC-I delivery from the
570 endosomal recycling compartment to allow cross-presentation. *Cell* 158, 506–521

571 61 Gros, M. and Amigorena, S. (2019) Regulation of Antigen Export to the Cytosol During Cross-
572 Presentation. *Front. Immunol.* 10, 41

573 62 Kwok, L.-Y. *et al.* (2003) The induction and kinetics of antigen-specific CD8 T cells are
574 defined by the stage specificity and compartmentalization of the antigen in murine
575 toxoplasmosis. *J. Immunol. Baltim. Md 1950* 170, 1949–1957

576 63 Gregg, B. *et al.* (2011) Subcellular antigen location influences T-cell activation during acute
577 infection with *Toxoplasma gondii*. *PloS One* 6, e22936

578 64 Dupont, C.D. *et al.* (2014) Parasite fate and involvement of infected cells in the induction of
579 CD4+ and CD8+ T cell responses to *Toxoplasma gondii*. *PLoS Pathog.* 10, e1004047

580 65 Grover, H.S. *et al.* (2014) Impact of regulated secretion on antiparasitic CD8 T cell responses.
581 *Cell Rep.* 7, 1716–1728

582 66 Gubbels, M.-J. *et al.* (2005) Class I major histocompatibility complex presentation of antigens
583 that escape from the parasitophorous vacuole of *Toxoplasma gondii*. *Infect. Immun.* 73, 703–
584 711

585 67 Blanchard, N. *et al.* (2008) Immunodominant, protective response to the parasite *Toxoplasma*
586 *gondii* requires antigen processing in the endoplasmic reticulum. *Nat. Immunol.* 9, 937–944

587 68 Goldszmid, R.S. *et al.* (2009) Host ER-parasitophorous vacuole interaction provides a route of
588 entry for antigen cross-presentation in *Toxoplasma gondii*-infected dendritic cells. *J. Exp. Med.*
589 206, 399–410

590 69 Cebrian, I. *et al.* (2011) Sec22b regulates phagosomal maturation and antigen crosspresentation
591 by dendritic cells. *Cell* 147, 1355–1368

592 70 Buaille, C. *et al.* (2017) MHC I presentation of *Toxoplasma gondii* immunodominant antigen
593 does not require Sec22b and is regulated by antigen orientation at the vacuole membrane. *Eur.*
594 *J. Immunol.* 47, 1160–1170

595 71 Feliu, V. *et al.* (2013) Location of the CD8 T cell epitope within the antigenic precursor
596 determines immunogenicity and protection against the *Toxoplasma gondii* parasite. *PLoS*
597 *Pathog.* 9, e1003449

598 72 Lopez, J. *et al.* (2015) Intravacuolar Membranes Regulate CD8 T Cell Recognition of
599 Membrane-Bound *Toxoplasma gondii* Protective Antigen. *Cell Rep.* 13, 2273–2286

600 73 Cebrian, I. *et al.* (2016) Rab22a controls MHC-I intracellular trafficking and antigen cross-
601 presentation by dendritic cells. *EMBO Rep.* 17, 1753–1765

602 74 Galluzzi, L. *et al.* (2017) Endoplasmic reticulum stress and unfolded protein response in
603 infection by intracellular parasites. *Future Sci. OA* 3, FSO198

604 75 Cubillos-Ruiz, J.R. *et al.* (2015) ER Stress Sensor XBP1 Controls Anti-tumor Immunity by
605 Disrupting Dendritic Cell Homeostasis. *Cell* 161, 1527–1538

606 76 Grootjans, J. *et al.* (2016) The unfolded protein response in immunity and inflammation. *Nat.*
607 *Rev. Immunol.* 16, 469–484

608 77 Osorio, F. *et al.* (2018) Antigen presentation unfolded: identifying convergence points between
609 the UPR and antigen presentation pathways. *Curr. Opin. Immunol.* 52, 100–107

610 78 Yamamoto, M. *et al.* (2011) ATF6beta is a host cellular target of the *Toxoplasma gondii*
611 virulence factor ROP18. *J. Exp. Med.* 208, 1533–1546

612 79 Salvioni, A. *et al.* (2019) Robust Control of a Brain-Persisting Parasite through MHC I
613 Presentation by Infected Neurons. *Cell Rep.* 27, 3254–3268.e8

614 80 Leroux, L.-P. *et al.* (2015) Parasite Manipulation of the Invariant Chain and the Peptide Editor
615 H2-DM Affects Major Histocompatibility Complex Class II Antigen Presentation during
616 *Toxoplasma gondii* Infection. *Infect. Immun.* 83, 3865–3880

617 81 Lüder, C.G.K. *et al.* (2003) Toxoplasma gondii inhibits MHC class II expression in neural
618 antigen-presenting cells by down-regulating the class II transactivator CIITA. *J.*
619 *Neuroimmunol.* 134, 12–24

620 82 Lang, C. *et al.* (2006) Diverse mechanisms employed by Toxoplasma gondii to inhibit IFN- γ -
621 induced major histocompatibility complex class II gene expression. *Microbes Infect.* 8, 1994–
622 2005

623 83 Kim, S.-K. *et al.* (2007) Toxoplasma gondii Dysregulates IFN- γ -Inducible Gene Expression in
624 Human Fibroblasts: Insights from a Genome-Wide Transcriptional Profiling. *J. Immunol.* 178,
625 5154–5165

626 84 McKee, A.S. *et al.* (2004) Functional inactivation of immature dendritic cells by the
627 intracellular parasite Toxoplasma gondii. *J. Immunol. Baltim. Md 1950* 173, 2632–2640

628 85 Grover, H.S. *et al.* (2012) The Toxoplasma gondii peptide AS15 elicits CD4 T cells that can
629 control parasite burden. *Infect. Immun.* 80, 3279–3288

630 86 Liu, E. *et al.* (2015) Atg5 but not Atg7 in dendritic cells enhances IL-2 and IFN- γ production
631 by Toxoplasma gondii-reactive CD4+ T cells. *Microbes Infect.* 17, 275–284

632 87 Lee, H.K. *et al.* (2010) In vivo requirement for Atg5 in antigen presentation by dendritic cells.
633 *Immunity* 32, 227–239

634 88 Séguin, R. and Kasper, L.H. (1999) Sensitized lymphocytes and CD40 ligation augment
635 interleukin-12 production by human dendritic cells in response to Toxoplasma gondii. *J. Infect.*
636 *Dis.* 179, 467–474

637 89 Tait, E.D. *et al.* (2010) Virulence of Toxoplasma gondii is associated with distinct dendritic cell
638 responses and reduced numbers of activated CD8+ T cells. *J. Immunol. Baltim. Md 1950* 185,
639 1502–1512

640 90 Straw, A.D. *et al.* (2003) CD154 plays a central role in regulating dendritic cell activation
641 during infections that induce Th1 or Th2 responses. *J. Immunol. Baltim. Md 1950* 170, 727–734

642 91 Schulz, O. *et al.* (2000) CD40 triggering of heterodimeric IL-12 p70 production by dendritic
643 cells in vivo requires a microbial priming signal. *Immunity* 13, 453–462

644 92 Courret, N. *et al.* (2006) CD11c- and CD11b-expressing mouse leukocytes transport single
645 *Toxoplasma gondii* tachyzoites to the brain. *Blood* 107, 309–316

646 93 Konradt, C. *et al.* (2016) Endothelial cells are a replicative niche for entry of *Toxoplasma*
647 *gondii* to the central nervous system. *Nat. Microbiol.* 1, 16001

648 94 Lambert, H. *et al.* (2006) Induction of dendritic cell migration upon *Toxoplasma gondii*
649 infection potentiates parasite dissemination. *Cell. Microbiol.* 8, 1611–1623

650 95 Lambert, H. *et al.* (2009) The *Toxoplasma gondii*-shuttling function of dendritic cells is linked
651 to the parasite genotype. *Infect. Immun.* 77, 1679–1688

652 96 Kanatani, S. *et al.* (2015) Infection by *Toxoplasma gondii* Induces Amoeboid-Like Migration
653 of Dendritic Cells in a Three-Dimensional Collagen Matrix. *PloS One* 10, e0139104

654 97 Lambert, H. *et al.* (2011) Migratory responses of leukocytes infected with *Toxoplasma gondii*.
655 *Microbes Infect.* 13, 96–102

656 98 Bhandage, A.K. and Barragan, A. (2019) Calling in the CaValry-*Toxoplasma gondii* Hijacks
657 GABAergic Signaling and Voltage-Dependent Calcium Channel Signaling for Trojan horse-
658 Mediated Dissemination. *Front. Cell. Infect. Microbiol.* 9, 61

659 99 Persat, F. *et al.* (2012) A synthetic peptide derived from the parasite *Toxoplasma gondii*
660 triggers human dendritic cells' migration. *J. Leukoc. Biol.* 92, 1241–1250

661 100 Weidner, J.M. *et al.* (2016) Migratory activation of parasitized dendritic cells by the protozoan
662 *Toxoplasma gondii* 14-3-3 protein. *Cell. Microbiol.* 18, 1537–1550

663 101 Bierly, A.L. *et al.* (2008) Dendritic cells expressing plasmacytoid marker PDCA-1 are Trojan
664 horses during *Toxoplasma gondii* infection. *J. Immunol. Baltim. Md 1950* 181, 8485–8491

665 102 Braun, L. *et al.* (2013) A *Toxoplasma* dense granule protein, GRA24, modulates the early
666 immune response to infection by promoting a direct and sustained host p38 MAPK activation.
667 *J. Exp. Med.* 210, 2071–2086

668 103 Dalod, M. *et al.* (2014) Dendritic cell maturation: functional specialization through signaling
669 specificity and transcriptional programming. *EMBO J.* 33, 1104–1116

670 104 Evans, R.J. *et al.* (2018) The Interplay of Host Autophagy and Eukaryotic Pathogens. *Front.*
671 *Cell Dev. Biol.* 6, 118

672 105 Subauste, C.S. (2019) Interplay Between *Toxoplasma gondii*, Autophagy, and Autophagy
673 Proteins. *Front. Cell. Infect. Microbiol.* 9, 139

674 106 Corcino, Y.L. *et al.* (2019) Epidermal growth factor receptor promotes cerebral and retinal
675 invasion by *Toxoplasma gondii*. *Sci. Rep.* 9, 669

676 107 Ghislat, G. and Lawrence, T. (2018) Autophagy in dendritic cells. *Cell. Mol. Immunol.* 15, 944–
677 952

678 108 Wang, Y. *et al.* (2009) Host cell autophagy is induced by *Toxoplasma gondii* and contributes to
679 parasite growth. *J. Biol. Chem.* 284, 1694–1701

680 109 Yarovinsky, F. *et al.* (2006) Toll-like receptor recognition regulates immunodominance in an
681 antimicrobial CD4+ T cell response. *Immunity* 25, 655–664

682 110 Draheim, M. *et al.* (2017) Profiling MHC II immunopeptidome of blood-stage malaria reveals
683 that cDC1 control the functionality of parasite-specific CD4 T cells. *EMBO Mol. Med.* 9, 1605–
684 1621

685 111 Fuks, J.M. *et al.* (2012) GABAergic signaling is linked to a hypermigratory phenotype in
686 dendritic cells infected by *Toxoplasma gondii*. *PLoS Pathog.* 8, e1003051

687 112 Kanatani, S. *et al.* (2017) Voltage-dependent calcium channel signaling mediates GABAA
688 receptor-induced migratory activation of dendritic cells infected by *Toxoplasma gondii*. *PLoS*
689 *Pathog.* 13, e1006739

690 113 Ólafsson, E.B. *et al.* (2018) *Toxoplasma gondii* infection shifts dendritic cells into an amoeboid
691 rapid migration mode encompassing podosome dissolution, secretion of TIMP-1, and reduced
692 proteolysis of extracellular matrix. *Cell. Microbiol.* 20,
693 114 Weidner, J.M. *et al.* (2013) Rapid cytoskeleton remodelling in dendritic cells following
694 invasion by *Toxoplasma gondii* coincides with the onset of a hypermigratory phenotype. *Cell.*
695 *Microbiol.* 15, 1735–1752

696

697 Glossary

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

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

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

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

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

711 **Exosome:** extracellular vesicle originated from the endocytic compartment involved in cell to cell
712 communication.

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

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

718 **Intravacuolar network (IVN):** network of membrane tubules and vesicles present into the vacuolar
719 space of the parasitophorous vacuole that interconnect the parasites and also connect the parasites to
720 the vacuole limiting membrane.

721 **Parasitophorous vacuole (PV):** intracellular vacuole in which the parasites replicate protected from
722 the host defense mechanisms.

723 **Pathogen-associated molecular pattern (PAMP):** small molecular motifs found in microbial
724 molecules recognized by pattern recognition receptors.

725 **Pattern recognition receptor (PRR):** host sensors that detect PAMPs and trigger signaling
726 pathways involved in innate immune responses.

727 **Phagocytosis:** mechanism by which phagocytes engulf and digest large particles and
728 microorganisms.

729 **Rhoptry (ROP):** specific apicomplexan secretory organelle containing proteins involved in parasite
730 invasion, PV formation and modulation of host cell responses.

731 **Unfolded protein response (UPR):** ER stress response induced by an accumulation of misfolded
732 proteins in the ER, changes in lipid homeostasis, TLR stimulation and oxidative stresses. It is
733 characterized by the activation of three ER sensors: ATF6, PERK and IRE1 α which induce
734 transcriptional programs aiming to restore cell homeostasis but can lead to the induction of cell
735 apoptosis.

736

737

Box 1: Dendritic cell functions and subsets

Dendritic cells (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 semi-mature 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 Major Histocompatibility Complex (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).

Table I. Dendritic cell subsets.

DC subsets	Monocyte DC	cDC1	cDC2	pDC
Type	Migratory DC	Lymphoid resident DC (CD8 α^+) Migratory DC (CD103 $^+$)	Lymphoid resident DC	Lymphoid resident DC
Mouse markers	CD11b $^+$ Lyc6C $^{hi/lo}$ CD64 $^+$	CD11c $^+$ MHC-II $^+$ XCR1 $^+$ CLEC9A $^+$ FLT3 $^+$ CD11b $^-$ CD8 α^+ or CD103 $^+$	CD11c $^+$ MHC-II $^+$ CD11b $^+$ CD24 $^+$ CD172a $^+$ CD8 α^-	CD11c int MHC-II int B220 $^+$ PDCA1 $^+$ SiglecH $^+$ Lyc6C $^+$
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 IL12 production TLR3 induced Cross-presentation on MHC-I CD8 T cell response	Against extracellular pathogens IL12, TNF α production Presentation on MHC-II CD4 T cell response	IFN I/III, IL12, TNF α , IL6 production TLR7, TLR9 induced CD4 and CD8 T cell response

754

755 **Box 2: *Tg* modulation of autophagy in DCs**

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

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

776

777 **Box 3: Mechanisms of MHC-II presentation by *T. gondii***

Two natural CD4⁺ T cell antigens from *Tg* were described so far; the AS15 peptide derives from the TgCD4Ag28m protein [85], predicted to be a secreted protein; the second one derives from *Tg*PRF [109]. Based on the fact that the *Tg* CD4Ag28m-derived AS15 peptide is robustly presented even when DC are fed with heat-killed parasites, it is likely that both secreted and non-secreted 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. DC 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-1 β or TNF- α [86], suggesting that it may be linked to a reduced delivery of antigens to the MHC-II presentation pathway.

790

Box 4: Modulation of DC migratory functions by *T. gondii*

In vitro, BMDC and human MDCC 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 DC 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_v1.3. More recently, the Barragan laboratory further identified a TIMP-1-CD63-ITGB1-FAK signaling axis hijacked by *Tg* to drive high-speed amoeboid 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 amoeboid-like motility

803 [96,114]. These morphological changes require parasite invasion of the DC but are independent of
804 GABAergic signaling, in agreement with the hypermotility phenotype being independent of
805 chemotactic cues [114]. In particular, the initial hypermotility phenotype of infected DCs does not
806 depend on CCR7 or CCR5, but infected DCs up-regulate CCR7 to potentiate their chemotactic
807 migration [111,114].

808

809 **Legends**

810 **Figure 1, Key Figure: Innate sensing and modulation of signaling pathways in *Tg*-infected**
811 **DCs. (a)** Internalization of parasite debris, STAg (Soluble Tachyzoite Antigen), *Tg*PRF (*Tg* profilin)
812 or *Tg* exosomes stimulate IRF8-mediated production of IL-12 via a TLR11/12 and MyD88-
813 dependent pathway in mice. **(b)** Phagocytosis of live or killed parasites results in TLR (Toll Like
814 Receptors) activation after phagolysosomal fusion. TLR11 and TLR12 play a major role in triggering
815 cytokine response in mice. However, other endosomal TLRs, such as TLR 3/7/8/9 may be also
816 stimulated, notably in human DCs, which lack functional TLR11 and TLR12 genes. Parasite
817 degradation and partial disruption of the phagosomal membrane may lead to the release of
818 RNA/DNA from the parasite sensed by cytosolic PRRs (Pattern Recognition Receptors) described to
819 induce type I IFN production. **(c)** Innate sensing of type I parasites that have actively invaded DC
820 relies on MyD88-dependent activation of TLRs, yet to be fully uncovered. Possible additional
821 mechanisms may involve the RIG-1 and STING sensors localized at the host mitochondria and
822 Endoplasmic Reticulum, respectively, two compartments tightly associated with the parasitophorous
823 vacuole (PV). IFN- γ stimulation triggers the phosphorylation of STAT1, but the transcriptional
824 activation of Interferon-stimulated gene (ISG), notably IRGs, is impaired in type I-infected DC,
825 likely by a *Tg*IST-mediated process. The *Tg* ROP5/17/18 effectors also block IRG (Immune-Related
826 GTPases)-induced vacuole damage upon IFN- γ stimulation, while *Tg* ROP18 (*Tg* ROP18) triggers
827 ATF6 β phosphorylation and degradation, resulting in decreased CD8⁺ T cell activation. Also, MHC-

828 I, MHC-II and co-stimulatory molecule surface expression are down-regulated by type I parasites.
829 (d) The innate sensing of type II parasites also relies on MyD88-dependent activation of TLR.
830 Distinct sensing mechanisms compared to type I parasites may be induced, correlating with partial
831 vacuole disruption. Upon IFN γ stimulation, PV damage and antigen escape to the cytosol is mediated
832 by autophagy proteins (ATG and p62) and IRG / GBP recruitment at the PV membrane. Other ISG
833 genes may be also induced in a *Tg*GRA15II- and NF κ B-dependent manner. Possible leakage of
834 parasite RNA/DNA upon PV disruption may activate cytosolic PRRs. In addition, ISG15 induces IL-
835 1 β production in infected cDC1. A putative activation of the inflammasome has not been
836 investigated. Type II infection itself also triggers the phosphorylation of STAT1 but *Tg*IST effector
837 likely reduces STAT1-mediated IRG/GBP expression. *Tg*IST is also likely involved in the down-
838 regulation of MHC-II molecule surface expression upon IFN- γ stimulation.

839

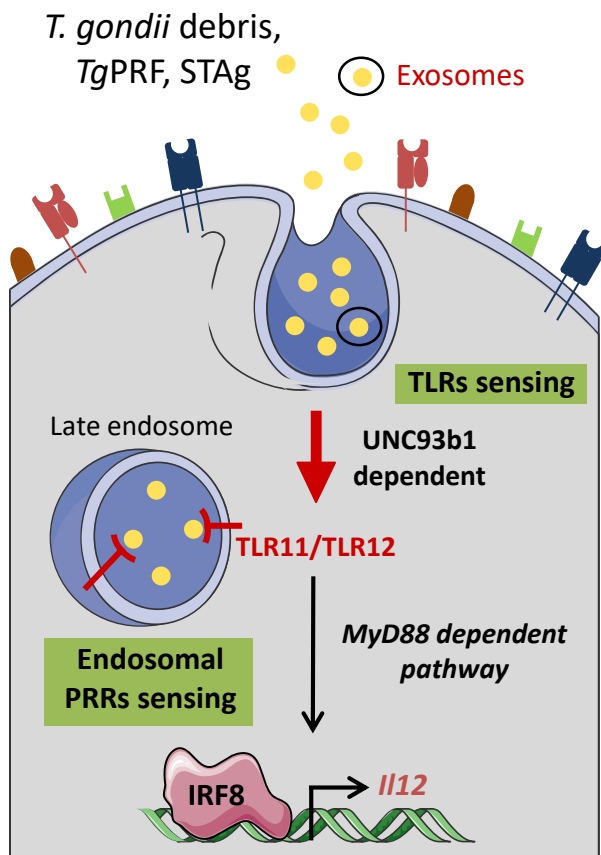
840 **Figure 2: Modulation of antigen presentation by *Tg* effectors.** The intravacuolar tubulovesicular
841 network (IVN) reduces GRA6 localization at the vacuole limiting membrane, therefore impeding
842 access of the C-terminal fragment that contains the antigenic peptide into the host cytosol. This
843 ultimately reduces presentation of the GRA6-derived peptide by MHC-I molecules on the cell
844 surface. *Tg*ROP18 down-regulates CD8 T cell activation by a yet unknown mechanism that involves
845 *Tg*ROP18-mediated phosphorylation and degradation of the ER sensor ATF6 β . *Tg* infection
846 represses H2-DM expression and induces CD74/li accumulation in the ER, thereby impairing MHC-
847 II antigen presentation and CD4 T cell activation.

848

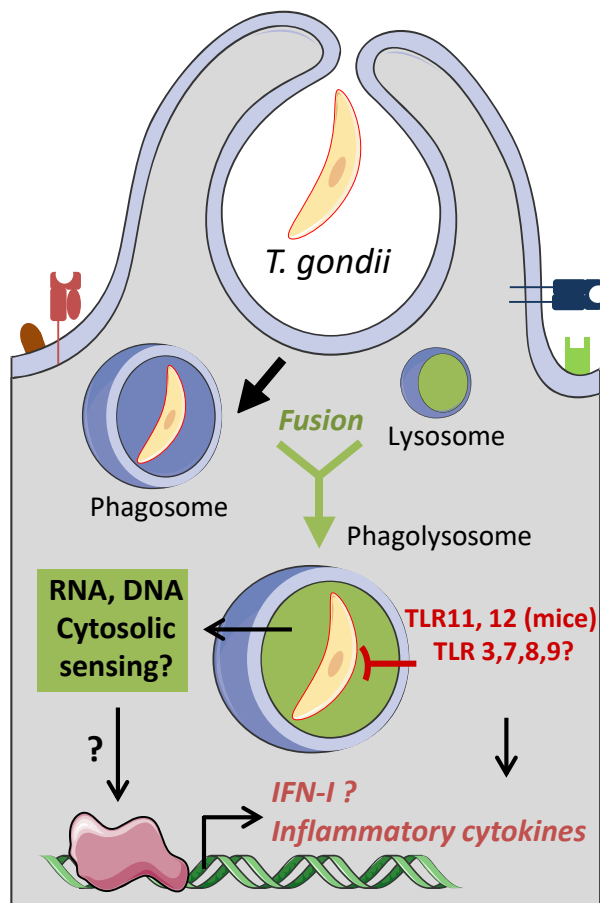
849 **Figure 3. Modulation of DC functions during *Tg* infection.** (a) Scheme summarizing the
850 modulation of DC functions in mice infected by the *Tg* type I and type II strains during the different
851 steps of the infection from parasite entry into the intestine, to their migration in secondary lymphoid
852 organs. *Tg* parasites can also disseminate to distant organs such as the brain and muscles, possibly by

853 using DC as shuttle leucocytes or as extracellular parasites in the blood. For simplification,
854 modulations of human DC functions by the infection were not included in this figure. **(b)** Table
855 summarizing the functions of murine DCs that are up-regulated (red) or down-regulated (green)
856 compared to unchallenged DCs, by live *Tg* type I and type II strain infection, killed parasites or
857 STAgS. When identified, the parasite effectors and targeted host pathways were indicated, as well as
858 whether these findings were obtained *in vitro* in the context of differentiated or isolated murine DCs
859 (#) or *in vivo* in infected mice (§).

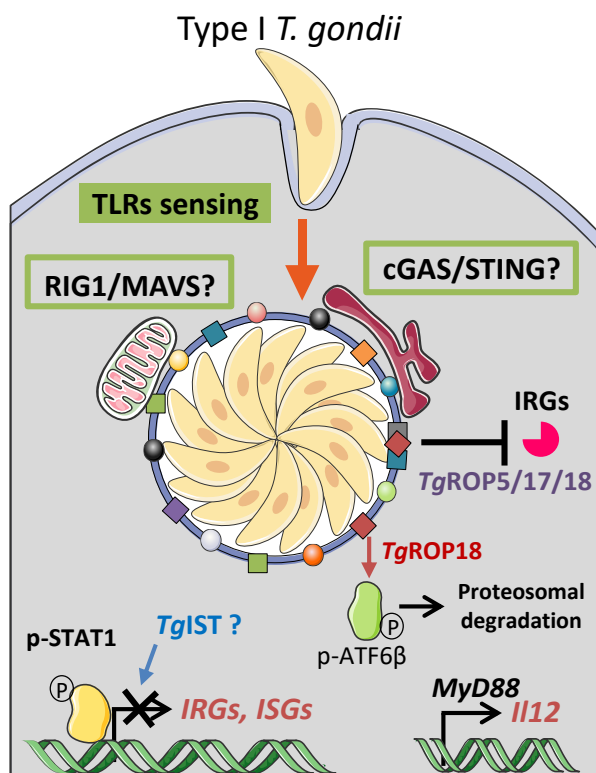
A Endocytosis



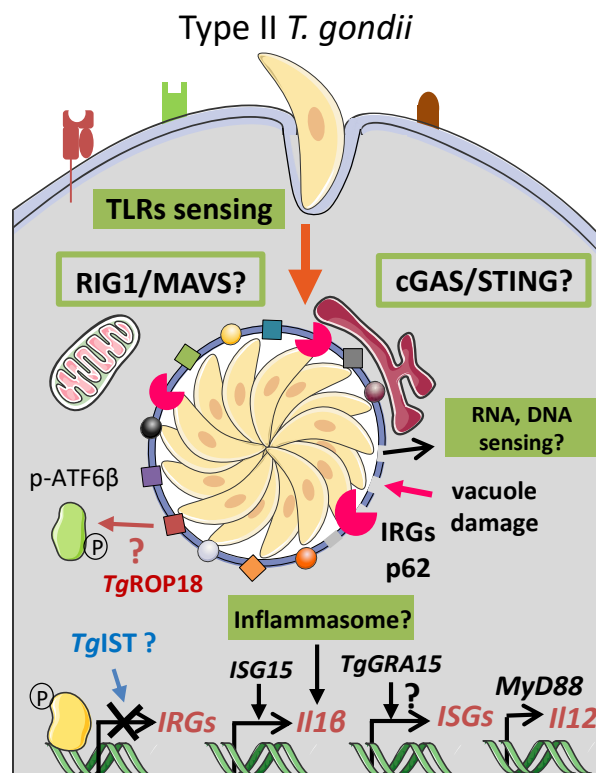
B Phagocytosis



C Active invasion with type I



D Active invasion with type II



Legend:

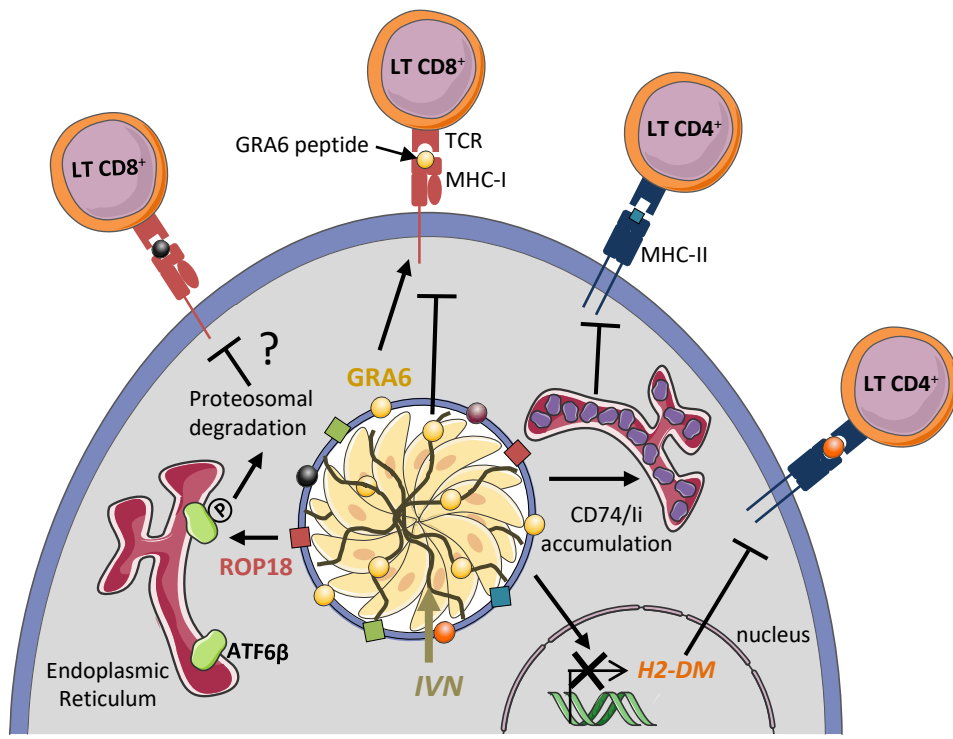
- *T. gondii* ROP protein
- *T. gondii* GRA protein

- CD80/86
- CD40

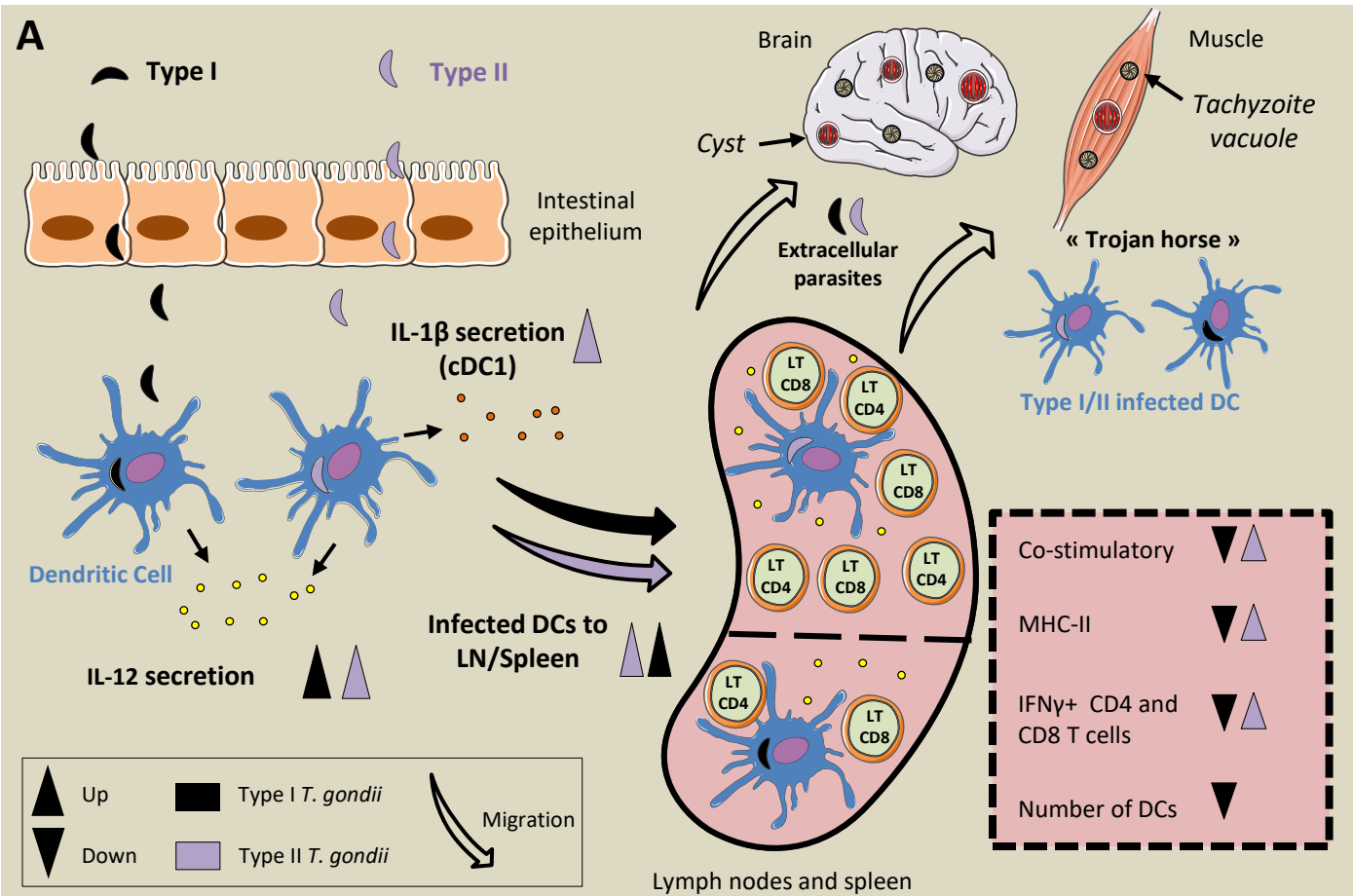
MHC-I MHC-II

- MHC-I
- MHC-II

- Mitochondria
- Endoplasmic reticulum



- GRA6 associated to intravacuolar network
- Tg ROP proteins
- CD74/li
- GRA6 associated to vacuolar membrane
- Tg GRA proteins



Modulation of DC functions	Type I		Type II		Killed parasites	STAgS
	<i>Tg</i> effector	Host target / pathway	<i>Tg</i> effector	Host target		
IL12 secretion	<i>Tg</i> PRF # § <i>Tg</i> MIC1/MIC4 # <i>Tg</i> Cyclophilin #	TLR11/12 # § TLR2 # CCR5 #	<i>Tg</i> PRF # § <i>Tg</i> Cyclophilin #	TLR11/12 # § CCR5 #	#	#
IL1 β secretion	nd	nd	§		nd	nd
STAT1 pathway	#		#		nd	nd
IFN γ -inducible gene transcription	<i>Tg</i> IST? #	STAT1 # MHC-II ? #	<i>Tg</i> IST? #	STAT1 # MHC-II ? #	nd	nd
Co-stimulatory molecule expression	# §		§		#	#
T cell priming/activation	<i>Tg</i> ROP18 §	ATF6 β §	#		nd	nd
Motility / Chemotaxis	<i>Tg</i> 14-3-3 # <i>Tg</i> GRA5 #	GABAergic signaling # CCR7 # TIMP-1-CD63-FAK #	# §		#	#

■ up
 ■ down
 ■ no modulation
 nd : no data
 # : in vitro
 § : in vivo