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Toxoplasma and Dendritic Cells

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

2

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11

12 **Keywords**

13 *Toxoplasma gondii*, dendritic cells, innate sensing, antigen presentation, migration

14

15 **Abstract**

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

24

25

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-

76 mediated microbial detection. Similar to MyD88^{-/-} animals, mice in which MyD88 deletion is restricted to
77 CD11c⁺ DCs fail to control type II *Tg* systemic infection and exhibit a strong decrease in IFN- γ production
78 [10,11], demonstrating the major role of DCs in inducing a protective Th1 immunity. In contrast to
79 macrophages, IL-12 production in DCs downstream of MyD88 was found to be independent from the NF κ B
80 pathway but to rely on the transcription factor IRF8 [12] (Figure 1a). TLR11 and TLR12, two endo-
81 lysosomal TLRs, which are able to form heterodimers and are stimulated in an UNC93B1-dependent
82 manner, were then identified as the major players in innate sensing of *Tg* by DCs, via the recognition
83 of *Tg* profilin (*Tg*PRF) (Figure 1a) [13]. Mice deficient in these sensing molecules display a drastic
84 defect in IL-12 production by splenic DCs. Consequently, IFN- γ production is severely impaired and
85 mice become highly susceptible to infection [13–17]. There seems to be a functional specificity
86 between both TLRs, since TLR12 but not TLR11 is involved in IL-12 production by plasmacytoid
87 DCs (pDCs) (Box 1). Similar to DC-MyD88^{-/-} mice, TLR12^{-/-} mice display a more severe
88 susceptibility to infection compared to TLR11^{-/-} mice, which is due to a defect in the early activation
89 of IFN- γ production by NK cells and impaired parasite clearance in the peritoneum [16]. Of note,
90 TLR11/TLR12-mediated recognition of *Tg*PRF only operates in mice and not in humans, in which
91 the ortholog TLR11/12 genes are not functional.

92

93 *Sensing Tg beyond TLR11 and TLR12*

94 TLR2 and TLR4 represent major mammalian surface TLRs that recognize lipoproteins and
95 liposaccharides derived from bacteria, viruses, fungi and parasites. They can also signal from
96 endosomes after internalization of ligand/receptor complexes. In contrast to macrophages [18], TLR2
97 and TLR4 were proposed to not operate in murine DCs in the sensing of *Tg* **pathogen-associated**
98 **molecular patterns (PAMPs)** based on the observation that IL-12 production is not altered in
99 splenic DCs of TLR2^{-/-} and TLR4^{-/-} mice injected with soluble tachyzoite antigens (STAg) [19].
100 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

151 immunity, suggesting that *Tg* exosome internalization by DCs contributes to MHC-I and MHC-II
152 antigen presentation [29,30]. Furthermore, in correlation with the detection of *Tg*PRF by immune
153 cells “at distance” of infected cells, bystander splenic DCs, which do not contain phagocytosed or
154 invaded parasites, induce high amounts of IL-12 [31]. Yet, one may envision that while TLR11/12
155 stimulation by *Tg*PRF is critical to trigger IL-12 secretion, active invasion by parasites may activate
156 distinct endosomal TLRs or cytosolic PRRs (Figure 1c and 1d). Cytosolic PRR stimulation would
157 imply a process of *Tg* PAMP export from the PV to the host cytosol, in particular nucleic acids.
158 Although speculative, it is logical to consider that this process may be facilitated by vacuolar
159 membrane breakdown following IFN- γ and STAT1-induced **immunity-related GTPase (IRG)** and
160 **guanylate-binding proteins (GBP)** recruitment at the PV membrane (PVM) [6,32]. Supporting this
161 hypothesis, polymorphic *Tg* ROP18, ROP5 and ROP17 proteins are essential secreted factors
162 localized at the PVM that disrupt IRG/GBP-mediated parasite restriction, albeit in a strain-specific
163 manner (Figure 1c). Type II strains possess an inactive ROP5 allele, which hampers blockade of PV
164 disruption in IFN- γ -activated macrophages (Figure 1d). It is likely that similar mechanisms exist in
165 DCs. In support of this hypothesis, infection of IFN- γ -primed BMDCs by type II, but not by type I,
166 parasites induces the recruitment of ubiquitin and the P62 sequestosome (SQSTM1) at the PVM, via
167 a process dependent on the IRG-regulatory proteins *Irgm1/Irgm3* and the autophagy-related proteins
168 (ATG) *ATG3*, *ATG5*, *ATG7* and *ATG16L1* (Box 2). Accumulation of p62 on parasite-containing
169 vacuoles occurs after partial damage by IFN- γ -inducible IRG and GBP and contribute to the
170 cytosolic release of luminal antigens that activate CD8⁺ T cell responses [33]. In addition, in
171 BMDCs, the infection itself is sufficient to trigger STAT1 phosphorylation and nuclear translocation
172 in a strain-independent manner and these responses are accentuated by the addition of IFN- γ [34]
173 (Figure 1c and 1d). However, parasites impede STAT1 activity by inducing the formation of aberrant
174 transcriptional complexes at the promoter of IFN-stimulated genes (ISGs), such as *Irf1* and *Igtp* [34]
175 (Figure 1c). In macrophages, this inhibitory process is mediated by the *Tg*-secreted effector IST

176 (inhibitor of STAT1 transcriptional activity) in both type I and type II strain-infected cells [35–37].
177 Most likely, similar *Tg*IST-mediated inhibitory mechanisms take place in infected DCs, however this
178 remains to be formally established. As previously mentioned, upon IFN- γ stimulation of BMDCs,
179 type II parasites, but not type I parasites, induce *Irgm1/Irgm3* recruitment at the PVM, which is not
180 sufficient to induce parasite clearance but triggers partial PV disruption [33]. Although this
181 difference is likely linked to the inactive *ROP5-II* allele, it is worth mentioning that the distinct *Tg*
182 strains can differentially modulate specific aspects of IFN γ signaling through the polymorphic
183 effector proteins *ROP16* and *GRA15*. In particular, *GRA15* from type II strains was reported to up-
184 regulate a certain set of ISGs in human fibroblasts in a STAT1-independent but NF- κ B-dependent
185 manner [38] [39].

186 Therefore, strain-specific induced PV damage may promote *Tg* PAMP export to the host cytosol,
187 resulting in strain-specific innate sensing mechanisms and activation of corresponding downstream
188 pathways. Of particular interest is the activation of the **inflammasome**. NLRP3 activation and IL-1 β
189 production in human monocytes and bone marrow-derived macrophages were reported to be
190 specifically triggered by type II strains and dependent on the secretion of *TgGRA15* into host cells
191 [40,41]. Whether the inflammasome is activated in *Tg*-infected DCs remains to be addressed.

192 Finally, although not yet explored in *Tg*-infected immune cells, other mechanisms of microbial
193 sensing may control the immune response outcome. The mitochondrial antiviral-signaling protein
194 (MAVS), stimulated downstream of the nucleic acid cytosolic sensor RIG-I (retinoic acid-inducible
195 gene I) as well as STING (stimulator of interferon genes), downstream of the cGAS sensor, are
196 localized at the mitochondrial membrane and the **endoplasmic reticulum (ER)**, respectively [42]
197 (Figure 1c and 1d). Interestingly, these two compartments tightly associate with the PVM, therefore
198 are likely in direct contact with *Tg* PAMPs. Of note, only type I and type III strains, but not type II
199 strains, were reported to trigger the recruitment of mitochondria at the PVM in human fibroblasts
200 [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.

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

238 To conclude, while in mice, *Tg*PRF detection by TLR11/12 is critical to trigger the secretion of IL-
239 12 and the IFN- γ response for early parasite clearance, active invasion of DCs by parasites and
240 parasite phagocytosis in human cells likely stimulate additional innate sensing mechanisms, which
241 are not fully unraveled (Figure 1). These distinct mechanisms of *Tg* detection may not only regulate
242 DC secretion of various chemokines and cytokines, but also modulate other DC functions including
243 antigen presentation and T cell priming [58–60]. In addition, it is possible that *Tg* secretes yet to be
244 identified, strain-specific factors that target PRR-induced signaling pathways, impacting on the
245 outcome of inflammatory responses. Finally, based on the diversity of DC phenotypes and functions,
246 it appears essential to explore in more depth the mechanisms of *Tg* innate sensing in the different
247 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]. *Tg*IST, 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,

326 immunization with the *Tg* AS15 peptide, a natural CD4⁺ T cell antigen (Box 3), lowered cyst burden
327 and parasite load in the brain of infected mice [85].

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

333

334 *Modulation of DC maturation by Tg*

335 Contrasting results were obtained *in vitro* on the modulation of co-stimulatory molecule expression
336 following *Tg* infection. Infection by type I parasites leads to the activation of human DCs by
337 increasing the expression of CD40, CD80, CD86 and HLA-DR [26,88]. In contrast, immature
338 murine BMDCs are activated after exposure to HK parasites or STAg (increased CD40, CD80,
339 CD86 and MHC-II expression) but not after invasion by type I parasites, which also render them
340 resistant to subsequent activation by TLR ligands [84] (Figure 3). Importantly, Tait *et al.* reported
341 that infection by type I parasites is correlated with a decrease in the number of DCs in the PEC and
342 LN, compared to type II infection [89]. DCs recruited at the site of infection display a reduced
343 expression of CD80 and MHC-I compared to DCs analyzed during type II infection. As expected,
344 the diminished number of activated DCs altered the generation of specific CD8⁺ T cell responses. In
345 addition, IL-12 treatment during type I infection only modestly increased the number of antigen-
346 specific CD8⁺ T cells, reinforcing the hypothesis that type I parasites restrict CD8⁺ T cell priming.
347 The observed DC reduction upon type I infection may be a result of parasite-triggered cell death or
348 impaired recruitment in response to altered chemokine environment. Overall, this may well explain
349 the higher virulence of type I strains in comparison to type II strains and suggest that type II parasite
350 virulence may be efficiently controlled by murine DCs. Supporting this hypothesis, systemic

351 infection of mice by type II parasites resulted in increased expression of CD40, CD80, CD86 and
352 MHC-II markers and elevated IL-12 production by both CD8 α^- and CD8 α^+ splenic DCs [90] (Figure
353 3). In addition, CD40-CD154 interactions were found to be essential to maintain splenic DC
354 activation during infection [90]. In agreement, CD40 is upregulated in cDC1 following STAg
355 injection in mice [91]. Therefore, strain-dependent modulation of DC maturation appears critical to
356 shape the generation of *Tg* antigen-specific T cells and determine the outcome of infection in mice.
357 Nonetheless, the precise mechanisms by which the parasite interferes with key DC functions, such as
358 antigen presentation, DC maturation and survival need to be elucidated. Also, an improved
359 characterization of *Tg* antigen presentation pathways in human DCs as well as in distinct tissue-
360 resident DC subtypes is an important goal for future studies.

361

362 ***Tg* modulation of DC migratory functions**

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

368 Supporting the “Trojan horse’ hypothesis”, type I strain-infected murine BMDCs display enhanced
369 ability to disseminate *in vivo* compared to free tachyzoites [94]. Phagocytosis of HK parasites or
370 incubation with *Tg* secretory fraction do not enhance migration of DCs *in vitro* [94,95] suggesting a
371 modulation of host signaling by secreted effectors. In line, DCs infected by type II parasites, but not
372 bystander cells, migrate significantly longer distances compared to type I-infected DCs in a 3D
373 matrix [96]. Similarly, an enrichment of migratory type II-infected DCs compared to type I strain-
374 infected DCs was monitored in spleen and MLN 16 hours after inoculation of pre-infected BMDCs
375 [95]. Importantly, infection of monocytes, NK, B and T cells fail to induce an enhanced migratory

376 phenotype [97], indicating a DC-specific modulation of host responses by the parasite via regulatory
377 mechanisms that have been partially uncovered and recently expertly reviewed [98]. It is proposed
378 that a transition from mesenchymal to amoeboid migration mode enables high-speed locomotion of
379 the parasite through interstitial tissues and across biological barriers, which in conjunction with
380 enhanced chemotaxis, promotes *Tg* dissemination (Box 4).

381 Nonetheless, parasite effectors that stimulate the migratory ability of DCs need to be characterized.
382 *TgGRA5*, secreted and inserted at the PVM during parasite intracellular replication, was identified as
383 a factor stimulating migration of human DCs *in vitro* [99]. The authors further pinpointed a peptide
384 derived from the type I-*TgGRA5* hydrophilic N-terminal region, which, after internalization by
385 micropinocytosis, triggers the expression of CCR7 and enhances DC directional migration via a
386 JNK-dependent signaling [99]. However, how this peptide acts at the PVM during infection by live
387 parasites remains to be determined. In addition to *TgGRA5*, *Tg14-3-3*, a protein located in the
388 vacuolar space, was shown to induce *in vitro* DC hypermotility and enhanced chemotaxis via an
389 unknown mechanism that may involve sequestration of host 14-3-3 at the PVM [100] (Figure 3).
390 Thus, it is likely that a synergistic effect of several parasite effectors contribute to the modulation of
391 distinct signaling pathways and transcriptional responses altering DC migration. The identification
392 and subsequent depletion of these effectors will provide a useful mean to assess the importance of
393 parasite-driven activation of DC migration in *Tg* virulence and the establishment of latent infections
394 in mice. Noteworthy, after intraperitoneal type I parasite injection, *Tg* was reported to preferentially
395 target pDCs in the peritoneum and to enhance their migration towards the marginal zone and T cell
396 areas of the spleen. This process was found to be partially dependent on CCR2 [101]. Therefore,
397 further *in vivo* investigations are required to clearly identify which DC subset(s) are targeted by the
398 parasite at the site of infection after oral infection [8], and whether a differential impact on DC
399 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.

426 Up to now, no type II-specific effector that manipulates host cell responses to eventually promote
427 parasite latency and host maintenance has been identified. Hence, identifying the key DC subsets
428 operating in the gut of orally infected mice or the brain and muscular tissues during parasite latency,
429 and the characterization of their responses upon virulent *versus* latent infection are important goals
430 for future studies. Based on the specificity of DC functions compared to macrophages, in-depth
431 exploration of DC-*Toxoplasma* interactions is needed to open novel therapeutic avenues that would
432 reduce inflammation-induced pathology and boost antigen-specific T cell responses, which could
433 ultimately ameliorate the clinical outcome of cerebral chronic toxoplasmosis.

434

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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

738 **Box 1: Dendritic cell functions and subsets**

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

753 **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***

778 Two natural CD4⁺ T cell antigens from *Tg* were described so far; the AS15 peptide derives from the
779 TgCD4Ag28m protein [85], predicted to be a secreted protein; the second one derives from *Tg*PRF
780 [109]. Based on the fact that the *Tg* CD4Ag28m-derived AS15 peptide is robustly presented even
781 when DC are fed with heat-killed parasites, it is likely that both secreted and non-secreted parasite
782 antigens have access to the MHC-II antigen degradative compartments where peptide loading on
783 MHC-II molecules occurs [85,110]. The mechanisms promoting antigen entry into the degradative
784 compartments in the case of replicating parasites residing in a vacuole are still elusive. DC deficient
785 in the autophagy protein ATG5, but not ATG7, induce diminished CD4⁺ T cell production of IL-2
786 and IFN- γ after type II strain infection [86,87]. This defect is not accompanied by changes in MHC-
787 II and co-stimulatory molecule expression or impaired production of IL-12, IL-1 β or TNF- α [86],
788 suggesting that it may be linked to a reduced delivery of antigens to the MHC-II presentation
789 pathway.

790

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

792 *In vitro*, BMDC and human MDCC secrete GABA after infection with type I, II or III strains [111].
793 GABA secretion activates, via an autocrine effect, functional GABA_A receptors, which induce the *in*
794 *vitro* transmigration and chemotaxis of type II infected murine DC and promote parasite
795 dissemination after their adoptive transfer in mice [111,112]. The signaling molecule calcium also
796 plays a central role for this migratory activation as signal transduction following GABAergic
797 activation is mediated via the L-type voltage-dependent calcium channel (L-VDCC) subtype Ca_v1.3.
798 More recently, the Barragan laboratory further identified a TIMP-1-CD63-ITGB1-FAK signaling
799 axis hijacked by *Tg* to drive high-speed amoeboid migration [113]. Moreover, infected DC enhanced
800 migration is preceded by morphological changes. Human and murine DCs infected by type II
801 parasites display cytoskeletal actin remodeling, exhibit a process of podosome dissolution and CD18
802 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 TL12 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

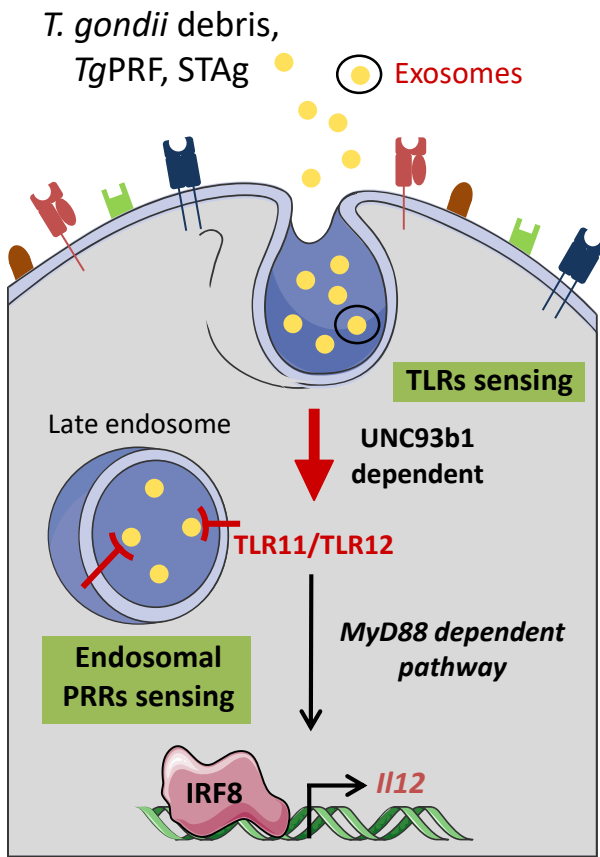
840 **Figure 2: Modulation of antigen presentation by *Tg* effectors.** The intravacuolar tubulovesicular
841 network (IVN) reduces GRA6 localization at the vacuole limiting membrane, therefore impeding
842 access of the C-terminal fragment that contains the antigenic peptide into the host cytosol. This
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

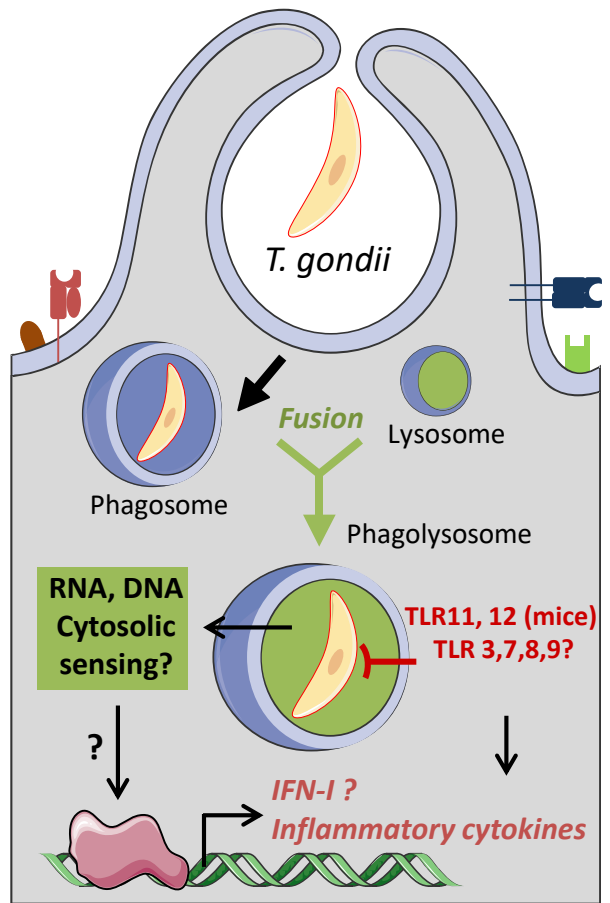
849 **Figure 3. Modulation of DC functions during *Tg* infection.** (a) Scheme summarizing the
850 modulation of DC functions in mice infected by the *Tg* type I and type II strains during the different
851 steps of the infection from parasite entry into the intestine, to their migration in secondary 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 (§).

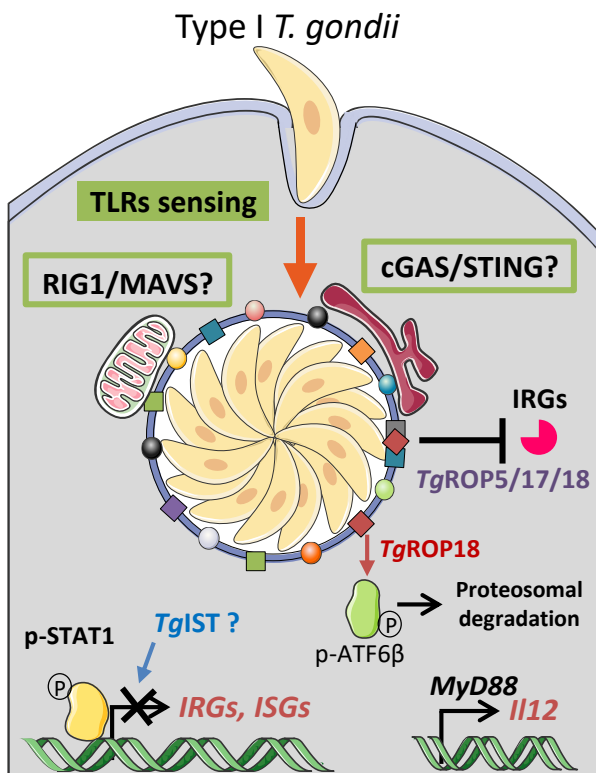
A Endocytosis



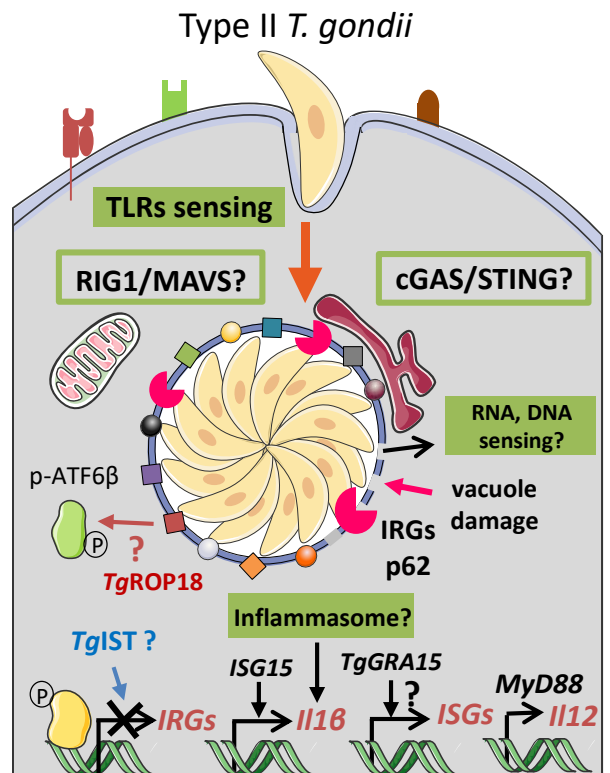
B Phagocytosis



C Active invasion with type I



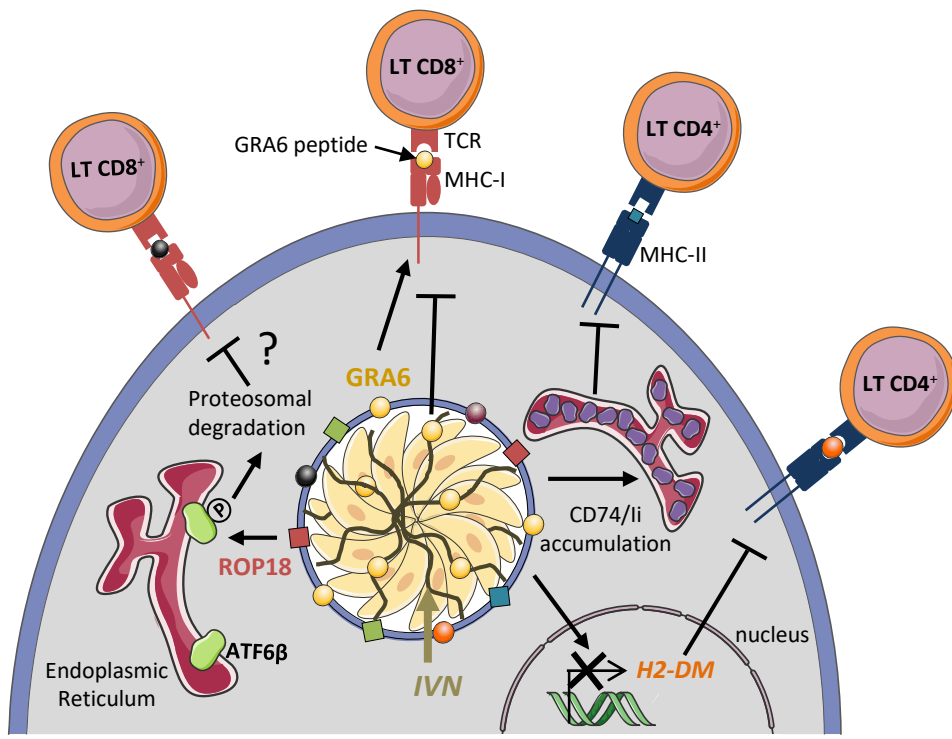
D Active invasion with type II








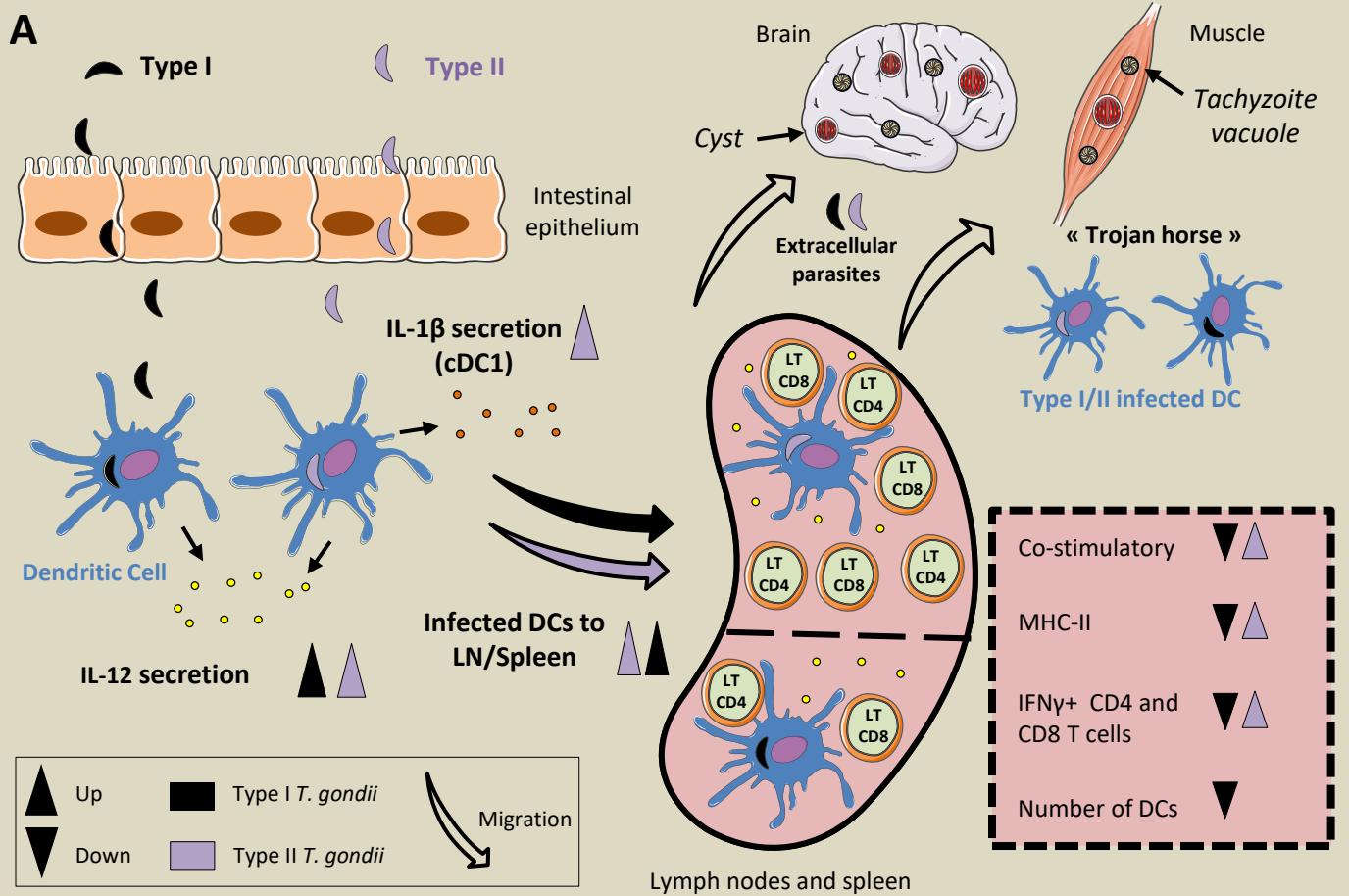
Legend:

- *T. gondii* ROP protein
- CD80/86
- *T. gondii* GRA protein
- CD40

- 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



B

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