



**HAL**  
open science

# The pathogenicity and virulence of *Toxoplasma gondii*

Syrian G Sanchez, Sébastien Besteiro

► **To cite this version:**

Syrian G Sanchez, Sébastien Besteiro. The pathogenicity and virulence of *Toxoplasma gondii*. *Virulence*, 2021, 12 (1), pp.3095 - 3114. 10.1080/21505594.2021.2012346 . inserm-03479520

**HAL Id: inserm-03479520**

**<https://inserm.hal.science/inserm-03479520>**

Submitted on 14 Dec 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## The pathogenicity and virulence of *Toxoplasma gondii*

Syrian G. Sanchez & Sébastien Besteiro

To cite this article: Syrian G. Sanchez & Sébastien Besteiro (2021) The pathogenicity and virulence of *Toxoplasma gondii*, *Virulence*, 12:1, 3095-3114, DOI: [10.1080/21505594.2021.2012346](https://doi.org/10.1080/21505594.2021.2012346)

To link to this article: <https://doi.org/10.1080/21505594.2021.2012346>



© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 11 Dec 2021.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)

## The pathogenicity and virulence of *Toxoplasma gondii*

Syrian G. Sanchez and Sébastien Besteiro 

Lphi, UMR5235, Univ Montpellier, CNRS, Montpellier, France

### ABSTRACT

*Toxoplasma gondii* is a parasitic protist infecting a wide group of warm-blooded animals, ranging from birds to humans. While this infection is usually asymptomatic in healthy individuals, it can also lead to severe ocular or neurological outcomes in immunocompromised individuals or in developing fetuses. This obligate intracellular parasite has the ability to infect a considerable range of nucleated cells and can propagate in the intermediate host. Yet, under the pressure of the immune system it transforms into an encysted persistent form residing primarily in the brain and muscle tissues. Encysted parasites, which are resistant to current medication, may reactivate and give rise to an acute infection. The clinical outcome of toxoplasmosis depends on a complex balance between the host immune response and parasite virulence factors. Susceptibility to the disease is thus determined by both parasite strains and host species. Recent advances on our understanding of host cell-parasite interactions and parasite virulence have brought new insights into the pathophysiology of *T. gondii* infection and are summarized here.

### ARTICLE HISTORY

Received 13 August 2021  
Revised 24 November 2021  
Accepted 25 November 2021

### KEYWORDS

*Toxoplasma gondii*; chronic toxoplasmosis; acute toxoplasmosis; parasite transmission; secreted virulence factors; immune evasion

### Introduction

*Toxoplasma gondii* belongs to the phylum Apicomplexa, a diverse group of protists that are mostly intracellular parasites and can cause potentially serious disease in animals and humans. *T. gondii* is arguably the most widespread of these, being able to infect almost any warm-blooded vertebrate, and thus a common cause of infection in wild and domestic animals, in addition to an estimated one-third of the human world population [1]. Seropositivity rates in the human population are evolving, and they range from less than 10% to over 90% depending on the country or region considered, varying in part because of regional socioeconomic parameters and population habits [2]. There is, for example, a higher prevalence in South America, Central America, and continental Europe, than in the United States of America or the United Kingdom. Seroprevalence is also widespread in wild and domestic animals, which are important as reservoirs for *T. gondii*, but also as sources for human contamination through meat consumption [3]. Toxoplasmosis in farm animals is not only a problem for human contamination but it also has a considerable burden on the livestock (on milk production and reproductive performance for instance) and thus represents a significant cost for the industry [4].

While a single species has been described for the genus *Toxoplasma*, there are several clonal lineages that differ in their pathogenicity. In Europe and

North America the population structure of *T. gondii* is dominated by four main clonal types (I, II, III, and XII) [5–7]. In Europe, type II strains (as well as type III, although to a lesser extent) are the most prevalent in a wild and domestic environment [8,9]. In North America, domestic isolates are similar to those in Europe (types II and III), but in the wild environment strains belonging to type XII predominate [7,10]. In other parts of the world, the situation is more contrasted. In South America, for example, there is a much greater genetic diversity [11–13], suggesting a greater occurrence of recombination. Strain virulence is typically defined by the outcome of infection in the mouse model, in which type I strains are much more virulent, than type II and III strains [14]. The type of *T. gondii* strain also has a considerable impact on the pathogenicity, with, for example, severe cases of acquired toxoplasmosis in immunocompetent patients caused by highly pathogenic South American strains from the wild [15].

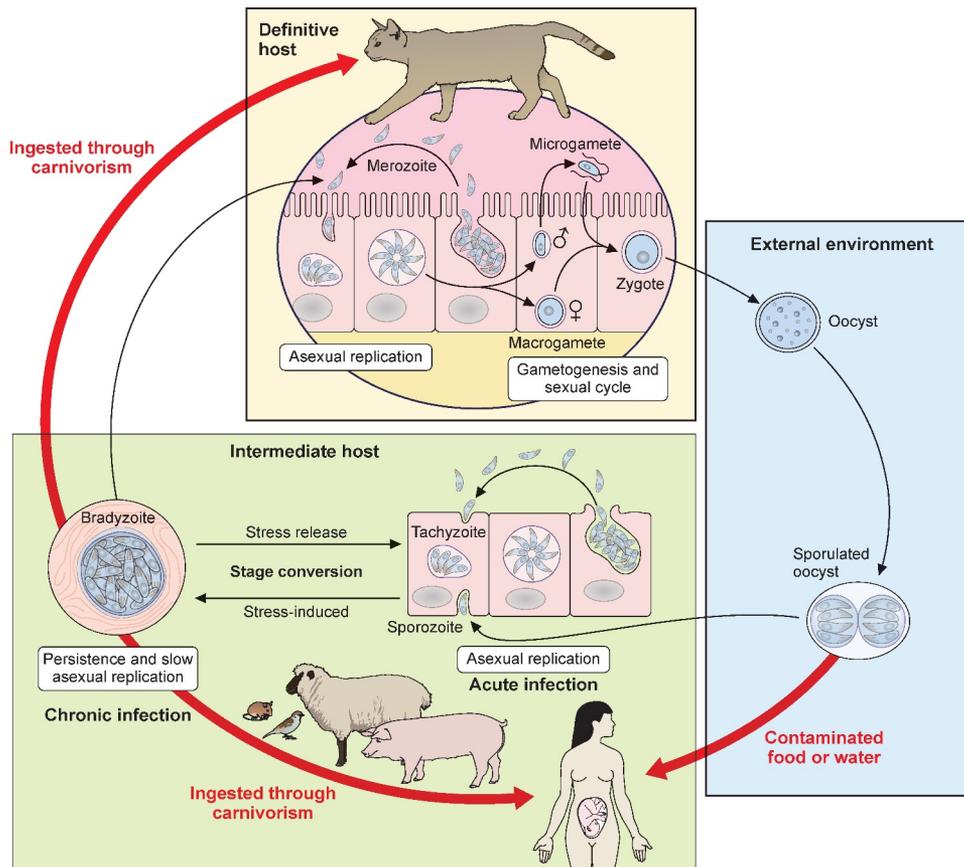
Upon infection by *T. gondii*, disease development also depends on the type of host, its genetic background, and of course its immune status. Several species seem to be naturally resistant to *T. gondii* infection, while others are very susceptible, due in part to factors including the proximity of their habitat with the definitive hosts of the parasite [16]. Yet, one of the most critical factors influencing susceptibility to *T. gondii*

remains the host immune system and the way it is modulated by parasite factors [17–20]. In this review, we will summarize the recent findings on the host- and parasite-dependent factors that not only govern the outcome of infection, but also give clues as to why *T. gondii* is such a highly successful parasite.

### *T. gondii* life cycle and routes of transmission

The life cycle of *T. gondii* (Figure 1) involves both sexual replication in felids (definitive hosts), and asexual replication in a variety of vertebrate hosts (intermediate hosts). Felids ingest the parasite by preying on infected intermediate hosts that contain encysted bradyzoites. Bradyzoites are released from cysts under the action of intestinal enzymes and acid digestion, and invade the epithelial cells of the small intestine. Although the parasite may disseminate throughout the definitive host's body and give rise to clinical signs, it is rarely the case [21]. More frequently, in the intestine and within the course of a few days, bradyzoites will develop into different morphological enteroepithelial stages (or schizonts) to finally reach the merozoite

stage [22]. In turn, after a few rounds of asexual division merozoites will differentiate into male (micro-) and female (macro-) gametes. Male and female gametes will then fuse to produce diploid oocysts, which will be encapsulated in a thick impermeable wall. Millions of these will be shed in the feces of the felid and contaminate the environment. There, oocysts undergo a sporulation process involving meiosis and mitosis to generate mature and infectious haploid sporozoites within now so-called sporulated oocysts [23]. The oocysts are remarkably resistant and can persist in the environment for a long period of time, which allow their dissemination in the terrestrial or aquatic environments [24,25]. Intermediate hosts ingest sporulated oocysts through contaminated food (such as produce) or water. Sporozoites will invade host cells and occupy a transient parasitophorous vacuole (PV) in which they quickly differentiate into the tachyzoite form [26]. Tachyzoites are highly proliferative and invasive forms that will disseminate in the host, and they are responsible for the symptoms of acute toxoplasmosis. They can travel through blood vessels or the lymphatic system and reach a number of different locations, like



**Figure 1.** Life cycle of *T. gondii*. Schematic representation of the infective stages and their modes of transmission and replication in their respective hosts.

visceral organs, muscle, and nervous tissue. The hijacking of host immune cells allows parasite dissemination through the body [27], and this way (but also using paracellular entry and transcellular migration [28,29]) they can also cross non-permissive biological barriers, like the blood–brain barrier, to reach immune-privileged organs like the brain. In fact, immunocompetent individuals will eventually control this acute phase of infection, but coincident to the emergence of the host immune response, fast-replicating tachyzoites will differentiate into slow-growing encysted forms called bradyzoites that will remain largely hidden from the immune system [30]. These persistent forms reside primarily in the central nervous system and muscle [31], where they may remain for a very long time [32]. This ensures parasite transmission to the definitive host to complete the cycle, at least when felids can prey on the intermediate host. When intermediate hosts are not typical preys of felids, the parasites can still be transmitted to other intermediate hosts by carnivorism, maintaining a parasite transmission cycle without need of sexual replication.

Human contamination can thus happen through food consumption: by ingestion of uncooked or undercooked cyst-containing meat, or of sporulated oocysts-containing vegetables, fruits or water [33]. For this reason, adequate sewage or water treatment, and washing of produce and proper cooking of meat, are important to prevent foodborne toxoplasmosis [34]. Other routes of transmission include congenital transmission of tachyzoites from a primarily infected mother to the developing fetus through the placenta [35]. Preventative measures for limiting the contact of the mother with known routes of transmission during pregnancy, as well as prompt diagnosis, and rapid initiation of medical treatment at the onset of infection, are critical for managing congenital toxoplasmosis [36]. Another potential source of contamination in humans, although rare, is from blood transfusion [37] or organ transplantation [38] from infected donors.

### Clinical manifestations of toxoplasmosis

Toxoplasmosis is an infection that is usually asymptomatic or may result in a mild, self-limiting illness in immunocompetent individuals. However, in immunocompromised individuals or fetuses it can lead to much more serious clinical manifestations [39].

Congenital toxoplasmosis can occur when there is primary maternal infection during pregnancy, as during the parasite dissemination phase it may cross the placental barrier to contaminate the developing fetus. It can cause neurological, ocular, or systemic damage with

variable severity, which depends on the gestational age at the time of primary maternal infection. For instance, first-trimester maternal infection can lead to more severe manifestations [40]. The most important sequelae for the newborn include hydrocephalus, mental retardation, epilepsy, and blindness, although some of these can also occur later in life [41].

In adults, immunodeficiency can also lead to severe toxoplasmosis, which is most often the result of reactivation of latent infection, even if acute acquired infection may also occur. Individuals who are immunocompromised or immunosuppressed (in the context of HIV infection [42], or for cancer patients [43] and transplant recipients) are particularly at risk. The most serious outcome in this context is arguably toxoplasmic encephalitis, in which recurrence of toxoplasmosis from parasites encysted in the central nervous system can lead to substantial tissue damage and inflammation [44]. Common symptoms include headache, fever, ataxia, or seizure, but this cerebral form of toxoplasmosis can be potentially life-threatening if not treated.

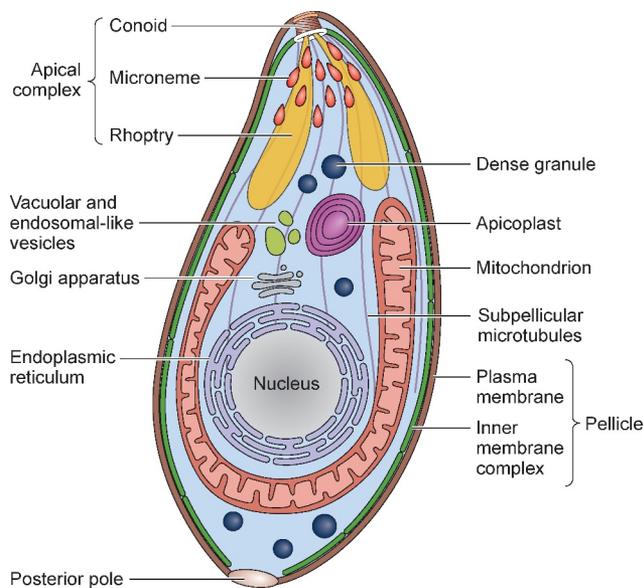
There is also an ocular presentation of the disease called ocular toxoplasmosis, a progressive necrotizing retinitis, that may lead to vision-threatening complications [45]. This can happen both in the context of congenital or acquired toxoplasmosis. As with the brain and muscles, the eyes are one of the organs the tachyzoites can disseminate to upon initial infection. There, they can cause self-limiting lesions, but might encyst and be able to subsequently reactivate if host immunity becomes impaired. Although this is for the moment poorly understood, recurrences may also occur in immunocompetent subjects, where *T. gondii* is a major cause of posterior uveitis worldwide [46]. Of note, higher severity and frequency of ocular toxoplasmosis in South America compared with Europe is probably due to exposure to more virulent strains [47,48].

In addition to the obvious deleterious effects of acute toxoplasmosis, chronic toxoplasmosis (i.e. the long-term persistence of the parasite in the form of tissue cysts), especially as it targets the central nervous system, may also have an important impact on behavioral changes and psychiatric disorders [49]. One famous example of behavioral alteration is how the parasite presence in the brain of the intermediate hosts may decrease felid aversion, or at least induce a general boldness (including toward predators), which may facilitate the completion of the life cycle and thus favor the reproductive efficiency of the parasite. This has been observed in laboratory settings for rodents [50,51], and even in the wild for larger animals [52]. Epidemiological studies and meta-analyses have shown

that *T. gondii* seropositivity can be associated with a number of mental health disorders, including schizophrenia, but also epilepsy and neurodegenerative diseases [53], although causality has not been firmly established. So far, there is no clear experimental demonstration of the direct effects of *T. gondii* on neurons and their functions, and few evidence of the cellular mechanisms that are dysregulated by the long-term establishment of this parasite in the brain [54].

### Typical ultrastructure of a *T. gondii* invasive stage

*T. gondii* is an obligate intracellular parasite that has the remarkable ability to invade a variety of nucleated cells. The parasite encounters multiple host cell and tissue types during its life cycle, which involves four different invasive forms: the tachyzoite, bradyzoite, sporozoite and merozoite [23]. All the infectious stages of the parasite present the same overall organization, and are highly polarized cells displaying an elongated shape (Figure 2). They contain universal eukaryotic organelles such as a nucleus, endoplasmic reticulum and Golgi apparatus, but also have some more original features. For instance, *T. gondii* tachyzoites harbor two organelles of endosymbiotic origin: a mitochondrion, essentially found as a single ramified organelle [55], although its morphology is dynamic in nature [56]; and a non-photosynthetic plastid named the apicoplast,

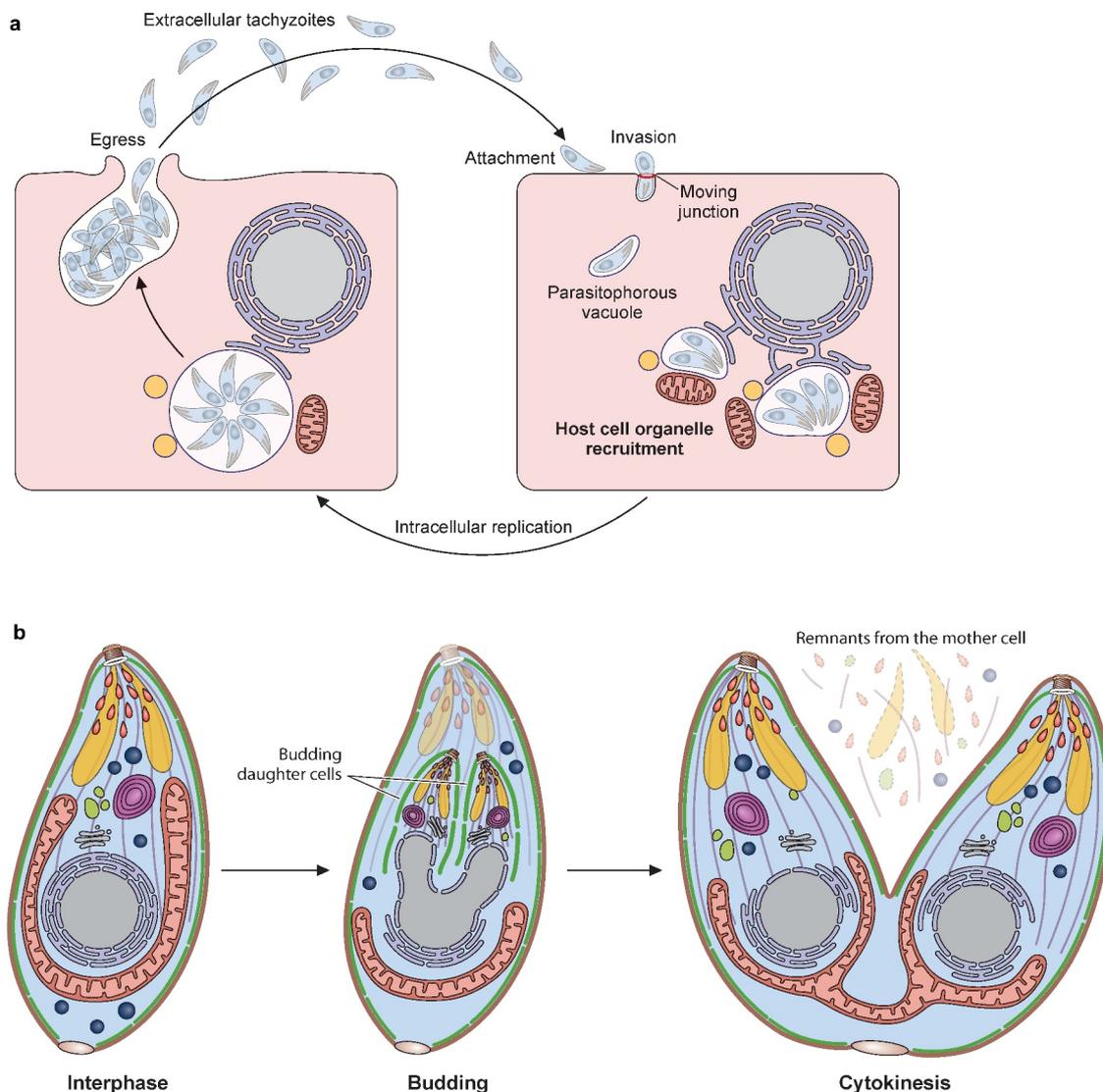


**Figure 2.** Ultrastructure of a *T. gondii* tachyzoite. As displayed on this schematic representation tachyzoites, like other *T. gondii* invasive zoite stages, are highly polarized cells and contain specialized organelles involved in the secretion of virulence factors.

originating from a secondary endosymbiotic event, and thus enclosed by four membranes [57]. Both organelles have important metabolic functions for the parasite [58]. Another similarity with plants is the vacuolar/lytic compartment of tachyzoites, that may also be involved in osmoregulation [59,60]. Tachyzoites are enclosed by a trilaminar membrane structure termed the pellicle, that apicomplexan parasites share with other alveolates (like ciliates and dinoflagellates) [61]. This structure comprises the plasma membrane and the underlying inner membrane complex, which is made of flattened membrane sacs. It is supported on its cytoplasmic face by a complex and highly organized network of intermediate filament-like proteins and by a subpellicular network of microtubule cytoskeleton, which is instrumental in driving the gliding motility of the parasite [62]. The apical complex, which gave its name to the Apicomplexa phylum, comprises a cytoskeletal structure called the conoid. It is an assembly of spirally arranged fibers originating from the preconoidal rings, at the distal tip of the structure, but also the polar ring, from which the 22 subpellicular microtubules originate, and two short intraconoidal microtubule [63,64]. This structure is closely associated with two different types of specialized apical secretory organelles called micronemes [65] and rhoptries [66,67], that secrete virulence factors that are essential for dissemination and survival of the parasites. Dense granules are another type of specialized secretory organelle that will later on release important factors for the intracellular establishment of the parasite [68], although these particular vesicles are not restricted to its apical part [69].

### Host cell invasion and parasite development: The example of tachyzoites

Host cell invasion followed by rapid asexual multiplication are key steps of the *T. gondii* life cycle, which allow for instance population expansion in the definitive or intermediate hosts, through the merozoites and tachyzoites developmental stages, respectively (Figure 1). The invasion process, and subsequent steps of intracellular asexual replication and exit (or egress) from the host cell, collectively referred to as the “lytic cycle” [70], is mostly studied for tachyzoites (Figure 3a). Host cell invasion mechanism involves the formation of a PV that constitutes a unique replicative niche, providing some protection from the host cell and access to nutrient sources [71,72]. Invasion and subsequent establishment of the parasite in the PV is possible thanks to the sequential exocytosis of the three aforementioned specialized secretory organelles: micronemes, rhoptries,



**Figure 3.** Asexual replication of *T. gondii* tachyzoites. A) Schematic representation of the lytic cycle of *T. gondii* tachyzoites, which comprises three main steps: invasion, intracellular replication and egress. B) Schematic representation of endodyogeny, the process by which *T. gondii* tachyzoites replicate intracellularly. It involves the coordinated assembly and internal budding of two daughter cells within a mother cell. The daughter-forming material is either synthesized *de novo* or recycled from the mother cell.

and dense granules [73]. Micronemes are rod-like organelles clustered at the apical pole of the parasites (Figure 2), they contain a large array of proteins (MICs), many of which are important for the invasion process. Several MICs secreted by extracellular tachyzoites are adhesins that can bind to a number of different host cell surface components that comprise proteins, but also carbohydrates [74]. This mediates an important initial attachment step for the parasites at the surface of the host cell and provides an anchor for gliding motility, which is crucial to the invasion process [75]. Some MICs seem also important for controlling the exocytosis of the rhoptries [76], which are also apical secretory organelles, and are acting downstream in the process of invasion. Rhoptries are club-

shaped organelles whose protein content localizes to discrete sub-compartments: the bulbous part, containing proteins called ROPs that are involved in the subversion of host cell functions; and the neck, containing proteins called RONs that are more specifically associated with host cell invasion [67]. Rhoptries and micronemes are both involved in the secretion of factors that will constitute the moving junction (MJ), a structure formed by a MIC ligand and a RON receptor protein complex secreted by the parasite into its host cell plasma membrane to anchor itself firmly prior to entering [77]. The MJ also constitutes a physical barrier that likely restricts the incorporation of host plasma membrane proteins into the nascent PV membrane (PVM) that forms as the parasite enters the cell

[78,79]. This selective incorporation of host material is critical for rendering the PV nonfusogenic with the host endolysosomal system, and thus preventing parasite degradation by lysosomal acidification [78].

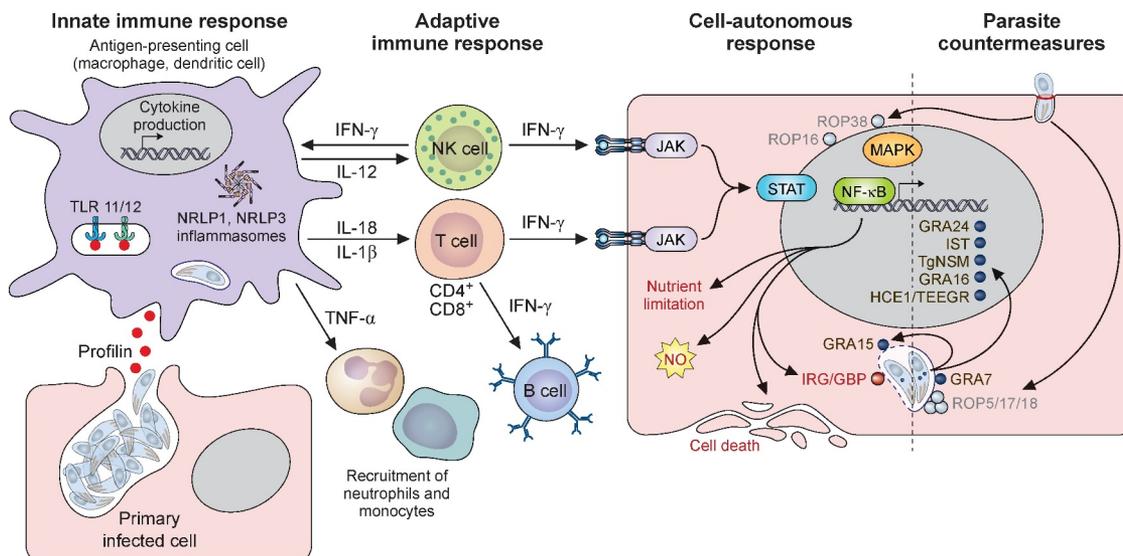
Later during the invasion process, or once they are intracellular, the parasites will secrete other factors that will help modifying the host cell in order to facilitate replication [80]. These include ROPs and dense granule effectors (GRAs), which are secreted in the vacuolar space or beyond, like the PVM, the host cytosol, or even the host nucleus [81]. Some GRAs are involved in the genesis of an intravacuolar membrane network [82] as early as 10–20 minutes post-invasion, or the establishment of pores in the PV to function as molecular sieves [83]. These modifications are important to the intracellular growth of the parasites, as they will allow the scavenging of essential nutrients from the host. *T. gondii* is auxotroph for a number of important metabolites, including amino acids, nucleotide precursors, essential co-factors and lipids. Thus to survive and ensure its division it must acquire these molecules from its host [84]. One striking feature that also quickly follows invasion is the recruitment of host organelles (Figure 3a) around the parasite-containing PV. These include part of the endoplasmic reticulum, mitochondria, but also multivesicular bodies and transport vesicles, Golgi ministacks and lipid droplets [85], and GRAs are instrumental in this process. For instance, the anchorage of host mitochondria to the PVM is mediated by a GRA protein called MAF1 (for mitochondrial association factor 1) [86]. Recruitment of host organelles by the parasites may serve several purposes including the scavenging of nutrients, but also the counteracting of harmful host functions. Interestingly, like several other host-related features controlled by GRA and ROP effectors (which are described later in this review), the organelle recruitment capacity shows some difference between different strains [86], which may also account for strain-specific differences in pathogenesis.

Tachyzoites replicate asexually through a process called endodyogeny (Figure 3b), in which two daughter cells are assembled inside a mother cell that ends up being consumed as they are formed, leaving behind only a small residual body [87]. In this particular form of replication, daughter cell budding and DNA replication are coordinated, and while some organelles are synthesized *de novo* (like rhoptries and micronemes), others like the apicoplast or the Golgi apparatus are duplicated and segregated into nascent daughter buds in a process coordinated by the centrosomes [87,88]. After successive rounds of division (usually 5 or 6, at least *in vitro* [89,90])

tachyzoites will actively egress the PV and the host cell [91,92], thereby causing its destruction. Like invasion, egress is highly dependent on microneme secretion, which is regulated through calcium release [93,94], itself depending on a signaling cascade sensing local concentration of phosphatidic acid [95,96]. Among the microneme-dependent factors important for egress are those governing parasite motility [62], but also specific factors like the PLP1 perforin that destabilizes membranes and facilitates the exit of the parasites [97]. Although some laboratory-adapted strains show some short-term survival (a few hours) in the extracellular environment, once they have egressed, extracellular tachyzoites must invade a new host cell in order to survive and initiate a new lytic cycle (Figure 3a).

### Replication of other developmental stages

As tachyzoites can be easily propagated *in vitro* their division process is well characterized, but the multiplication of other parasite stages is often less studied, although they are also critical for the pathogenicity of *T. gondii* (Figure 1). The bradyzoites, the latent and persistent stage found in the intermediate host, also replicates asexually, albeit much more slowly and asynchronously than the tachyzoite stage [98]. It does so by a combination of endodyogeny (like for tachyzoites) and also, occasionally, by a process called endopolygeny, by which more than two daughter cells form within the mother cell [99]. The asexual expansion in enterocytes of the small intestine of the definitive host is also an important prerequisite to the formation of gametes and for the hundreds of millions of oocysts that will be shed in the environment subsequently. It involves a complex differentiation through five morphologically-different stages, some of which divide by endodyogeny, whereas others multiply by endopolygeny, or schizogony (with multinucleated intermediates, or schizonts). At the end of this division process, the daughter parasites will bud from the periphery to produce infective merozoites [22]. After this asexual multiplication, the sexual cycle starts by gametogenesis and the formation of macro- and microgamonts, that will later develop into gametes. Macro- and microgametes are not equilibrated in numbers, and the rate of macrogamete fertilization is unknown. However, *T. gondii* has clearly managed to achieve maximum oocyst output, with hundreds of millions of them potentially being shed by a single felid [100]. Many questions still remain regarding these understudied stages [100,101]. Yet, the recent discovery that linoleic acid is critical in conferring the specificity of felids as



**Figure 4.** Host immune response to *T. gondii* infection and examples of parasite evasion mechanisms. Schematic view of typical components of the murine immune response to *T. gondii* upon initial infection. Cells involved in the innate and adaptive immune response, through the secretion of pro-inflammatory cytokines like IL-12, will elicit an IFN- $\gamma$ -dependent activation of various cell-autonomous pathways for limiting parasite growth, which include parasitophorous vacuole destruction by immunity-related GTPases, nitric oxide production, nutrient limitation and host cell death. Virulent parasite strains can in turn secrete factors from their rhoptries or dense granules, that will interfere with nucleus-located upstream transcriptional regulators of the immune response, or with parasitophorous vacuole-located host effectors.

definitive hosts has allowed using mice as efficient oocysts spreaders when their diet is supplemented with an excess of this fatty acid [102]. This new experimental model will likely help solving some of the existing conundrums regarding the part of the *T. gondii* life cycle that takes place in the definitive hosts.

The conversion between the different developmental stages is also key for the pathogenicity, and our understanding of gene regulation across these life stages has recently advanced. For example, recent studies have identified major transcriptional regulators controlling sexual commitment [103] or encystation [104], providing access to new tools for characterizing stage-specific transcriptomes. Transcriptomic and proteomic analyses have also highlighted that different *T. gondii* infectious zoite stages express specific repertoires of genes [105–109]. Noticeably stage-specific effectors include different subsets of GRAs (some of which are for example involved in the formation of the cyst wall that surrounds bradyzoites [110]), MICs and ROPs, which are important in attachment, invasion, and host cell modification. This is perhaps unsurprising, as it may reflect the capacity for the different zoite stages to invade and develop into different host cell types. From this point of view, tachyzoites are clearly the most versatile, and yet these stages also show some strain-specific differences in the virulence factors they express. Some of these

virulence factors inhibit host defense mechanisms and thus contribute to the relative differences in virulence during primary infection.

### Immune response against *T. gondii* in the intermediate host

The ability of *T. gondii* to persist in a wide range of intermediate hosts is the result of a balance between the host immune system and the parasite's own escape mechanisms. Noticeably, cell responses to infection are dependent on species and cell types infected by the parasite. It is also known that the different parasite strains will not induce the same immune response depending on the presence and the polymorphism of their effectors. Most of the *in vivo* infection data for *T. gondii* were generated in mice, not only because they are well-characterized models for mammalian immune function in general, but also because they are natural hosts of the parasite. Yet, although the findings described in this part mostly focus on mice as the archetypal model for mammalian response to *T. gondii*, it should be kept in mind that there are marked differences between humans and mice in sensor and effector proteins that determine host resistance to this parasite.

### Initial recognition of *T. gondii*

In the early stages of *T. gondii* infection, dendritic cells (DCs), macrophages, and monocytes are the first host cells to respond (Figure 4). Classically, during pathogen infection the host will first identify the “non-self” via receptors called PRRs (pattern recognition receptors) located on the cell surface or inside the cell (like Toll-like receptors – TLR-). These receptors will generally recognize components of microbes or pathogens called MAMPs or PAMPs (microbes/pathogens associated molecular patterns). This way, innate immune cells will trigger the production of IL-12, a cytokine that plays an early and major role in the resistance to bacterial and parasitic infections [111]. In mice, the main mechanism driving IL-12 production in response to *T. gondii* infection is through the recognition of *T. gondii* profilin by TLR11 and TLR12 [112–114]. However, other proteins or parasite molecules, such as glycosylphosphatidylinositols, can also activate TLRs [115–117]. Noticeably, mice deficient for myeloid differentiation primary response 88 (MyD88), an important adaptor for signaling by most TLRs, are highly susceptible to *T. gondii* infection [118]. Humans do not have functional equivalents to all murine TLRs, and thus may not use the exact same mechanism for parasite sensing [119], which might involve sensing of parasite-derived RNA and DNA instead of profilin [120]. Yet, importantly, IL-12 is also produced by human innate immune cells in response to *T. gondii* [121].

IL-12-producing cells such as DCs and macrophages are important actors for controlling the parasite at the early onset of infection, but various other cell types, including neutrophils and inflammatory monocytes, are also involved (Figure 4). This also depends on the parasite tropism and site of infection. For instance, when intermediate hosts are infected through the ingestion of contaminated food, primary infected cells include intestinal epithelial cells and peritoneal cells. Innate lymphoid cells (ILCs) produce high levels of interferon-gamma (IFN- $\gamma$ ) and tumor necrosis factor, and help protecting against *T. gondii* infections in the intestine and in secondary lymphoid organs [122,123].

Other components of the innate immune system that can limit parasite growth are multiprotein complexes called the inflammasomes. They include sensor proteins that can detect a number of environmental and microbial danger signals, and will then in turn activate caspase-1, a protease that will allow cleavage and release of proinflammatory cytokines such as IL-1 $\beta$  and IL-18, but also induce host cell death by a specific process called pyroptosis [124,125]. NLRP1 (nucleotide-binding

domain leucine-rich repeat pyrin domain containing 1) and NLRP3 are PRRs that were found to be important *in vivo* regulators of *T. gondii* proliferation [126,127]. NLRPs and the inflammasome cascades they induce have essentially been described in DCs and macrophages, but they are also present in other cell types including intestinal epithelial cells or polynuclear neutrophils. As *T. gondii* can potentially infect many nucleated cell types, the inflammasome is thus likely important for limiting parasite growth and dissemination in several tissues.

### The adaptive response to infection

Pathogen-activated antigen-presenting cells, particularly macrophages and DCs will induce the proliferation and stimulation of natural killer (NK) cells through IL-12 production in conjunction with TNF- $\alpha$  [128], and this is enhanced by IL-18 production [129] (Figure 4). This will trigger a typical T helper type 1 (Th1) effector response, with IFN- $\gamma$ -producing CD4 + T cells, and cytotoxic CD8 + T cells. IFN- $\gamma$  is clearly the main mediator for resistance to *T. gondii* [130]. A robust IFN- $\gamma$ -dependent adaptive Th1-immune response is likely important in both mouse and humans for controlling parasite proliferation [130,131]. Moreover, it is not only crucial for the resolution of acute infections, but also for the control of the latent chronic infections [132].

It should be noted that besides the paramount importance of cellular immunity, in the context of murine toxoplasmosis, humoral immunity, and antibodies-generating B cells have also been shown to contribute at least in part to the control of long-term parasite persistence and vaccination-induced resistance to the infection [133–135].

### The cell-autonomous immune response against *T. gondii*

Cytokines help mounting an efficient anti-parasitic response by activating cells through specific receptors. One of the best-characterized pathway is the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway, which can lead to the transcriptional modulation of hundreds of IFN-regulated genes [136,137]. STAT1 and STAT4, for instance, influence the transcription of pro- but also anti-inflammatory molecules, and are important for innate NK and adaptive T cell responses involved in the resistance to *T. gondii* [138,139]. Yet, beyond the well-known and specialized set of immune cells, most cell lineages are able

to defend themselves against infection through a number of processes collectively termed cell-autonomous immunity [140] (Figure 4), for which IFN stimulation plays a considerable role [141]. One of the best characterized IFN- $\gamma$ -stimulated process of *T. gondii* elimination in mice is through the action of two types of GTPases: Immunity Regulated GTPases (IRGs) and Guanylate Binding Proteins (GBPs). These proteins are recruited to the PVM through a complex and sequential process involving several autophagy-related proteins (acting through an atypical non-degradative function [142]), as well as ubiquitin and p62 [143]. The GTPases seem to disrupt the vacuolar membrane by a process that is still not completely characterized, subsequently exposing the parasites for degradation [144]. Human cells have a wide repertoire of GBPs, but they do not express the IRGs usually found in mice [145], so there is a difference between these two hosts in the GTPase effectors involved in the control of parasite growth. There is generally less information available on the human cell-autonomous response against *T. gondii*. For example how the IFN- $\gamma$ -stimulated non-canonical autophagy pathway mediates parasite growth restriction in HeLa cells is not completely elucidated [146,147].

Cell-autonomous defense mechanisms also include nutrient limitation and other anti-parasitic strategies [141], although they have been mostly characterized in macrophages. For instance, in IFN- $\gamma$ -stimulated murine macrophages, nitric oxide (NO) limits the replication of *T. gondii* [148], while other reactive oxygen species can be used for the clearance of some of the less virulent parasite strains, even by non-stimulated macrophages [149]. IFN-dependent stimulation of indoleamine 2,3-dioxygenases (IDOs) leads to tryptophan depletion (an amino acid the parasite is auxotrophic for) and restricts parasite growth; this has been characterized in human macrophages [150], but also fibroblasts [151]. Yet again, IDOs do not seem to have such a strong implication in *T. gondii* clearance in the mouse model [152,153], highlighting possible differences between hosts in parasite control strategies. As another striking example of this, limitation of iron supply, but not tryptophan, seems to be the main nutrient deprivation restricting parasite growth in IFN- $\gamma$ -stimulated primary rat enterocytes [154]. It should thus be noted that many parasite-restricting processes have been studied in only a few mammalian hosts and certain cell-types, so to which extent they can be extrapolated to other cell types remains uncertain.

## Parasite countermeasures to the host immune response

As we have seen before, *T. gondii* contains several secretory organelles that release virulence factors crucial for host cell invasion and establishment of the parasite in a PV. As early as the invasion process begins, one of the first protective measure is the formation of the MJ that, as a molecular sieve, ensures that the nascent PV will be nonfusogenic with host lysosomes and endosome [78], and thus maintains a neutral pH [155]. Then, the rhoptries and later the dense granules, subsequently help the *T. gondii* tachyzoites modulating the host immune response and defense mechanisms by secreting GRA and ROP effectors, respectively, beyond the PVM (selected factors are highlighted on Figure 4, but more details can be found in recent reviews on this topic [17–20]). Because of the broad host range of *T. gondii*, it must be equipped to intersect with a wide variety of evolutionarily distinct immune systems and host cell types. Again, it should be noted that most of the studies on parasite modulation of the host immune response were performed in the mouse model, and likely involve a different set of effectors depending on the host and the cell type. Moreover, many of these factors, which are instrumental in defining the virulence profile of the parasites for mice, act in a strain-specific manner (Table 1).

### ROP effectors

The first wave of effectors to be released are ROP proteins, which are discharged from the rhoptries into the cytoplasm of the host cell as invasion starts. Several ROPs are kinases that will be exported to the host cell nucleus in order to rapidly subvert signal transduction pathways governing the immune response or apoptosis. One example is ROP16, a tyrosine kinase that can phosphorylate host STAT3/STAT6 [181], which results in the inhibition of cytokine and NO production to favor parasite growth [182]. Another exported kinase, ROP38 is able to modulate the mitogen-activated protein kinase (MAPK) signaling pathway [183]. Several of the ROP effectors, once secreted into the host cytoplasm, associate with the cytoplasmic face of the PV to counteract effectors of host-cell autonomous immunity. The ROP5 pseudokinase, and its serine/threonine kinase partners ROP17/18 can for instance collaborate to phosphorylate IRGs and prevent their recruitment to the PV, again in a strain-dependent manner [184].



**Table 1.** Secreted *T. gondii* ROP and GRA effectors and their strain-specific impact on the host. Abbreviations for cellular localizations: ER, endoplasmic reticulum; IVN, intravacuolar membrane network; PV, parasitophorous vacuole; PVM, PV membrane; Acronyms for host target proteins: ASC, Apoptosis-associated Speck-like protein containing a CARD (caspase activation and recruitment domain); ATF, activating transcription factor; CAMLG, calcium-modulating ligand; CCCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand, DP1, E2F dimerization partner 1; E2F, E2 transcription factor; GBP, guanylate-binding protein; GSK3, glycogen synthase kinase 3; HAUSP, herpesvirus-associated ubiquitin-specific protease; IRG, immunity-related GTPase; MAPK, mitogen-activated protein kinase; MIB complex, mitochondrial intermembrane space bridging complex; NCOR1, nuclear receptor corepressor 1; NuRD, nucleosome-remodeling deacetylase; PP2A, protein phosphatase 2A; STAT, signal transducers, and activators of transcription; TRAF, TNF receptor associated factor.

Effector name	ToxoDB Accession Number (Type I)	Localization	Target host protein	Role	Type I	Type II	Type III	Ref
<b>ROP effectors</b>								
ROP5	TGGT1_308090	PVM	Irga6	Inhibition of PVM IRG coating and activation of ROP18	Active	Less active (ROP18 activity not enhanced)	Active	[156–159]
ROP16	TGGT1_262730	Nucleus	STAT3/6	Activation of STAT3/6 inducing a decrease of IL-12 expression and suppressing TH1 response. Reduces the PVM coating of GBPs	Active	Less active (no sustained STAT3/6 activation)	Active	[160,181,204]
ROP17	TGGT1_258580	PVM	Irga6/Irgb6	Enhances ROP18 activity and binds IRGs (preferentially Irgb6) for disassembly	Active	Not studied	Not studied	[184]
ROP18	TGGT1_205250	PVM/ER	IRGs, ATF6β	Binds IRGs for disassembly (preferentially Irga6). Also targets host transcription factor ATF6β, reducing antigen presentation	Active	Active	Less active (low expression)	[160–164,200–202]
ROP38	TGGT1_242110	IVN/PVM	Unknown	Inhibits MAPK/NF-κB pathways, controlling apoptosis in infected cells and IL-18 secretion. Expression levels vary between <i>T. gondii</i> strains	Potentially less active (low expression)	Active	Active (high expression)	[165–167,183]
ROP54	TGGT1_210370	PVM	GBP2	Inhibits GBP2 coating at the PVM	Less active? (no virulence phenotype for the KO)	Active	Not studied	• [168]
<b>GRA effectors</b>								
GRA6	TGGT1_275440	IVN/PVM	CAMLG	Activates host transcription factor NFAT4 via CAMLG, leading to the expression of CCCL2/CXCL2 and neutrophil/monocyte recruitment. In type II parasites, has an epitope eliciting T-cell response	Active	Less active but has an epitope inducing a strong T-cell response	Active	[169,170]
GRA7	TGGT1_203310	PVM	Irga6, TRAF6, ASC	Accelerates the turnover of Irga6 by interacting with the ROP5/ROP18 complex. The GRA7 protein can also stimulate the immune system through TRAF6/ NF-κB activation and inflammasome activation through the ASC adaptor.	Active	NF-κB pathway and macrophage activation	Not studied	[164,171,197,199]
GRA12	TGGT1_288650	IVN/PVM	Unknown	Inhibits IFN-γ mediated parasite killing	Active	Active	Not studied	• [172]
GRA14	TGGT1_239740	PV/PVM/IVN	Unknown	Activation of NF-κB pathway and recruitment of macrophages in type II parasites, potentially in other strains too	Active	Active	Not studied	[173,199]
GRA15	TGGT1_275470	PVM	TRAF2/ TRAF6 GBP1	In type II parasites, activation of NF-κB pathway via TRAF2/6 interaction leading to IL-12 and IL-18 expression. Also linked to the inhibition of lysosomes fusion and GBP loading to the PVM	Truncated (and thus inactive) in some strains	Active	Active, but less than type II parasites	[174,198,199,205,206]
GRA16	TGGT1_208830	Nucleus	PP2A-B55, HAUSP	Modulates the expression of host cell genes involved in the control of cell cycle progression, p53 signaling, steroids and lipids metabolism	Less active? (no virulence phenotype for the KO)	Active	Not studied	[189]

(Continued)

Table 1. (Continued).

Effector name	ToxodB Accession Number (Type I)	Localization	Target host protein	Role	Type I	Type II	Type III	Ref
GRA18	TGGT1_288840	Cytoplasm	PP2A, GSK3, $\beta$ -catenin	Activation of $\beta$ -Catenin inducing upregulation of IFN- $\beta$ , CCL24 and anti-inflammatory chemokines CCL22 and CCL17	Active (secreted, but no functional study)	Active	Not studied	[191]
GRA24	TGGT1_230180	Nucleus	p38 $\alpha$ /MAPK	Activation of p38 $\alpha$ /MAPK, inducing TH1/M1 polarization and cytokines/chemokines secretion	Active	Active	Not studied	[175]
GRA25	TGGT1_290700	PV	Unknown	Allow secretion of CXCL1 and CCL2 chemokines by infected macrophages	Not studied	Active	Less active	[176]
GRA28	TGGT1_231960	Nucleus	Unknown	Induces CCL22 secretion	Active	Likely active	Likely active	[177]
GRA60	TGGT1_204270	PVM	Unknown	Inhibits Irga6 and Irgb10 recruitment at the PVM	Active	Active	Not studied	[178]
HCE1/TEEGR	TGGT1_239010	Nucleus	E2Fs/DPI	Inhibits NF- $\kappa$ B induced cytokines by interacting with host transcription factors and controls host Cyclin E expression by interacting with DP1	Active	Active	Not studied	[195,196]
TgJST	TGGT1_240060	Nucleus	NuRD, STAT1/2	Blocks signaling through type I interferon by recruiting the NuRD repressor and binding to STAT1/STAT2 heterodimers	Active	Active	Not studied	[193,194]
TgNSM	TGGT1_235140	Nucleus	NCoR	Inhibits interferon-regulated genes involved in cell death	Not studied	Active	Not studied	[190]
MAG1	TGGT1_270240	PVM/ Cytoplasm	Unknown	Inhibits IL-1 $\beta$ secretion in macrophages	Not studied	Active	Not studied	[179]
MAF1b	TGGT1_220950	PVM	MIB complex	Induces host mitochondria association with the PVM and modulates the cytokine response	Active	Inactive	Active	[86,180]

## GRA effectors

After the initial secretion of ROP factors, and as *T. gondii* tachyzoites establish themselves into the protective haven of their PV, they secrete GRA effectors. Most of those will remain within the confines of the PV (within the PV lumen or at the PVM) and will noticeably contribute to long-term nutrient acquisition [82,83]. Yet, some will be secreted into the host cell to act as modulators of important host pathways. Successful export of GRA effectors beyond the PVM usually depends on proteolytic processing by the ASP5 aspartyl protease, and then translocation through the PVM by a complex composed of MYR proteins [81,185,186]. Although translocation itself is not completely elucidated at the molecular level, it involves the aforementioned secreted and PVM-located ROP17 kinase [187], highlighting a collaborative effort between rhoptries and dense granules effectors to subvert host cell functions.

Six MYR-dependent secreted effectors have been described so far: GRA16, GRA18, GRA24, HCE1/TEEGR, TgNSM and TgIST [188]. These effectors will usually act in the host cell nucleus (with the exception of GRA18, that functions in the host cytoplasm), to modulate several pathways involved in parasite control. GRA16 impacts cell cycle-associated pathways through p53 modulation, and potentially promotes host cell survival under stress conditions [189]. Conversely, TgNSM inhibits IFN-regulated genes involved in host cell death, again likely promoting parasite growth and dissemination [190]. GRA18 induces a switch from a Th1 (cellular) to a Th2 (humoral) immune response, more likely to promote parasite survival [191]. GRA24 alters IL-12 levels and the IFN $\gamma$  response through modulation of the p38 MAPK [192]. TgIST is also able to inhibit IFN-dependent signaling by acting as an inhibitor of the transcriptional activator STAT1 [193,194]. Finally, HCE1/TEEGR promotes parasite persistence by antagonizing the nuclear factor-kappa B (NF- $\kappa$ B) pathway [195,196], which plays a key role in modulating innate immunity, inflammation, but also cell death.

Some PVM-located GRAs also have important pro-survival functions for tachyzoites, like GRA7 that promotes IRG turnover [197]. Yet, the same protein, along with other PVM-located GRAs like GRA14 and GRA15, can also be an activator of the NF- $\kappa$ B pathway and promote macrophage activation in strains of *T. gondii* which are less virulent for mice [198,199].

## Selected examples of effector polymorphism and how strain-specificity influences *T. gondii* virulence

Strain-specific differences in *T. gondii* virulence can be linked to the fact that many effectors are highly

polymorphic and thus have different outcomes depending on the strain (summarized on Table 1), but also of the host [17]. Type I *T. gondii* strains are much more virulent for mice than type II and III strains [14]. Taking advantage of these differences, powerful forward genetics approaches such as quantitative trait locus mapping have been used to identify strain-specific *T. gondii* virulence factors, including rhoptry kinases/pseudokinases ROP5, ROP16 and ROP18 [200]. As mentioned before, *T. gondii* interferes with the host immune response by a number of strategies ranging from direct local action on the host immune effectors, to modulation of upstream transcriptional programs in the host nucleus.

Direct interference with host anti-*T. gondii* factors is exemplified by ROP18 from type I strains, which is able to phosphorylate specific residues in the GTPase domain of IRGs to prevent their oligomerization and loading on the PVM, thus preventing its degradation [201,202]. Type III strains, on the contrary, do not express ROP18; thus, they are very sensitive to mouse IRGs [201,203]. Intriguingly, type II strains are efficiently eliminated by IRGs although they express ROP18, but it has been subsequently shown that ROP18 action is potentialized by the action of the ROP5 pseudokinase, which is less active in type II parasites [184]. If anything, this shows strain-specific virulence is the result of a complex and intricate interaction between several polymorphic effectors.

Two of the most striking examples of how strain-specific polymorphism modulates the inflammatory response in mice at the transcriptional level are ROP16 [181] and GRA15 [198]. In type I parasites ROP16 activates the SAT3/STAT6 pathway to favor parasite growth [182], while type II parasites contain a polymorphic form of ROP16 that is a poor STAT activator [204]. GRA15, which is more expressed in type II than in type III parasites, can stimulate the NF- $\kappa$ B-dependent production of inflammatory cytokines and activates macrophages [198,199], but is truncated and thus inactive in the type I lab-adapted RH strain. GRA15 also stimulates the recruitment of GBP1 to the PVM through an unknown mechanism [205]. However, it should be noted that the type I GT1 strain expresses a fully functional GRA15 [198,206]. This shows intra-clonal group variations can be observed and again, this highlights the complex and multifactorial nature of host modulation by parasite effectors, whose differences include sequence polymorphism, but also changes in their expression levels.

The increasing number of effector proteins characterized over the recent years highlights the very complex nature of the host–pathogen interactions

underlying *T. gondii* virulence in the mouse model. In any case, the lethal acute infection by type I parasites or chronic establishment of type II strains in mice constitute an archetypal and certainly useful model to study the balance of both pro- and anti-inflammatory modulation by various parasite proteins. The current state of knowledge shows there is a diversity of mechanisms governing this balance, allowing *T. gondii* to adapt to its host for disseminating without killing it. However, once again, how these effectors may co-opt host functions in other intermediate hosts, in particular in humans, certainly deserves to be investigated further. While murine immunity to *T. gondii* has been extensively studied, data available on the human immune response to the parasite shows common features, but also marked differences [207]. Because the hosts vary in some key innate immune pathways, parasite countermeasures are also likely adapted [208]. To refer to specific parasite effectors mentioned above, the function of the ROP5/17/18 complex on IRG recruitment to the PV is for example not conserved in the human host model [203], as humans lack the extensive IRG repertoire found in mice. While studies in the murine models have revealed a variety of parasite defense mechanisms and intricate relationship between *T. gondii* and its host, they need to be extended further to human cells. This may provide new insights into potential therapeutic strategies.

## Conclusions

*T. gondii* rarely cause serious disease in immunocompetent individuals, yet the high prevalence of this ubiquitous parasite makes it an important zoonotic pathogen. There is likely a coevolution between the parasite and its intermediate hosts, creating a balance between the acute and the chronic phases of the disease that favors parasite transmission. The domestic cat/mouse transmission cycle for *T. gondii*, that arose about 11,000 years ago as a consequence of human agricultural development and cat domestication, has likely been instrumental in selecting parasite strains infecting humans in the Old World [209,210]. However, humans themselves being rare or inaccessible prey for felids, they may be considered a dead-end for parasite transmission to the definitive hosts. While transmission between intermediate hosts by feeding, or vertical transmission to the progeny allow the parasite to bypass its sexual cycle in intermediate hosts felids cannot prey on, this limits genetic diversity and potentially reinforces the clonal population pattern. Perhaps as a result of all this, European infections are in large part attributed to strains that are few,

homogenous, and mildly virulent. On the other hand, there is a greater diversity and pathogenicity of South American strains, which clearly contain more virulent variants. Human contamination with some of these strains originated from wild felids with a forest-based cycle, can cause significant damage and even death in adults who are not particularly immunocompromised, perhaps due to poor adaptation of the parasite to the host [15,211]. This is a sharp reminder that the balance between parasite virulence and adaptation for persistence in its hosts can be fragile.

First-line therapy against toxoplasmosis is usually a combination of pyrimethamine and sulfadiazine (drugs targeting folic acid metabolism), and it is used both for the treatment of congenital toxoplasmosis, or toxoplasmic encephalitis and ocular toxoplasmosis in adults [212]. Although relatively efficient in stopping tachyzoite proliferation, and thus acute toxoplasmosis, these treatments are inefficient against the encysted forms of the parasite [30]. More generally, drugs able to simultaneously target both developmental stages have not yet been identified. Also, there is currently no effective vaccine for human clinical use, which is another major tool unfortunately lacking in our arsenal to combat toxoplasmosis [213]. In any case, as we have seen in this review, *T. gondii* is master in the art of subverting the host immune system for ensuring its long-term persistence. Hence, although there is usually a robust cell-mediated immune response to primary infection in immunocompetent individuals, it controls but does not completely clear the parasite. Thus, so far no immunity sufficient for complete *T. gondii* elimination has been demonstrated in humans. While these are important challenges, there are prospects for developing new approaches for the control of human toxoplasmosis [214]. Recent progress on genetic manipulation of the parasites has allowed identifying novel factors conferring fitness and virulence to *T. gondii* *in vivo* [215–217]. Genetic tools, when coupled to computational modeling [218] or novel imaging techniques [219,220], can also reveal new potential drug targets. Besides, new *in vivo* or *in vitro* differentiation models [102,221–223] offer interesting perspectives for investigating different developmental stages that were largely understudied until now. All this will certainly allow an even broader understanding of the biology of *T. gondii*, which could lead to the identification of new anti-parasitic strategies.

## Acknowledgments

We tried to keep this review as accessible and concise as possible and we apologize to the many investigators whose work we have

not cited. Work in our laboratory is funded by the Agence Nationale de la Recherche (ANR-19-CE15-0023 grant to S.B.)

## Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was supported by the Agence Nationale de la Recherche [ANR-19-CE15-0023].

## ORCID

Sébastien Besteiro  <http://orcid.org/0000-0003-1853-1494>

## References

- [1] Robert-Gangneux F, Dardé M-L. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev.* 2012;25(2):264–296.
- [2] Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int J Parasitol.* 2009;39(12):1385–1394.
- [3] Schlüter D, Däubener W, Schares G, et al. Animals are key to human toxoplasmosis. *Int J Med Microbiol.* 2014;304(7):917–929.
- [4] Dubey JP, Murata FHA, Cerqueira-Cézar CK, et al. Economic and public health importance of *Toxoplasma gondii* infections in sheep: 2009–2020. *Vet Parasitol.* 2020;286:109195.
- [5] Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis.* 1995;172(6):1561–1566.
- [6] Lorenzi H, Khan A, Behnke MS, et al. Local admixture of amplified and diversified secreted pathogenesis determinants shapes mosaic *Toxoplasma gondii* genomes. *Nat Commun.* 2016;7(1):10147.
- [7] Khan A, Dubey JP, Su C, et al. Genetic analyses of atypical *Toxoplasma gondii* strains reveal a fourth clonal lineage in North America. *Int J Parasitol.* 2011;41(6):645–655.
- [8] Ajzenberg D, Yera H, Marty P, et al. Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. *J INFECT DIS.* 2009;199(8):1155–1167.
- [9] Verma SK, Ajzenberg D, Rivera-Sanchez A, et al. Genetic characterization of *Toxoplasma gondii* isolates from Portugal, Austria and Israel reveals higher genetic variability within the type II lineage. *Parasitology.* 2015;142(7):948–957.

- [10] Jiang T, Shwab EK, Martin RM, et al. A partition of *Toxoplasma gondii* genotypes across spatial gradients and among host species, and decreased parasite diversity towards areas of human settlement in North America. *Int J Parasitol.* 2018;48(8):611–619.
- [11] Khan A, Fux B, Su C, et al. Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. *Proc Nat Acad Sci.* 2007;104(37):14872–14877.
- [12] Lehmann T, Marcet PL, Graham DH, et al. Globalization and the population structure of *Toxoplasma gondii*. *Proc Nat Acad Sci.* 2006;103(30):11423–11428.
- [13] Su C, Khan A, Zhou P, et al. Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. *Proc Nat Acad Sci.* 2012;109(15):5844–5849.
- [14] Sibley LD, Boothroyd JC. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature.* 1992;359(6390):82–85.
- [15] Galal L, Hamidović A, Dardé ML, et al. Diversity of *Toxoplasma gondii* strains at the global level and its determinants. *Food Waterborne Parasitol.* 2019;15:e00052.
- [16] Innes EA. Toxoplasmosis: comparative species susceptibility and host immune response. *Comp Immunol Microbiol Infect Dis.* 1997;20(2):131–138.
- [17] Mukhopadhyay D, Arranz-Solís D, Saeij JPJ. Influence of the host and parasite strain on the immune response during *Toxoplasma* infection. *Front Cell Infect Microbiol.* 2020;10:580425.
- [18] Ihara F, Nishikawa Y. *Toxoplasma gondii* manipulates host cell signaling pathways via its secreted effector molecules. *Parasitol Int.* 2021;83:102368.
- [19] Tomita T, Guevara RB, Shah LM, et al. Secreted effectors modulating immune responses to *Toxoplasma gondii*. *Life.* 2021;11(9):988.
- [20] Hakimi M-A, Olias P, Sibley LD. *Toxoplasma* effectors targeting host signaling and transcription. *Clin Microbiol Rev.* 2017;30(3):615–645.
- [21] Calero-Bernal R, Gennari SM. Clinical toxoplasmosis in dogs and cats: an update. *Front Vet Sci.* 2019;6:54.
- [22] Speer CA, Dubey JP. Ultrastructural differentiation of *Toxoplasma gondii* schizonts (types B to E) and gamonts in the intestines of cats fed bradyzoites. *Int J Parasitol.* 2005;35(2):193–206.
- [23] Speer CA, Clark S, Dubey JP. Ultrastructure of the oocysts, sporocysts, and sporozoites of *Toxoplasma gondii*. *J Parasitol.* 1998;84(3):505–512.
- [24] Shapiro K, Bahia-Oliveira L, Dixon B, et al. Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. *Food Waterborne Parasitol.* 2019;15:e00049.
- [25] Torrey EF, Yolken RH. *Toxoplasma* oocysts as a public health problem. *Trends Parasitol.* 2013;29(8):380–384.
- [26] Tilley M, Fichera ME, Jerome ME, et al. *Toxoplasma gondii* sporozoites form a transient parasitophorous vacuole that is impermeable and contains only a subset of dense-granule proteins. *Infect Immun.* 1997;65(11):4598–4605.
- [27] Lambert H, Hitziger N, Dellacasa I, et al. Induction of dendritic cell migration upon *Toxoplasma gondii* infection potentiates parasite dissemination. *Cell Microbiol.* 2006;8(10):1611–1623.
- [28] Barragan A, Sibley LD. Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. *J Exp Med.* 2002;195(12):1625–1633.
- [29] Konradt C, Ueno N, Christian DA, et al. Endothelial cells are a replicative niche for entry of *Toxoplasma gondii* to the central nervous system. *Nat Microbiol.* 2016;1:16001.
- [30] Cerutti A, Blanchard N, Besteiro S. The bradyzoite: a key developmental stage for the persistence and pathogenesis of toxoplasmosis. *Pathogens.* 2020;9(3). DOI:10.3390/pathogens9030234
- [31] Remington JS, Cavanaugh EN. Isolation of the encysted form of *Toxoplasma gondii* from human skeletal muscle and brain. *N Engl J Med.* 1965;273(24):1308–1310.
- [32] Rougier S, Montoya JG, Peyron F. Lifelong persistence of *Toxoplasma* cysts: a questionable dogma? *Trends Parasitol.* 2017;33(2):93–101.
- [33] Belluco S, Simonato G, Mancin M, et al. *Toxoplasma gondii* infection and food consumption: a systematic review and meta-analysis of case-controlled studies. *Crit Rev Food Sci Nutr.* 2018;58(18):3085–3096.
- [34] Thebault A, Kooh P, Cadavez V, et al. Risk factors for sporadic toxoplasmosis: a systematic review and meta-analysis. *Microb Risk Anal.* 2021;17:100133.
- [35] Borges M, Magalhães Silva T, Brito C, et al. How does toxoplasmosis affect the maternal-foetal immune interface and pregnancy? *Parasite Immunol.* 2019;41(3):e12606.
- [36] McLeod R, Lykins J, Gwendolyn Noble A, et al. Management of congenital toxoplasmosis. *Curr Pediatr Rep.* 2014;2(3):166–194.
- [37] Foroutan-Rad M, Majidani H, Dalvand S, et al. Toxoplasmosis in blood donors: a systematic review and meta-analysis. *Transfus Med Rev.* 2016;30(3):116–122.
- [38] Dard C, Marty P, Brenier-Pinchart M-P, et al. Management of toxoplasmosis in transplant recipients: an update. *Expert Rev Anti Infect Ther.* 2018;16(6):447–460.
- [39] Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet.* 2004;363(9425):1965–1976.
- [40] Dunn D, Wallon M, Peyron F, et al. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. *Lancet.* 1999;353(9167):1829–1833.
- [41] McAuley JB. Congenital toxoplasmosis. *J Pediatric Infect Dis Soc.* 2014;3(Suppl 1):S30–35.
- [42] Wang Z-D, Wang S-C, Liu H-H, et al. Prevalence and burden of *Toxoplasma gondii* infection in HIV-infected people: a systematic review and meta-analysis. *Lancet HIV.* 2017;4(4):e177–e188.
- [43] Ali MI, Abd El Wahab WM, Hamdy DA, et al. *Toxoplasma gondii* in cancer patients receiving chemotherapy: seroprevalence and interferon gamma level. *J Parasit Dis.* 2019;43(3):464–471.
- [44] Blanchard N, Dunay IR, Schlüter D. Persistence of *Toxoplasma gondii* in the central nervous system: a fine-tuned balance between the parasite, the brain and the immune system. *Parasite Immunol.* 2015;37(3):150–158.

- [45] Park Y-H, Nam H-W. Clinical features and treatment of ocular toxoplasmosis. *Korean J Parasitol.* 2013;51(4):393–399.
- [46] Greigert V, Bittich-Fahmi F, Pfaff AW. Pathophysiology of ocular toxoplasmosis: facts and open questions. *PLoS Negl Trop Dis.* 2020;14(12):e0008905.
- [47] Gilbert RE, Freeman K, Lago EG, et al.; For The European Multicentre Study on Congenital Toxoplasmosis (EMSCOT). Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. *PLoS Negl Trop Dis.* 2008;2(8):e277.
- [48] Pfaff AW, De-la-torre A, Rochet E, et al. New clinical and experimental insights into old world and neotropical ocular toxoplasmosis. *Int J Parasitol.* 2014;44(2):99–107.
- [49] Tyebeji S, Seizova S, Hannan AJ, et al. Toxoplasmosis: a pathway to neuropsychiatric disorders. *Neurosci Biobehav Rev.* 2019;96:72–92.
- [50] Berdoy M, Webster JP, Macdonald DW. Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc R Soc Lond B.* 2000;267(1452):1591–1594.
- [51] Vyas A, Kim S-K, Giacomini N, et al. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc Nat Acad Sci.* 2007;104(15):6442–6447.
- [52] Gering E, Laubach ZM, Weber PSD, et al. *Toxoplasma gondii* infections are associated with costly boldness toward felids in a wild host. *Nat Commun.* 2021;12(1):3842.
- [53] Ngô HM, Zhou Y, Lorenzi H, et al. *Toxoplasma* modulates signature pathways of human epilepsy, neurodegeneration & cancer. *Sci Rep.* 2017;7(1). DOI:10.1038/s41598-017-10675-6
- [54] Johnson HJ, Koshy AA. Latent toxoplasmosis effects on rodents and humans: how much is real and how much is media hype? *mBio.* 2020;11(2). DOI:10.1128/mBio.02164-19
- [55] Melo EJ, Attias M, De Souza W. The single mitochondrion of tachyzoites of *Toxoplasma gondii*. *J Struct Biol.* 2000;130(1):27–33.
- [56] Ovcariškova J, Lemgruber L, Stilger KL, et al. Mitochondrial behaviour throughout the lytic cycle of *Toxoplasma gondii*. *Sci Rep.* 2017;7(1):42746.
- [57] van Dooren GG, Striepen B. The algal past and parasite present of the apicoplast. *Annu Rev Microbiol.* 2013;67:271–289.
- [58] Sheiner L, Vaidya AB, McFadden GI. The metabolic roles of the endosymbiotic organelles of *Toxoplasma* and *Plasmodium* spp. *Curr Opin Microbiol.* 2013;16(4):452–458.
- [59] Parussini F, Coppens I, Shah PP, et al. Cathepsin L occupies a vacuolar compartment and is a protein maturase within the endo/exocytic system of *Toxoplasma gondii*. *Mol Microbiol.* 2010;76(6):1340–1357.
- [60] Miranda K, Pace DA, Cintron R, et al. Characterization of a novel organelle in *Toxoplasma gondii* with similar composition and function to the plant vacuole. *Mol Microbiol.* 2010;76(6):1358–1375.
- [61] Wolters J. The troublesome parasites — molecular and morphological evidence that Apicomplexa belong to the dinoflagellate-ciliate clade. *Biosystems.* 1991;25(1–2):75–83.
- [62] Frénal K, Dubremetz J-F, Lebrun M, et al. Gliding motility powers invasion and egress in Apicomplexa. *Nat Rev Microbiol.* 2017;15(11):645–660.
- [63] Hu K, Johnson J, Florens L, et al. Cytoskeletal components of an invasion machine—the apical complex of *Toxoplasma gondii*. *PLoS Pathog.* 2006;2(2):e13.
- [64] Dos Santos Pacheco N, Tosetti N, Koreny L, et al. Evolution, composition, assembly, and function of the conoid in Apicomplexa. *Trends Parasitol.* 2020;36(8):688–704.
- [65] Dubois DJ, Soldati-Favre D. Biogenesis and secretion of micronemes in *Toxoplasma gondii*. *Cell Microbiol.* 2019;21(5):e13018.
- [66] Sparvoli D, Lebrun M. Unraveling the elusive rhoptry exocytic mechanism of Apicomplexa. *Trends Parasitol.* 2021;37(7):622–637.
- [67] Ben Chaabene R, Lentini G, Soldati-Favre D. Biogenesis and discharge of the rhoptries: key organelles for entry and hijack of host cells by the Apicomplexa. *Mol Microbiol.* 2021;115(3):453–465.
- [68] Mercier C, Cesbron-Delauw M-F. *Toxoplasma* secretory granules: one population or more? *Trends Parasitol.* 2015;31(2):60–71.
- [69] Heaslip AT, Nelson SR, Warshaw DM. Dense granule trafficking in *Toxoplasma gondii* requires a unique class 27 myosin and actin filaments. *Mol Biol Cell.* 2016;27(13):2080–2089.
- [70] Blader IJ, Coleman BI, Chen C-T, et al. Lytic cycle of *Toxoplasma gondii*: 15 years later. *Annu Rev Microbiol.* 2015;69(1):463–485.
- [71] Martin AM, Liu T, Lynn BC, et al. The *Toxoplasma gondii* parasitophorous vacuole membrane: transactions across the border. *J Eukaryot Microbiol.* 2007;54(1):25–28.
- [72] Clough B, Frickel E-M. The *Toxoplasma* parasitophorous vacuole: an evolving host–parasite frontier. *Trends Parasitol.* 2017;33(6):473–488.
- [73] Carruthers VB, Sibley LD. Sequential protein secretion from three distinct organelles of *Toxoplasma gondii* accompanies invasion of human fibroblasts. *Eur J Cell Biol.* 1997;73(2):114–123.
- [74] Carruthers VB, Tomley FM. Microneme proteins in apicomplexans. *Subcell Biochem.* 2008;47:33–45.
- [75] Whitelaw JA, Latorre-Barragan F, Gras S, et al. Surface attachment, promoted by the actomyosin system of *Toxoplasma gondii* is important for efficient gliding motility and invasion. *BMC Biol.* 2017;15(1):1.
- [76] Kessler H, Herm-Götz A, Hegge S, et al. Microneme protein 8 – a new essential invasion factor in *Toxoplasma gondii*. *J Cell Sci.* 2008;121(7):947–956.
- [77] Besteiro S, Dubremetz J-F, Lebrun M. The moving junction of apicomplexan parasites: a key structure for invasion. *Cell Microbiol.* 2011;13(6):797–805.
- [78] Mordue DG, Desai N, Dustin M, et al. Invasion by *Toxoplasma gondii* establishes a moving junction that selectively excludes host cell plasma membrane proteins on the basis of their membrane anchoring. *J Exp Med.* 1999;190(12):1783–1792.
- [79] Charron AJ, Sibley LD. Molecular partitioning during host cell penetration by *Toxoplasma gondii*: membrane

- dynamics at the moving junction. *Traffic*. 2004;5(11):855–867.
- [80] Coppens I, Romano JD. Hostile intruder: Toxoplasma holds host organelles captive. *PLoS Pathog*. 2018;14(3):e1006893.
- [81] Rastogi S, Cygan AM, Boothroyd JC. Translocation of effector proteins into host cells by Toxoplasma gondii. *Curr Opin Microbiol*. 2019;52:130–138.
- [82] Sibley LD, Niesman IR, Parmley SF, et al. Regulated secretion of multi-lamellar vesicles leads to formation of a tubulo-vesicular network in host-cell vacuoles occupied by Toxoplasma gondii. *J Cell Sci*. 1995;108(4):1669–1677.
- [83] Gold DA, Kaplan AD, Lis A, et al. The Toxoplasma dense granule proteins GRA17 and GRA23 mediate the movement of small molecules between the host and the parasitophorous vacuole. *Cell Host Microbe*. 2015;17(5):642–652.
- [84] Blume M, Seeber F. Metabolic interactions between Toxoplasma gondii and its host. *F1000Res*. 2018;7. DOI:10.12688/f1000research.16021.1.
- [85] Nolan SJ, Romano JD, Coppens I. Host lipid droplets: an important source of lipids salvaged by the intracellular parasite Toxoplasma gondii. *PLoS Pathog*. 2017;13(6):e1006362.
- [86] Pernas L, Adomako-Ankomah Y, Shastri AJ, et al. Toxoplasma effector MAF1 mediates recruitment of host mitochondria and impacts the host response. *PLoS Biol*. 2014;12(4):e1001845.
- [87] Hu K, Mann T, Striepen B, et al. Daughter cell assembly in the protozoan parasite Toxoplasma gondii. *Mol Biol Cell*. 2002;13(2):593–606.
- [88] Nishi M, Hu K, Murray JM, et al. Organellar dynamics during the cell cycle of Toxoplasma gondii. *J Cell Sci*. 2008;121(Pt 9):1559–1568.
- [89] Alvarez CA, Suvorova ES. Checkpoints of apicomplexan cell division identified in Toxoplasma gondii. *PLoS Pathog*. 2017;13(7):e1006483.
- [90] Radke JR, Striepen B, Guerini MN, et al. Defining the cell cycle for the tachyzoite stage of Toxoplasma gondii. *Mol Biochem Parasitol*. 2001;115(2):165–175.
- [91] Moudy R, Manning TJ, Beckers CJ. The loss of cytoplasmic potassium upon host cell breakdown triggers egress of Toxoplasma gondii. *J Biol Chem*. 2001;276(44):41492–41501.
- [92] Caldas LA, Attias M, de Souza W. A structural analysis of the natural egress of Toxoplasma gondii. *Microbes Infect*. 2018;20(1):57–62.
- [93] Bisio H, Soldati-Favre D. Signaling cascades governing entry into and exit from host cells by Toxoplasma gondii. *Annu Rev Microbiol*. 2019;73(1):579–599.
- [94] Vella SA, Moore CA, Li Z-H, et al. The role of potassium and host calcium signaling in Toxoplasma gondii egress. *Cell Calcium*. 2021;94:102337.
- [95] Bisio H, Lunghi M, Brochet M, et al. Phosphatidic acid governs natural egress in Toxoplasma gondii via a guanylate cyclase receptor platform. *Nat Microbiol*. 2019;4(3):420–428.
- [96] Jia Y, Marq J-B, Bisio H, et al. Crosstalk between PKA and PKG controls pH-dependent host cell egress of Toxoplasma gondii. *EMBO J*. 2017;36(21):3250–3267.
- [97] Kafsack BFC, Pena JDO, Coppens I, et al. Rapid membrane disruption by a perforin-like protein facilitates parasite exit from host cells. *Science*. 2009;323(5913):530–533.
- [98] Watts E, Zhao Y, Dhara A, et al. Novel approaches reveal that Toxoplasma gondii Bradyzoites within tissue cysts are dynamic and replicating entities in vivo. *MBio*. 2015;6(5):e01155–01115.
- [99] Dziarszinski F, Nishi M, Ouko L, et al. Dynamics of Toxoplasma gondii differentiation. *Eukaryot Cell*. 2004;3(4):992–1003.
- [100] Tomasina R, Francia ME. The structural and molecular underpinnings of gametogenesis in Toxoplasma gondii. *Front Cell Infect Microbiol*. 2020;10:608291.
- [101] Ferguson DJP. Toxoplasma gondii and sex: essential or optional extra? *Trends Parasitol*. 2002;18(8):351–355.
- [102] Martorelli Di Genova B, Wilson SK, Dubey JP, et al. Intestinal delta-6-desaturase activity determines host range for Toxoplasma sexual reproduction. *PLoS Biol*. 2019;17(8):e3000364.
- [103] Farhat DC, Swale C, Dard C, et al. A MORC-driven transcriptional switch controls Toxoplasma developmental trajectories and sexual commitment. *Nat Microbiol*. 2020;5(4):570–583.
- [104] Waldman BS, Schwarz D, Wadsworth MH, et al. Identification of a master regulator of differentiation in Toxoplasma. *Cell*. 2020:S0092867419313753. DOI:10.1016/j.cell.2019.12.013
- [105] Pittman KJ, Aliota MT, Knoll LJ. Dual transcriptional profiling of mice and Toxoplasma gondii during acute and chronic infection. *BMC Genomics*. 2014;15:806.
- [106] Garfoot AL, Wilson GM, Coon JJ, et al. Proteomic and transcriptomic analyses of early and late-chronic Toxoplasma gondii infection shows novel and stage specific transcripts. *BMC Genomics*. 2019;20(1):859.
- [107] Buchholz KR, Fritz HM, Chen X, et al. Identification of tissue cyst wall components by transcriptome analysis of in vivo and in vitro Toxoplasma gondii bradyzoites. *Eukaryot Cell*. 2011;10(12):1637–1647.
- [108] Fritz HM, Buchholz KR, Chen X, et al. Transcriptomic analysis of toxoplasma development reveals many novel functions and structures specific to sporozoites and oocysts. *PLoS ONE*. 2012;7(2):e29998.
- [109] Hehl AB, Basso WU, Lippuner C, et al. Asexual expansion of Toxoplasma gondii merozoites is distinct from tachyzoites and entails expression of non-overlapping gene families to attach, invade, and replicate within feline enterocytes. *BMC Genomics*. 2015;16:66.
- [110] Tu V, Mayoral J, Sugi T, et al. Enrichment and Proteomic characterization of the cyst wall from in vitro Toxoplasma gondii cysts. *mBio*. 2019;10(2):e00469–19.
- [111] Gee K, Guzzo C, Che Mat NF, et al. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. *Inflamm Allergy Drug Targets*. 2009;8(1):40–52.
- [112] Koblansky AA, Jankovic D, Oh H, et al. Recognition of profilin by Toll-like receptor 12 is critical for host resistance to Toxoplasma gondii. *Immunity*. 2013;38(1):119–130.
- [113] Yarovinsky F, Zhang D, Andersen JF, et al. TLR11 activation of dendritic cells by a protozoan

- profilin-like protein. *Science*. 2005;308(5728):1626–1629.
- [114] Plattner F, Yarovinsky F, Romero S, et al. Toxoplasma profilin is essential for host cell invasion and TLR11-dependent induction of an interleukin-12 response. *Cell Host Microbe*. 2008;3(2):77–87.
- [115] Sardinha-Silva A, Mendonça-Natividade FC, Pinzan CF, et al. The lectin-specific activity of Toxoplasma gondii microneme proteins 1 and 4 binds Toll-like receptor 2 and 4 N-glycans to regulate innate immune priming. *PLoS Pathog*. 2019;15(6):e1007871.
- [116] Ricci-Azevedo R, Mendonça-Natividade FC, Santana AC, et al. Microneme proteins 1 and 4 from Toxoplasma gondii induce IL-10 production by macrophages through TLR4 endocytosis. *Front Immunol*. 2021;12:655371.
- [117] Debierre-Grockiego F, Campos MA, Azzouz N, et al. Activation of TLR2 and TLR4 by glycosylphosphatidylinositols derived from Toxoplasma gondii. *J Immunol*. 2007;179(2):1129–1137.
- [118] Scanga CA, Aliberti J, Jankovic D, et al. Cutting Edge: myD88 is required for resistance to Toxoplasma gondii infection and regulates parasite-induced IL-12 production by dendritic cells. *J Immunol*. 2002;168(12):5997–6001.
- [119] Sher A, Tosh K, Jankovic D. Innate recognition of Toxoplasma gondii in humans involves a mechanism distinct from that utilized by rodents. *Cell Mol Immunol*. 2017;14(1):36–42.
- [120] Andrade WA, Souza MDC, Ramos-Martinez E, et al. Combined action of nucleic acid-sensing Toll-like receptors and TLR11/TLR12 heterodimers imparts resistance to Toxoplasma gondii in mice. *Cell Host Microbe*. 2013;13(1):42–53.
- [121] Aldebert D, Durand F, Mercier C, et al. Toxoplasma gondii triggers secretion of interleukin-12 but low level of interleukin-10 from the THP-1 human monocytic cell line. *Cytokine*. 2007;37(3):206–211.
- [122] Beck JR, Rodriguez-Fernandez IA, de Leon JC, et al. A novel family of Toxoplasma IMC proteins displays a hierarchical organization and functions in coordinating parasite division. *PLoS Pathog*. 2010;6(9):e1001094.
- [123] Ivanova DL, Denton SL, Fettel KD, et al. Innate lymphoid cells in protection, pathology, and adaptive immunity during apicomplexan infection. *Front Immunol*. 2019;10:196.
- [124] Wang Y, Zhu J, Cao Y, et al. Insight into inflammatory signaling: implications for Toxoplasma gondii infection. *Front Immunol*. 2020;11:583193.
- [125] Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;526(7575):660–665.
- [126] Ewald SE, Chavarria-Smith J, Boothroyd JC. NLRP1 is an inflammasome sensor for Toxoplasma gondii. *Infect Immun*. 2014;82(1):460–468.
- [127] Gorfu G, Cirelli KM, Melo MB, et al. Dual role for inflammasome sensors NLRP1 and NLRP3 in murine resistance to Toxoplasma gondii. *mBio*. 2014;5(1). DOI:10.1128/mBio.01117-13
- [128] Sher A, Oswald IP, Hieny S, et al. Toxoplasma gondii induces a T-independent IFN-gamma response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-alpha. *J Immunol*. 1993;150(9):3982–3989.
- [129] Cai G, Kastelein R, Hunter CA. Interleukin-18 (IL-18) enhances innate IL-12-mediated resistance to Toxoplasma gondii. *Infect Immun*. 2000;68(12):6932–6938.
- [130] Suzuki Y, Orellana MA, Schreiber RD, et al. Interferon-gamma: the major mediator of resistance against Toxoplasma gondii. *Science*. 1988;240(4851):516–518.
- [131] Däubener W, Mackenzie C, Hadding U. Establishment of T-helper type 1- and T-helper type 2-like human Toxoplasma antigen-specific T-cell clones. *Immunology*. 1995;86(1):79–84.
- [132] Gazzinelli R, Xu Y, Hieny S, et al. Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with Toxoplasma gondii. *J Immunol*. 1992;149(1):175–180.
- [133] Kang H, Remington JS, Suzuki Y. Decreased resistance of B cell-deficient mice to infection with Toxoplasma gondii despite unimpaired expression of IFN- $\gamma$ , TNF- $\alpha$ , and inducible nitric oxide synthase. *J Immunol*. 2000;164(5):2629–2634.
- [134] Deshmukh AS, Gurupwar R, Mitra P, et al. Toxoplasma gondii induces robust humoral immune response against cyst wall antigens in chronically infected animals and humans. *Microb Pathog*. 2021;152:104643.
- [135] Johnson LL, Sayles PC. Deficient humoral responses underlie susceptibility to Toxoplasma gondii in CD4-deficient mice. *Infect Immun*. 2002;70(1):185–191.
- [136] Darnell J, Kerr I, Stark G. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*. 1994;264(5164):1415–1421.
- [137] Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol*. 2014;32:513–545.
- [138] Cai G, Radzanowski T, Villegas EN, et al. Identification of STAT4-dependent and independent mechanisms of resistance to Toxoplasma gondii. *J Immunol*. 2000;165(5):2619–2627.
- [139] Lieberman LA, Banica M, Reiner SL, et al. STAT1 plays a critical role in the regulation of antimicrobial effector mechanisms, but not in the development of Th1-type responses during toxoplasmosis. *J Immunol*. 2004;172(1):457–463.
- [140] Randow F, MacMicking JD, James LC. Cellular self-defense: how cell-autonomous immunity protects against pathogens. *Science*. 2013;340(6133):701–706.
- [141] MacMicking JD. Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nat Rev Immunol*. 2012;12(5):367–382.
- [142] Besteiro S. The role of host autophagy machinery in controlling Toxoplasma infection. *Virulence*. 2019;10(1):438–447.
- [143] Saeij JP, Frickel E-M. Exposing Toxoplasma gondii hiding inside the vacuole: a role for GBPs, autophagy and host cell death. *Curr Opin Microbiol*. 2017;40:72–80.
- [144] Martens S, Parvanova I, Zerrahn J, et al. Disruption of Toxoplasma gondii parasitophorous vacuoles by the

- mouse p47-resistance GTPases. *PLoS Pathog.* **2005**;1(3):e24.
- [145] Pilla-Moffett D, Barber MF, Taylor GA, et al. Interferon-inducible GTPases in host resistance, inflammation and disease. *J Mol Biol.* **2016**;428(17):3495–3513.
- [146] Selleck EM, Orchard RC, Lassen KG, et al. A noncanonical autophagy pathway restricts *Toxoplasma gondii* growth in a strain-specific manner in IFN- $\gamma$ -activated human cells. *MBio.* **2015**;6(5). DOI:10.1128/mBio.01157-15
- [147] Bhushan J, Radke JB, Perng Y-C, et al. ISG15 connects autophagy and IFN- $\gamma$ -dependent control of *Toxoplasma gondii* infection in human cells. *mBio.* **2020**;11(5):e00852–20.
- [148] Adams LB, Hibbs JB, Taintor RR, et al. Microbiostatic effect of murine-activated macrophages for *Toxoplasma gondii*. Role for synthesis of inorganic nitrogen oxides from L-arginine. *J Immunol.* **1990**;144(7):2725–2729.
- [149] Matta SK, Patten K, Wang Q, et al. NADPH oxidase and guanylate binding protein 5 restrict survival of avirulent type III strains of *Toxoplasma gondii* in naive macrophages. *mBio.* **2018**;9(4):e01393–18.
- [150] Schmitz JL, Carlin JM, Borden EC, et al. Beta interferon inhibits *Toxoplasma gondii* growth in human monocyte-derived macrophages. *Infect Immun.* **1989**;57(10):3254–3256.
- [151] Pfefferkorn ER. Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. *Proc Natl Acad Sci USA.* **1984**;81(3):908–912.
- [152] Ufermann C-M, Domröse A, Babel T, et al. Indoleamine 2,3-dioxygenase activity during acute toxoplasmosis and the suppressed T cell proliferation in mice. *Front Cell Infect Microbiol.* **2019**;9:184.
- [153] Divanovic S, Sawtell NM, Trompette A, et al. Opposing biological functions of tryptophan catabolizing enzymes during intracellular infection. *J Infect Dis.* **2012**;205(1):152–161.
- [154] Dimier IH, Bout DT. Interferon-gamma-activated primary enterocytes inhibit *Toxoplasma gondii* replication: a role for intracellular iron. *Immunology.* **1998**;94(4):488–495.
- [155] Sibley LD, Weidner E, Krahenbuhl JL. Phagosome acidification blocked by intracellular *Toxoplasma gondii*. *Nature.* **1985**;315(6018):416–419.
- [156] Behnke MS, Khan A, Wootton JC, et al. Virulence differences in *Toxoplasma* mediated by amplification of a family of polymorphic pseudokinases. *Proc Nat Acad Sci.* **2011**;108(23):9631–9636.
- [157] Behnke MS, Fentress SJ, Mashayekhi M, et al. The polymorphic pseudokinase ROP5 controls virulence in *Toxoplasma gondii* by regulating the active kinase ROP18. *PLoS Pathog.* **2012**;8(11):e1002992.
- [158] Fleckenstein MC, Reese ML, Könen-Waisman S, et al. A *Toxoplasma gondii* pseudokinase inhibits host IRG resistance proteins. *PLoS Biol.* **2012**;10(7):e1001358.
- [159] Murillo-León M, Müller UB, Zimmermann I, et al. Molecular mechanism for the control of virulent *Toxoplasma gondii* infections in wild-derived mice. *Nat Commun.* **2019**;10(1):1233.
- [160] Saeij JPJ, Boyle JP, Collier S, et al. Polymorphic secreted kinases are key virulence factors in Toxoplasmosis. *Science.* **2006**;314(5806):1780–1783.
- [161] Zhao Y, Ferguson DJP, Wilson DC, et al. Virulent *Toxoplasma gondii* evade immunity-related GTPase-mediated parasite vacuole disruption within primed macrophages. *J Immunol.* **2009**;182(6):3775–3781.
- [162] Khaminets A, Hunn JP, Könen-Waisman S, et al. Coordinated loading of IRG resistance GTPases on to the *Toxoplasma gondii* parasitophorous vacuole. *Cell Microbiol.* **2010**;12(7):939–961.
- [163] Yamamoto M, Ma JS, Mueller C, et al. ATF6 $\beta$  is a host cellular target of the *Toxoplasma gondii* virulence factor ROP18. *J Exp Med.* **2011**;208(7):1533–1546.
- [164] Hermanns T, Müller UB, Könen-Waisman S, et al. The *Toxoplasma gondii* rhoptry protein ROP18 is an Irga6-specific kinase and regulated by the dense granule protein GRA7. *Cell Microbiol.* **2016**;18(2):244–259.
- [165] Fox BA, Rommereim LM, Guevara RB, et al. The *Toxoplasma gondii* rhoptry kinome is essential for chronic infection. *mBio.* **2016**;7(3). DOI:10.1128/mBio.00193-16
- [166] Xu Y, Wang X, Liu J, et al. *Toxoplasma gondii* rhoptry protein38 (TgROP38) affects parasite invasion, egress, and induces IL-18 secretion during early infection. *Acta Biochim Biophys Sin (Shanghai).* **2018**;50(8):766–775.
- [167] Melo MB, Nguyen QP, Cordeiro C, et al. Transcriptional analysis of murine macrophages infected with different *Toxoplasma* strains identifies novel regulation of host signaling pathways. *PLoS Pathog.* **2013**;9(12):e1003779.
- [168] Kim EW, Nadipuram SM, Tetlow AL, et al. The rhoptry pseudokinase ROP54 modulates *Toxoplasma gondii* virulence and host GBP2 loading. *mSphere.* **2016**;1(2). DOI:10.1128/mSphere.00045-16
- [169] Ma JS, Sasai M, Ohshima J, et al. Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6. *J Exp Med.* **2014**;211(10):2013–2032.
- [170] Blanchard N, Gonzalez F, Schaeffer M, et al. Immunodominant, protective response to the parasite *Toxoplasma gondii* requires antigen processing in the endoplasmic reticulum. *Nat Immunol.* **2008**;9(8):937–944.
- [171] Yang C-S, Yuk J-M, Lee Y-H, et al. *Toxoplasma gondii* GRA7-induced TRAF6 activation contributes to host protective immunity. *Infect Immun.* **2016**;84(1):339–350.
- [172] Fox BA, Guevara RB, Rommereim LM, et al. *Toxoplasma gondii* parasitophorous vacuole membrane-associated dense granule proteins orchestrate chronic infection and GRA12 underpins resistance to host gamma interferon. *mBio.* **2019**;10(4). DOI:10.1128/mBio.00589-19
- [173] Rome ME, Beck JR, Turetzky JM, et al. Intervacuolar transport and unique topology of GRA14, a novel dense granule protein in *Toxoplasma gondii*. *Infect Immun.* **2008**;76(11):4865–4875.
- [174] Sangaré LO, Yang N, Konstantinou EK, et al. *Toxoplasma* GRA15 activates the NF- $\kappa$ B pathway

- through interactions with TNF receptor-associated factors. *mBio*. 2019;10(4):e00808–19.
- [175] Braun L, Brenier-Pinchart M-P, Yogavel M, et al. A *Toxoplasma* dense granule protein, GRA24, modulates the early immune response to infection by promoting a direct and sustained host p38 MAPK activation. *J Exp Med*. 2013;210(10):2071–2086.
- [176] Shastri AJ, Marino ND, Franco M, et al. GRA25 is a novel virulence factor of *Toxoplasma gondii* and influences the host immune response. *Infect Immun*. 2014;82(6):2595–2605.
- [177] Rudzki EN, Ander SE, Coombs RS, et al. *Toxoplasma gondii* GRA28 is required for placenta-specific induction of the regulatory chemokine CCL22 in human and mouse [Internet]; preprint; *mBio* - in press, 2020. <https://doi.org/10.1101/2020.10.14.335802>.
- [178] Nyonda MA, Hammoudi P, Ye S, et al. *Toxoplasma gondii* GRA60 is an effector protein that modulates host cell autonomous immunity and contributes to virulence. *Cell Microbiol*. 2021;23(2). DOI:10.1111/cmi.13278
- [179] Tomita T, Mukhopadhyay D, Han B, et al. *Toxoplasma gondii* matrix antigen 1 Is a secreted immunomodulatory effector. *mBio*. 2021;12(3). DOI:10.1128/mBio.00603-21
- [180] Blank ML, Xia J, Morcos MM, et al. *Toxoplasma gondii* association with host mitochondria requires key mitochondrial protein import machinery. *Proc Natl Acad Sci USA*. 2021;118(12):e2013336118.
- [181] Saeij JPJ, Collier S, Boyle JP, et al. *Toxoplasma* co-opts host gene expression by injection of a polymorphic kinase homologue. *Nature*. 2007;445(7125):324–327.
- [182] Butcher BA, Fox BA, Rommereim LM, et al. *Toxoplasma gondii* rhoptry kinase ROP16 activates STAT3 and STAT6 resulting in cytokine inhibition and arginase-1-dependent growth control. *PLoS Pathog*. 2011;7(9):e1002236.
- [183] Peixoto L, Chen F, Harb OS, et al. Integrative genomic approaches highlight a family of parasite-specific kinases that regulate host responses. *Cell Host Microbe*. 2010;8(2):208–218.
- [184] Etheridge RD, Alaganan A, Tang K, et al. The *Toxoplasma* pseudokinase ROP5 forms complexes with ROP18 and ROP17 kinases that synergize to control acute virulence in mice. *Cell Host Microbe*. 2014;15(5):537–550.
- [185] Marino ND, Panas MW, Franco M, et al. Identification of a novel protein complex essential for effector translocation across the parasitophorous vacuole membrane of *Toxoplasma gondii*. *PLoS Pathog*. 2018;14(1):e1006828.
- [186] Naor A, Panas MW, Marino N, et al. MYR1-dependent effectors are the major drivers of a host cell's early response to *Toxoplasma*, including counteracting MYR1-independent effects. *mBio*. 2018;9(2). DOI:10.1128/mBio.02401-17
- [187] Panas MW, Ferrel A, Naor A, et al. Translocation of dense granule effectors across the parasitophorous vacuole membrane in *Toxoplasma*-infected cells requires the activity of ROP17, a rhoptry protein kinase. *mSphere*. 2019;4(4):e00276–19.
- [188] Panas MW, Boothroyd JC. Seizing control: how dense granule effector proteins enable *Toxoplasma* to take charge. *Mol Microbiol*. 2021;115(3):466–477.
- [189] Bougdour A, Durandau E, Brenier-Pinchart M-P, et al. Host cell subversion by *Toxoplasma* GRA16, an exported dense granule protein that targets the host cell nucleus and alters gene expression. *Cell Host Microbe*. 2013;13(4):489–500.
- [190] Rosenberg A, Sibley LD. *Toxoplasma gondii* secreted effectors co-opt host repressor complexes to inhibit necroptosis. *Cell Host Microbe*. 2021;29(7):1186–1198.e8.
- [191] He H, Brenier-Pinchart M-P, Braun L, et al. Characterization of a *Toxoplasma* effector uncovers an alternative GSK3/ $\beta$ -catenin-regulatory pathway of inflammation. *Elife*. 2018;7:e39887.
- [192] Pellegrini E, Palencia A, Braun L, et al. Structural basis for the subversion of MAP kinase signaling by an intrinsically disordered parasite secreted agonist. *Structure*. 2017;25(1):16–26.
- [193] Gay G, Braun L, Brenier-Pinchart M-P, et al. *Toxoplasma gondii* TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN- $\gamma$ -mediated host defenses. *J Exp Med*. 2016;213(9):1779–1798.
- [194] Olias P, Etheridge RD, Zhang Y, et al. *Toxoplasma* effector recruits the Mi-2/NuRD complex to repress STAT1 transcription and block IFN- $\gamma$ -dependent gene expression. *Cell Host Microbe*. 2016;20(1):72–82.
- [195] Braun L, Brenier-Pinchart M-P, Hammoudi P-M, et al. The *Toxoplasma* effector TEEGR promotes parasite persistence by modulating NF- $\kappa$ B signalling via EZH2. *Nat Microbiol*. 2019;4(7):1208–1220.
- [196] Panas MW, Naor A, Cygan AM, et al. *Toxoplasma* controls host cyclin E expression through the use of a novel MYR1-dependent effector protein, HCE1. *mBio*. 2019;10(2):e00674–19.
- [197] Alaganan A, Fentress SJ, Tang K, et al. *Toxoplasma* GRA7 effector increases turnover of immunity-related GTPases and contributes to acute virulence in the mouse. *Proc Natl Acad Sci U S A*. 2014;111(3):1126–1131.
- [198] Rosowski EE, Lu D, Julien L, et al. Strain-specific activation of the NF- $\kappa$ B pathway by GRA15, a novel *Toxoplasma gondii* dense granule protein. *J Exp Med*. 2011;208(1):195–212.
- [199] Ihara F, Fereig RM, Himori Y, et al. *Toxoplasma gondii* dense granule proteins 7, 14, and 15 are involved in modification and control of the immune response mediated via NF- $\kappa$ B pathway. *Front Immunol*. 2020;11:1709.
- [200] Taylor S, Barragan A, Su C, et al. A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen *Toxoplasma gondii*. *Science*. 2006;314(5806):1776–1780.
- [201] Fentress SJ, Behnke MS, Dunay IR, et al. Phosphorylation of immunity-related GTPases by a *Toxoplasma gondii*-secreted kinase promotes macrophage survival and virulence. *Cell Host Microbe*. 2010;8(6):484–495.
- [202] Steinfeldt T, Könen-Waisman S, Tong L, et al. Phosphorylation of mouse immunity-related GTPase

- (IRG) resistance proteins is an evasion strategy for virulent *Toxoplasma gondii*. *PLoS Biol.* **2010**;8(12): e1000576.
- [203] Niedelman W, Gold DA, Rosowski EE, et al. The rhoptry proteins ROP18 and ROP5 mediate *Toxoplasma gondii* evasion of the murine, but not the human, interferon-gamma response. *PLoS Pathog.* **2012**;8(6):e1002784.
- [204] Jensen KDC, Hu K, Whitmarsh RJ, et al. *Toxoplasma gondii* rhoptry 16 kinase promotes host resistance to oral infection and intestinal inflammation only in the context of the dense granule protein GRA15. *Infect Immun.* **2013**;81(6):2156–2167.
- [205] Virreira Winter S, Niedelman W, Jensen KD, et al. Determinants of GBP recruitment to *Toxoplasma gondii* vacuoles and the parasitic factors that control it. *PLoS ONE.* **2011**;6(9):e24434.
- [206] Yang N, Farrell A, Niedelman W, et al. Genetic basis for phenotypic differences between different *Toxoplasma gondii* type I strains. *BMC Genomics.* **2013**;14(1):467.
- [207] Fisch D, Clough B, Frickel E-M. Human immunity to *Toxoplasma gondii*. *PLoS Pathog.* **2019**;15(12): e1008097.
- [208] Lima TS, Lodoen MB. Mechanisms of human innate immune evasion by *Toxoplasma gondii*. *Front Cell Infect Microbiol.* **2019**;9:103.
- [209] Müller UB, Howard JC. The impact of *Toxoplasma gondii* on the mammalian genome. *Curr Opin Microbiol.* **2016**;32:19–25.
- [210] Schwab EK, Saraf P, Zhu X-Q, et al. Human impact on the diversity and virulence of the ubiquitous zoonotic parasite *Toxoplasma gondii*. *Proc Natl Acad Sci USA.* **2018**;115(29):E6956–E6963.
- [211] Carme B, Demar M, Ajzenberg D, et al. Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. *Emerg Infect Dis.* **2009**;15(4):656–658.
- [212] Konstantinovic N, Guegan H, Stājner T, et al. Treatment of toxoplasmosis: current options and future perspectives. *Food Waterborne Parasitol.* **2019**;15:e00036.
- [213] Chu K-B, Quan F-S. Advances in *Toxoplasma gondii* vaccines: current strategies and challenges for vaccine development. *Vaccines (Basel).* **2021**;9(5):413.
- [214] Smith NC, Goulart C, Hayward JA, et al. Control of human toxoplasmosis. *Int J Parasitol.* **2021**;51(2–3):95–121.
- [215] Sidik SM, Huet D, Ganesan SM, et al. A genome-wide CRISPR screen in *Toxoplasma* identifies essential apicomplexan genes. *Cell.* **2016**;166(6):1423–1435.e12.
- [216] Young J, Dominicus C, Wagener J, et al. A CRISPR platform for targeted in vivo screens identifies *Toxoplasma gondii* virulence factors in mice. *Nat Commun.* **2019**;10(1):3963.
- [217] Wang Y, Sangaré LO, Paredes-Santos TC, et al. Genome-wide screens identify *Toxoplasma gondii* determinants of parasite fitness in IFN $\gamma$ -activated murine macrophages. *Nat Commun.* **2020**;11(1):5258.
- [218] Krishnan A, Kloehn J, Lunghi M, et al. Functional and computational genomics reveal unprecedented flexibility in stage-specific *Toxoplasma* metabolism. *Cell Host Microbe.* **2020**;S193131282030041X. DOI:10.1016/j.chom.2020.01.002.
- [219] Dos Santos Pacheco N, Soldati-Favre D. Coupling auxin-inducible degron system with ultrastructure expansion microscopy to accelerate the discovery of gene function in *Toxoplasma gondii*. *Methods Mol Biol.* **2021**;2369:121–137.
- [220] De Niz M, Nacer A, Frischknecht F. Intravital microscopy: imaging host–parasite interactions in the brain. *Cell Microbiol.* **2019**;21(5):e13024.
- [221] Ferreira-da-silva MDF, Takács AC, Barbosa HS, et al. Primary skeletal muscle cells trigger spontaneous *Toxoplasma gondii* tachyzoite-to-bradyzoite conversion at higher rates than fibroblasts. *Int J Med Microbiol.* **2009**;299(5):381–388.
- [222] Mouveaux T, Roger E, Gueye A, et al. Primary brain cell infection by *Toxoplasma gondii* reveals the extent and dynamics of parasite differentiation and its impact on neuron biology. *Open Biol.* **2021**. DOI:10.1098/rsob.210053
- [223] Christiansen C, Maus D, Melerowicz F, et al. A novel in vitro model for mature *Toxoplasma gondii* bradyzoites reveals their metabolome and a diminished role of the mitochondrial tricarboxylic acid cycle [Internet]; preprint. *BioRxiv.* **2021**. DOI:10.1101/2021.01.15.426845