

IFN- γ -induced Cell Autonomous Immunity to *Toxoplasma gondii*

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Abstract

Toxoplasma gondii is an obligate intracellular parasite that causes the disease toxoplasmosis. This highly successful parasite is able to infect virtually any warm blooded vertebrate host and host cell even though the definitive host is *felidae*. Here, we focus on IFN- γ -inducible cell autonomous immunity to *T. gondii* and mechanisms which the parasite has evolved to evade intracellular antimicrobial defenses. These are discussed in the context of co-evolution of *T. gondii* with its murine intermediate host.

The obligate intracellular pathogen *Toxoplasma gondii* is a highly successful parasite that can infect virtually any warm blooded animal even though its sexual cycle is restricted to *felidae*. Rodents, however, are a significant intermediate host and the parasite has evolved mechanisms to manipulate the behavior of *T. gondii* infected rodents to increase successful predation by *felidae* [1-4]. In addition to infecting a wide array of host species, *T. gondii* also infects virtually any vertebrate host cell as it uses a parasite actin-myosin motor to invade cells circumventing the need for phagocytosis [5,6]. Upon cell invasion, *T. gondii* forms its own unique intracellular compartment, or Parasitophorous Vacuole (PV), where it replicates, eventually lysing its host cell to egress and invade adjacent cells to resume its lytic cycle. Because *T. gondii* actively invades host cells forming a nascent PV in the process, it avoids even transient residence in a phagosome [7,8]. Likewise, the PV remains largely segregated from conventional host cell endocytic and exocytic trafficking pathways [9,10].

The relative contribution and efficacy of IFN- γ -inducible antimicrobial effectors varies depending on the intracellular niche occupied by the pathogen, the host species that is infected as well as the pathogen and strain-specific evasion mechanisms. In the case of *T. gondii*, its unique intracellular niche makes it impervious to antimicrobial mediators that operate strictly within the confines of a phagosome or on free microbes in the cytosol. However, host species have evolved IFN- γ -inducible mechanisms that are capable of acting on *T. gondii* within its segregated PV [11]. *T. gondii* has countered this, in part, by evolving a yet undefined mechanism to disrupt chromatin remodeling of STAT1 regulated promoters in infected cells; resulting in suppression of greater than 60% of IFN- γ induced transcripts [12-14]. Consequently, anti-*T. gondii* effector activity can differ depending on whether host cells are activated prior versus after parasite invasion. However, parasite downregulation of inducible nitric oxide synthase (iNOS) is not necessarily sufficient to avoid growth arrest by the residual nitric oxide (NO) produced even in infected cells and this may hold true for other antimicrobial effectors as well [15]. IFN- γ -inducible indoleamine 2, 3-dioxygenases (IDOs) mediate anti-*T. gondii* activity by restricting intracellular access to tryptophan; *T. gondii* is a tryptophan auxotroph [16,17]. IFN- γ -induced gasses such as reactive nitrogen (RNS) and oxygen species (ROS) are ancient and relatively conserved anti-microbial agents that can disrupt function of multiple processes in a microbe simultaneously and have the added benefits of acting synergistically and of being highly diffusible to enable contact with pathogens in diverse intracellular niches. In the case of *T. gondii*, inducible ROS generated predominantly by NADPH oxidase are capable of anti-*T. gondii* activity [18-20] during infection in both humans and mice. Similarly, nitric oxide generated by iNOS suppresses parasite replication independent of parasite genotype [21-

23]. However, iNOS^{-/-} mice retain the capacity to control acute, but not chronic, *T. gondii* infection although overall parasite numbers are increased compared to infected wild type mice [24-26]. This may, in part, be a consequence of *T. gondii* having evolved numerous mechanisms to withstand NO/RNS [27,28]. However, it more likely reflects the dominant and essential role of IFN- γ -induced immunity related p47 GTPases (IRGs) against Type II and III genotypes of the parasite in mice [29-33]. IRGs are sequentially and coordinately recruited (loaded) onto the PV rapidly following parasite invasion and break it down allowing destruction of the parasites within [34,35]. In contrast, the IRG family is largely absent in humans and do not contribute to the human immune response against *T. gondii* [36]. IFN- γ -inducible p65 Guanylate-Binding Proteins (GBPs) also contribute to anti-*T. gondii* activity at least in part by aiding the recruitment of IRGs to the PV [37,38]. GBPs unlike IRGs are well represented in humans and could play a role in parasite control in human infections.

Mice are important intermediate hosts for *T. gondii* and it is possible that the expanded family of IRGs in mice relative to humans may be an evolutionary adaptation to enable chronic versus lethal infection of mice with the parasite. It is evident that *T. gondii*-genotype-dependent mechanisms have evolved specifically to counter the action of IRGs. Type I genotypes of *T. gondii* share a phenotype of acute virulence in mice defined as an LD100 of a single parasite [39]. Two secreted parasite rhoptry proteins, ROP18 and ROP5, prevent loading of IRGs to PVs abolishing the effectiveness of IRGs against *T. gondii* [40-46]. Another parasite genotype-dependent adaptation, ROP16 from Type I and III strains, maintains constitutive activation of STAT 6 (IL-4 pathway) and STAT3 (IL-6 pathway) in macrophages dampening inflammatory cytokine production and possibly skewing infected macrophages to alternative (arginase) versus classical (iNOS) activation and a generally less potent antimicrobial state [47-49]. In contrast, the dense granule protein GRA15 from Type II strain parasites may contribute to classical activation of infected macrophages as it activates NF κ B resulting in increased IL-12 production [50]. Rats as well as peritoneal macrophages

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of rats are naturally more resistant to *T. gondii* infection compared to mice; a potential consequence of high levels of NO and low levels of arginase and polyamines in rats compared to mice [51]. Therefore, host and parasite-dependent variables impact both the effectiveness of IRGs and the relative ratio of iNOS and arginase in infected macrophages and exert effects on parasite survival.

Overall, it is clear that the potencies of individual antimicrobial effectors against the parasite are dependent on the cell type infected, the activation stimuli, the presence and effectiveness of antimicrobial mediators within different host species and parasite polymorphic differences between genotypes. It is also evident that mice as important intermediate hosts for *T. gondii* and humans, not only share similarities in their cell autonomous defenses against *T. gondii* but also have striking and critical differences that must be explored.

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