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Cell type and species-specific host responses to *Toxoplasma gondii* and its near relatives

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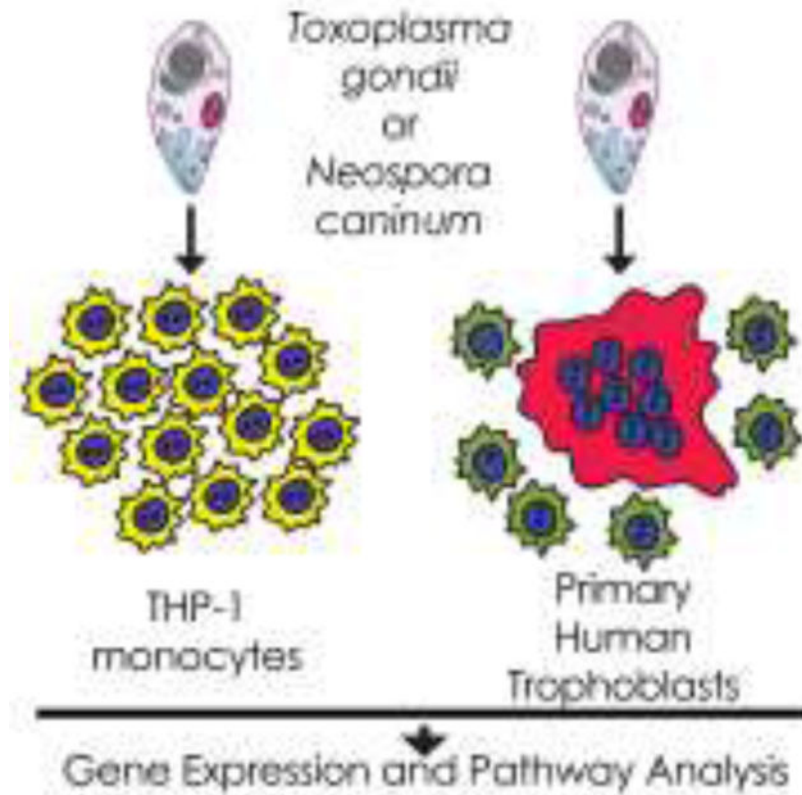
Abstract

Toxoplasma gondii is remarkably unique in its ability to successfully infect vertebrate hosts from multiple phyla and can successfully infect most cells within these organisms. The infection outcome in each of these species is determined by the complex interaction between parasite and host genotype. As techniques to quantify global changes in cell function become more readily available and precise, new data are coming to light about how i) different host cell types respond to parasitic infection and ii) different parasite species impact the host. Here we focus on recent studies comparing the response to intracellular parasitism by different cell types and insights into understanding host-parasite interactions from comparative studies on *T. gondii* and its close extant relatives.

Graphical Abstract

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Keywords

Toxoplasma gondii; *Hammondia hammondi*; *Neospora caninum*; Host response; Chemokines; Placenta

1. *Toxoplasma gondii*

Toxoplasma gondii, a member of the phylum Apicomplexa, has major implications in public health as it is estimated that one-third of the world's population is infected with this parasite (Halonen and Weiss, 2013). *Toxoplasma gondii* is an obligate intracellular parasite that can infect all warm-blooded animals and felines are the definitive host for sexual reproduction. *Toxoplasma gondii* infection can occur by ingestion of food or water contaminated with *T. gondii* tissue cysts or oocysts. The life cycle of *T. gondii* involves multiple hosts and developmental stages (reviewed in Hutchison, 1965; see also Dubey, 2009a). Given this complexity, like most parasites *T. gondii* encounters multiple host cell and tissue types during its life cycle. Following excystation of the oocyst, sporozoites primarily infect gut epithelial cells, and then differentiate into tachyzoites which disseminate throughout the organism after infecting circulating cells such as dendritic cells, natural killer cells, monocytes and macrophages (Dubey et al., 1997; Courret et al., 2006; Persson et al., 2009) Throughout this dissemination process, a robust immune response to the parasite is generated, ultimately leading to mobilization of cytotoxic T-cells and production of protective antibodies (Suzuki et al., 1988; Parker et al., 1991; Khan et al., 1994; Ely et al.,

1999) Coincident with the emergence of this host response, some tachyzoites differentiate into slow-growing bradyzoites which eventually become encased in a cyst wall. These tissue cysts can be found in a variety of tissues including heart, skeletal muscle, lung and the brain (Remington and Cavanaugh, 1965; Di Cristina et al., 2008). The adaptations that underlie the ability of *T. gondii* to replicate within, and persist in, such a wide variety of cell types is poorly understood, but this feature is critical for its ability to cause disease in humans. The bradyzoite-containing tissue cyst can effectively de-differentiate to the proliferative tachyzoite form in immunocompromised individuals such as those with HIV/AIDS and organ transplant patients (Luft et al., 1984; Gazzinelli et al., 1992; Derouin et al., 2008), causing disseminated disease and/or lethal encephalitis. The fact that *T. gondii* thrives in such a wide variety of tissues and cell types provides a unique opportunity to examine how different host cells respond to infection with the same parasite, and ultimately how these responses impact parasite growth, stage conversion, and survival.

2. *Toxoplasma gondii* modulation of host responses

Studies on *T. gondii*-mediated host gene expression regulation were first explored using cDNA microarrays (Blader et al., 2001; Gail et al., 2001; Chaussabel et al., 2003), and more recently RNA-sequencing (seq) (Garfoot et al., 2019; Li et al., 2019; Lu et al., 2019; Panas et al., 2019; Seizova et al., 2019). Genes found to be regulated by *T. gondii*, according to these studies, include genes encoding for many different processes including inflammation, apoptosis, metabolism, cell growth and differentiation (reviewed in Blader and Saeij, 2009). As an intracellular parasite, *T. gondii* is not able to replicate extracellularly. For invasion, *T. gondii* relies on the secretion of multiple effectors from the microneme and rhoptries (Alexander et al., 2005; Lebrun et al., 2005; Besteiro et al., 2009; Lamarque et al., 2011; Tyler and Boothroyd, 2011; Guerin et al., 2017), which are localized anteriorly for polarized anterior secretion (Nichols et al., 1983; Carruthers and Sibley, 1997). In addition to microneme and rhoptry proteins, the dense granules secrete proteins that can be found in multiple locations outside of the parasite, including soluble proteins in the parasitophorous vacuole (PV; Henriquez et al., 2005), associated with the PV tubulovesicular network (Labruyere et al., 1999), and the host cell cytoplasm and nucleus (Bonhomme et al., 1998; Rosowski et al., 2011; Bougdour et al., 2013; Braun et al., 2013; Ma et al., 2014).

Several parasite secreted effectors such as rhoptry- (ROP) and dense granules- (GRA) proteins are identified to be major players involved in interacting with host signaling pathways. Some *T. gondii* ROPs are essential for invasion of the host cell while several other ROPs have been shown to be important for *T. gondii* virulence and/or to co-opt host gene regulation (Taylor et al., 2006; Saeij et al., 2007). The ROP5/ROP18/GRA7 complex plays an important role in parasite virulence in vivo (Saeij et al., 2006; Taylor et al., 2006; Behnke et al., 2011; Reese and Boothroyd, 2011; Reese et al., 2011; Behnke et al., 2015; Hermanns et al., 2016). In murine hosts, TgROP18 from type 1 *T. gondii* strains (such as RH and GT-1) can disrupt the host interferon-gamma (IFN- γ) response by inhibiting the loading of immune-related GTPases (IRGs) onto the PV (Fentress et al., 2010; Khaminets et al., 2010). Interestingly *T. gondii* ROP18 was originally discovered as a virulence effector based on quantitative trait locus mapping in progeny derived from sexual crosses between either type 1 or type 2 strains and derivatives of the same type 3 isolate (CTG; (Taylor et al., 2006; Saeij

et al., 2007)). The basis for this virulence Quantitative trait locus (QTL) was found to be an insertion/deletion in the ROP18 promoter (Boyle et al., 2008) that was associated with dramatically reduced ROP18 transcript abundance in type 3 strains. This suggests that ROP18 itself is not required for *T. gondii* infection and its ability to infect a wide variety of hosts (since type 3) strains are found at a similar prevalence across the globe as other strain types; (Lehmann et al., 2006; Lorenzi et al., 2016)), but that it does have a dramatic influence on pathogenesis in the mouse model. Similarly, ROP5 was also identified based on the same or similar genetic crosses as those that led to the identification of ROP18, with the type 2 allele of ROP5 being associated with reduced virulence in the mouse model compared with the type 1 and type 3 alleles (Behnke et al., 2011; Reese et al., 2011). Interestingly, both ROP5 and ROP18 play critical roles in mouse virulence in South American *T. gondii* strains (Behnke et al., 2015), suggesting that their function in virulence is ancestral to the *T. gondii* lineage and that they have been subject to selection driven diversification and/or inactivation (in the case of type 3 ROP18) in canonical North American lineages (types 1, 2 and 3). We also discuss this issue below in regard to what is known about these genes in *Hammondia hammondi* and *Neospora caninum* below.

In addition to host-targeting ROPs that are mostly derived from the rhoptry bulb (rather than the rhoptry neck), parasites secrete proteins from the GRAs during and after invasion (Bonhomme et al., 1998). Effector GRA proteins (GRA15 (Rosowski et al., 2011), GRA16 (Bougdour et al., 2013), GRA24 (Braun et al., 2013), GRA28 (Ander et al., 2017), TgIST (Gay et al., 2016; Olias et al., 2016) and HCE1/TEEGR (Braun et al., 2019; Panas et al., 2019)) modulate multiple host pathways including necrosis factor (NF)- κ B, p53, mitogen-activated protein kinase (MAPK), cytokine signaling, signal transducer and activator of transcription (STAT)-regulated gene expression and the host cell cycle. What is particularly interesting about these effectors is that many of them have overlapping effects on the same host cell pathways.

During *T. gondii* infection, Types I and II IFN signaling are suppressed. Type II (IFN- γ)-dependent signaling is down-regulated by *T. gondii* Inhibition of STAT transcription (TgIST; (Gay et al., 2016; Olias et al., 2016; Matta et al., 2019)). The transcription factor STAT1 is the main signal transducer of the IFN- γ response to *T. gondii* infection (Zimmermann et al., 2006; Kim et al., 2007; Lang et al., 2012; Schneider et al., 2013; Rosowski et al., 2014) and subsequently impeding expression of genes for major histocompatibility (MHC) class II, inducible nitric oxide synthase (NOS2), class II transactivator (CIITA), IFN-inducible GTPases and chemokines such as CXCL9 and CXCL10 (Scharton-Kersten et al., 1997; Lüder et al., 2003; Kim et al., 2007; Lang et al., 2012; Rosowski and Saeij, 2012). When TgIST is secreted into the host cell, it translocates into the host cell nucleus and interacts with gamma-activated sequences (GASs) in the promoters of IFN-stimulated genes (ISGs) via an interaction with STAT1 homodimers. STAT1-mediated transcription of target genes is inhibited by IST due to its recruitment of the nucleosome remodeling and repressive (NuRD) complex (Gay et al., 2016; Olias et al., 2016). Recently, it was discovered that TgIST also associates with phosphorylated STAT2 and recruits the NuRD complex in response to IFN- β treatment, and represses a subset of Type I IFN response genes. Growth of parasites lacking TgIST is also restricted in host cells treated with IFN- β (Matta et al., 2019), suggesting that TgIST disrupts host responses to both Type I and Type II interferons.

In addition to TgIST, *T. gondii* HCE1/TEEGR also suppresses host responses during infection. HCE1/TEEGR partners with host E2F3/E2F4 transcription factors (Panas et al., 2019) and suppresses NF- κ B regulated TNF- α -cytokine signaling via interactions with the polycomb repressive complex 2 (PRC2) subunit EZH2 (Braun et al., 2019). Among these effectors only GRA15 has a strain-specific function, where Type II *T. gondii* induces an high level of NF- κ B activation compared with Type 1 and Type 3 parasites (Rosowski et al., 2011) and IL-1 β secretion in inflammatory monocytes (Gov et al., 2013). Interestingly HCE1/TEEGR of Type II *T. gondii* was found to not disrupt the activity of *T. gondii* type 2 strain GRA15 (Braun et al., 2019), suggesting that they are driving NF- κ B activation in distinct ways.

Interestingly, HCE1/TEEGR seems to have another role in modulating the host cell cycle by direct interaction with cyclins. *Toxoplasma gondii* HCE1/TEEGR associates with cyclin E (CCNE) by partnering with cell cycle transcription factor dimerization partner 1 (DP1) and ultimately forming a complex with E2F3/E2F4 proteins (Braun et al., 2019; Panas et al., 2019). DP1 and E2F proteins are part of the DREAM complex which plays a role in regulating host cell cycle regulation (reviewed in Engeland, 2018). The DP1/E2F complex binds to E2F binding sites to repress transcription during the early G₀/G₁ phase (Litovchick et al., 2007). Repression of transcription is released when the FOXM1 and B-MYB are recruited to the MuvB core when E2F4-5/DP and p107/p130 (pRB-like proteins) are dissociated from the DNA binding site (Mannefeld et al., 2009; Quaas et al., 2012; Sadasivam et al., 2012; Chen et al., 2013).

The ability of *T. gondii* to modulate the host cell cycle is not surprising as it has also been shown that *T. gondii* infection induces quiescent host cells to reenter the cell cycle (Holmes et al., 2019), or host cells in G₁ phase to enter into the S phase and arrest host cells at the G₂/M phase (Brunet et al., 2008; Molestina et al., 2008; Wong et al., 2019). While the impact of *T. gondii*-mediated host cell cycle modulation is poorly understood, early studies show that *T. gondii* may have a preference for infecting cells in the S phase (Lavine and Arrizabalaga, 2008) and it has been speculated that this is due to the fact that the microtubule-organizing centers (MTOCs) that are actively remodeled by *T. gondii* during an infection are not accessible at other host cell cycle stages (Coppens, 2006; Walker et al., 2008). In addition to the CCNE/E2F/DP1 complexes, other host factors have also been shown to be involved in *T. gondii*-mediated cell cycle regulation, including *UHRF1* (Brunet et al., 2008), p53 and CDKN1A (Bougdour et al., 2013). *Toxoplasma gondii*-mediated *UHRF1* gene expression causes host cells to arrest in the G₁ phase. When expression of UHRF1 was suppressed using small interfering RNA (siRNA), the growth of *T. gondii* was reduced in BeWo and normal human dermal fibroblast (NHDF) cells (Brunet et al., 2008). While the parasite effector responsible for host cell cycle regulation is unknown, the *T. gondii*-secreted effector GRA16 increases p53 and p21 levels in human host cells (Bougdour et al., 2013). The host transcription factor P53 can induce cell cycle arrest at either G₁/S or G₂/M checkpoints (Agarwal et al., 1995; Bunz et al., 1998) and p21 (gene product of *CDKN1A*) was the first transcriptional target identified for p53 (el-Deiry et al., 1993). While the exact mechanism of these parasite effector(s) in mediating host cell cycle regulation is not fully understood, these data suggest that *T. gondii* could also be modulating the p53-p21-

DREAM-E2F pathway as a parallel means to disrupt host cell cycle progression (more on this pathway in the section below)

An important recent advance in understanding of *T. gondii* manipulation of the host cell is the discovery of a complex of proteins that are required for secretion of multiple dense granule-derived effectors (including those described above). This complex, named after its founding member “Myc Regulation 1” (“MYR1”), was identified in a mutagenesis screen for *T. gondii* parasites that were deficient in inducing c-Myc upregulation in the host cell (Franco et al., 2016). This complex is now known to contain at least six dense granule proteins (Gay et al., 2016; Olias et al., 2016; Ander et al., 2017; He et al., 2018; Marino et al., 2018; Braun et al., 2019; Panas et al., 2019) and *T. gondii* MYR1 parasites are less able to regulate the host cell cycle compared with wild type parasites (Franco et al., 2016). Importantly, transcriptome data from host cells infected with wild type (WT) and MYR1 parasites suggests that MYR1-dependent effectors can have opposing effect on the same processes, including the cell cycle (Franco et al., 2016; Panas et al., 2019). The sum total of the response of a given host cell will depend on its sensitivity to each of these effectors, and this could provide a unique means for *T. gondii* to use the same effectors to mediate distinct outcomes in different cell types.

3. Response to intracellular parasitism by different cell types

Despite the fact that *T. gondii* resides in a variety of host cell types during infection of the definitive and intermediate host, including epithelial, endothelial, immune and neuronal cells, genome-wide data comparing the host response in different cell types are mostly lacking. Given their ease of cultivation, primary human foreskin fibroblasts (HFFs) are most commonly used in the study of many aspects of *T. gondii* biology including host responses to infection. TgGRA-mediated host modulation has been thoroughly investigated in HFFs and yielded important insights (Bougourd et al., 2013; Franco et al., 2016; Gay et al., 2016; Olias et al., 2016; Naor et al., 2018; Braun et al., 2019; Panas et al., 2019). However, in the few studies that have been performed there can be remarkably different responses to *T. gondii* between cell types. For example, *T. gondii* suppresses IL-1 β and lipopolysaccharide (LPS)-induced IL-1 β production in neutrophils, but fails to do so in monocytes (Lima et al., 2018). In our work, we have compared infection of HFFs with primary human trophoblast (PHT) cells, and discovered a cluster of genes that are induced in PHT cells but not in HFFs, including the transcription factor IRF4, the chemokines CCL22, CCL17, CCL20 and CCL1, and the chemokine receptor CCR7 (Ander et al., 2017). Importantly, some of these chemokines (CCL22 and CCL17, in particular) are also induced to be expressed in a variety of mouse macrophage cell types (He et al., 2018).

During the later stages of infection, parasite tissue cysts have a higher propensity to be found in neurons and muscle (Remington and Cavanaugh, 1965; Ferguson and Hutchison, 1987; Halonen et al., 1996; Fisher et al., 1997; Lüder et al., 1999, 2003; Dubey, 2009b; Cabral et al., 2016), although the reason for this is unknown. It has been speculated that i) the condition of immunity-related stress factors such as reactive oxygen and nitrogen species or nutrient depletion (Bohne et al., 1994; Bohne and Roos, 1997; Fox et al., 2004) and ii) the absence of exogenous stressors in neuronal and muscular cells provide a suitable

microenvironment for the development of bradyzoites and tissue cysts (Lüder et al., 1999; Ferreira-da-Silva Mda et al., 2008). Genome-wide transcriptomic comparative studies were performed on murine cells and identified highly divergent responses to *T. gondii* in different cell types (skeletal muscle cells (SkMCs), neurons, astrocytes and fibroblasts). Intriguingly, with only a small number of genes commonly regulated in these cell types (including only a few immune response-related genes), none of these genes were commonly regulated in all four cell types, suggesting that the host transcriptomic profile in relation to *T. gondii* infection is host cell type-specific rather than parasite-driven host cell manipulation (Swierzy et al., 2017).

In addition to differences between cell types within the same host, differences in expression profiles between different host cells (humans and mice) were also recognized (Channon et al., 2000; Blader et al., 2001; Chaussabel et al., 2003; Swierzy et al., 2017). *Toxoplasma gondii* infection in dendritic cells (DCs) and macrophages have a greater number of inflammation- and immunity-related genes being more prominently regulated (Chaussabel et al., 2003). Despite the differences in immune-related responses, in both human and mouse host cells, genes involved in translation and host cell cycle regulation were commonly regulated by *T. gondii*. In murine fibroblasts and astrocytes as well as human fibroblasts, *T. gondii* infection induces expression of genes involved in translation (Blader et al., 2001; Swierzy et al., 2017). Host cell cycle-related genes were regulated in human fibroblasts as well as murine SkMCs by *T. gondii*, with a lesser impact on murine neurons (Swierzy et al., 2017).

While this data will be helpful for investigation of *T. gondii*-mediated responses in the human host cells, a similar study would be useful to dissect the common and different responses in different human host cells and we have seen differential responses in human and mouse host cells by *T. gondii*, specifically in the regulation of IL-12 and IFN- γ (reviewed in Pifer and Yarovinsky, 2011). We expect new data examining transcriptomic and proteomic responses of different cell types to *T. gondii* to continue to emerge as RNAseq (both bulk and single cell) and label-free quantitative proteomics become more readily available to most research groups.

4. Host-pathogen interaction comparisons between *T. gondii* and its close relatives

Toxoplasma gondii is closely related to *N. caninum* and *H. hammondi*, and *T. gondii* is more closely related to *H. hammondi* than *N. caninum*. *Toxoplasma gondii* shares a high degree of gene-by-gene synteny with both species (>81% between *T. gondii* and *N. caninum* and >95% between *T. gondii* and *H. hammondi*) (Walzer et al., 2013; Adomako-Ankomah et al., 2014; Lorenzi et al., 2016). As tissue-dwelling coccidia, these parasite species share a number of life cycle features, with sexual stages occurring in either canine (for *N. caninum*) or feline (for *T. gondii* and *H. hammondi*) gut epithelial cells, and asexual reproduction and encystment occurring in intermediate host species. However, these species are divergent in a number of important phenotypes for which no molecular mechanisms are known. For example, *N. caninum* is not naturally transmitted by rodents and experimental infection has

shown that *N. caninum* is significantly less pathogenic in the mouse model compared with *T. gondii*, despite displaying highly similar growth profiles in HFFs in vitro (English et al., 2015; Coombs et al., 2020). *Hammondia hammondi* is naturally transmitted by rodents but is avirulent in laboratory mice, including mice that lack IFN γ -driven host responses. *Hammondia hammondi* also has a host range that is comparatively restricted compared with *T. gondii* as it is only known to naturally infect rodents, roe deer, and goats. However, experimentally *H. hammondi* is also capable of infecting dogs, pigs, monkeys and rabbits but fails to infect birds (Dubey and Sreekumar, 2003). Understanding the molecular mechanisms driving these phenotypic differences could provide new insights into host range determinants and virulence mechanisms that may be undetectable when studying only a single species. To this end, in laboratory settings these species can infect most, if not all, of the same cell types, allowing for rigorous interspecies comparisons of parasite modulation of host cell biology and responses to infection (Reid et al., 2012; Sokol et al., 2018). The outcomes of these infections are driven by the complex interplay between introduction of effectors into the host cell during infection and the host response. Here we focus on what is known regarding the *T. gondii* common and/or distinct impacts (as compared to *H. hammondi* or *N. caninum*) of parasite infection on the modulation of host signaling pathways, with a focus on IFN signaling and the host cell cycle.

4.1. *Neospora caninum*

Neospora caninum was first described in 1984 (Bjerkas et al., 1984) and is a major cause of abortion in cattle and therefore a significant threat to the cattle industry (Almería et al., 2017; Dubey et al., 2007). Similar to *T. gondii*, *N. caninum* can be transmitted by ingestion of sporulated oocysts from the environment or tissues harboring bradyzoite-containing tissue cysts. In bovines, *N. caninum* can also be transmitted vertically through the placenta during pregnancy (Dubey et al., 2007). The genomes of *T. gondii* and *N. caninum* are largely syntenic (DeBarry and Kissinger, 2011; Reid et al., 2012). In addition, corresponding orthologous genes are found elsewhere in the genome in regions where synteny is disrupted (Reid et al., 2012). Comparisons of transcript abundance between *T. gondii* and *N. caninum* tachyzoites show that SAG1-Related Sequences (SRSs), ROPs and AP2s are among the genes with higher expression in *T. gondii* relative to *N. caninum* (Reid et al., 2012).

In contrast to suppression of Type I and Type II IFN signaling pathway genes by *T. gondii*, *N. caninum* infection has been shown to induce robust Type I (IFN- α and - β ; (Beiting et al., 2014)) and Type II (IFN- γ) interferon signaling during infection (Baszler et al., 1999; Long and Baszler, 2000; Nishikawa et al., 2003). *Neospora caninum* infection induces a more potent expression of Type I IFN signaling pathway genes compared with *T. gondii* in vitro, and it appears that this response is actively suppressed by *T. gondii* since media from *T. gondii*-infected host cells can suppress *N. caninum*-induced Type I IFN responses (Beiting et al., 2014).

4.2. *Hammondia hammondi*

Hammondia hammondi is the closest known extant relative of *T. gondii*. Unlike *N. caninum*, both *H. hammondi* and *T. gondii* complete their sexual life cycle stage in the small intestine of cats. While *T. gondii* is identified as a major threat to human public health and animals, as

with *N. caninum*, *H. hammondi* has not been shown to be associated with any human clinical diseases (Dubey and Sreekumar, 2003). Despite having differential impact on parasite pathogenesis and infection outcomes, genetically *H. hammondi* is very similar to *T. gondii* with approximately 4% of molecular divergence in the first internal transcribed spacers (ITS-1) of rDNA (Ellis et al., 1999). *Hammondia hammondi* has also been previously referred to as *Toxoplasma hammondi* (Levine, 1977, 1985). A recent comparative analysis of the transcriptomic profiles of *T. gondii* and *H. hammondi* identified genetic profiles that might underlie differences in the in vitro developmental program and life cycle flexibility of these two parasites (Sokol et al., 2018).

Unlike *T. gondii*, *H. hammondi* replicates more slowly and is unable to be subcultured indefinitely in vitro in a variety of different host cells. Ultimately *H. hammondi* parasites spontaneously form bradyzoite-containing cysts that are infectious only to the definitive host (Sheffield et al., 1976; Riahi et al., 1995; Sokol et al., 2018). However, *H. hammondi* can infect and replicate in new intermediate host cells (in vitro and in vivo) for a limited time before the parasite begins to spontaneously form tissue cysts in vitro. Furthermore, *H. hammondi* and *T. gondii* have differential gene expression in vitro following sporozoite-initiated infections. One striking difference in gene expression between *T. gondii* and *H. hammondi* was the enrichment of merozoite and bradyzoite-related genes in both early tachyzoite (day 4) and late-tachy-early-bradyzoite life (day 15) in *H. hammondi* (Sokol et al., 2018). In *T. gondii* these transcriptional profiles are uniquely expressed in *T. gondii* bradyzoites and alkaline pH-treated *T. gondii* for tissue cyst formation induction (Jerome et al., 1998; Lyons et al., 2002; Behnke et al., 2008; Croken et al., 2014a, 2014b; Sokol et al., 2018). Despite being genetically closely related, the differential growth and virulence of *H. hammondi* in comparison to *T. gondii* has made *T. gondii/H. hammondi* a promising comparative model system to understand *T. gondii* virulence and pathogenesis.

Heterologous expression of orthologs of *H. hammondi* virulence factors in *T. gondii* has contributed to the understanding of some of the most important *T. gondii* virulence factors. When HhROP18/ROP5 are expressed heterologously in *T. gondii*, the orthologs are functional. Specifically HhROP5₂₋₁ ortholog expression in TgROP5 knockout mice caused higher mortality than the expression TgROP5 in the knockout mice (Reese et al., 2011; Walzer et al., 2013). Despite the fact that the HhROP18/ROP5 orthologs are i) expressed in *T. gondii*, and ii) can complement virulence defects in *T. gondii* knockouts, the fact that *H. hammondi* is not as virulent as *T. gondii* suggests that the differences in gene expression as these parasite species develop in vitro might be one of the contributing factors to the differential virulence observed between *T. gondii* and *H. hammondi* (Walzer et al., 2013). It is likely that *H. hammondi* is pre-programmed to develop into a bradyzoite as it appears to have a highly predictable window of infectivity and replicative capacity prior to terminally differentiating into tissue cysts. This type of strict, pre-defined development program resulting in 100% tissue cyst formation could underlie why *H. hammondi* is unable to be continually grown in cell culture or in intermediate hosts.

While transcriptomic studies on parasites at different life cycle stages have given insights into the biology of the parasites, the dynamic changes in host responses during infections of a virulent (*T. gondii*) and avirulent (*H. hammondi*) parasite could also contribute to

understanding of the host responses that *T. gondii* has to overcome and/or manipulate to ensure its intracellular survival. We have recently taken a comparative transcriptomic approach to analyze the global host response to parasite infections using dual RNA-seq to understand the interplay between parasite and host transcript regulatory network in parallel.

It was initially thought that *H. hammondi* and *T. gondii* would elicit similar host responses as these parasites are very similar genetically and morphologically, with cross-reactivity in serological tests (Frenkel and Dubey, 1975; Weiland et al., 1979), cross-reactivity of poly- and monoclonal antibodies (Araujo et al., 1984; Riahi et al., 1998; Riahi et al., 1999, 2000; Dumetre and Darde, 2007) and cross-protection against these two parasites in animals (Dubey, 1981; Munday and Dubey, 1988; Reddacliff et al., 1993). However, in the human acute monocytic leukemia THP-1 cell line (THP-1) (Tsuchiya et al., 1980), while both *T. gondii* (Type I-GT1, Type II-ME49, and Type III-VEG) and *H. hammondi* (HhEth1 and HhAmer) elicit a potent host response, *H. hammondi* induces it at a much higher magnitude than *T. gondii*. During *T. gondii* and *H. hammondi* sporozoite-initiated infections, the majority of the host responses are commonly regulated in both *T. gondii* and *H. hammondi* infection. As in *N. caninum* infection, some of the important *T. gondii*-initiated host responses such as the IFN γ and the TNF- α response (Suzuki et al., 1988; Yap and Sher, 1999) were more highly induced during *H. hammondi* infection in the monocytes compared with *T. gondii* infection (Wong et al., 2019).

Strikingly, the study showed that differential regulation of cell cycle-related control responses by *T. gondii* and *H. hammondi* might be one of the key players in determining parasite replication in vitro (Wong et al., 2019). Although both *T. gondii* and *H. hammondi* arrested host cell cycle progression, *T. gondii*-infected THP-1 cells were arrested at the G₂/M phase while *H. hammondi*-infected cells were arrested at G₁/S and, in some replicates, resembled cells without parasite infection. In addition, differential regulation of Myc and cell cycle pathways target genes, and activation of the forkhead transcription factor (*FOXM1*) during *T. gondii* infection also supports the host cell cycle state being at the G₂/M phase. TgGRA16 is shown to increase p53 and p21 levels in HFFs (Bougdoor et al., 2013), and THP-1 cells infected with *T. gondii* show decent levels of transcripts for these genes. However, *H. hammondi* infection induces higher levels of *p53* and *CDKN1A* gene transcripts relative to *T. gondii* infection (Wong et al., 2019). As E2F/DP1 and p53 are components of the p53-p21-DREAM-E2F pathway, in which activation of p53 increases p21-induced suppression of the transcription of DREAM target genes (reviewed in Engeland, 2018), it is likely that altering transcription of these genes is one of the mechanisms that *T. gondii* uses to regulate host cell cycle arrest. It is therefore likely that during *H. hammondi* infection the FOXM1-MMB complex was displaced during p53-mediated increased *p21/CDKN1A* expression during the DNA damage response and subsequently caused the formation of DREAM complex and p53-mediated G₁/S cell cycle arrest (Litovchick et al., 2007; Mannefeld et al., 2009; Quaas et al., 2012; Chen et al., 2013). While it was not identified whether formation of the DREAM complex during parasite infection is i) mediated by a *H. hammondi* secreted effector that is not functional in *T. gondii* or ii) a host response mechanism that is counter-balanced by *T. gondii* effector(s), both hypotheses are plausible as induction of the *CDKN1A* gene requires direct *H. hammondi* invasion and/or infection. It is also likely that interaction between E2F1/3/4/6 and DP-1 with

the *T. gondii* effector HCE1/TEEGR (Braun et al., 2019; Panas et al., 2019) might prevent formation of the DREAM complex (Wong et al., 2019).

4.3. An example head-to-head comparison examining species- and cell type-specific responses to infection

To further explore the impact of cell type and parasite species host transcriptional responses to intracellular parasitism, we performed head-to-head comparisons of the host transcriptional responses to *N. caninum* and *T. gondii* using two cell types: THP-1 cells and PHT cells cultivated from term placentas; (Ander et al., 2017). Note: *T. gondii* and *N. caninum* infection data in PHTs have been published previously (Ander et al., 2017) and are analyzed here in a different context. Our data show a clear difference in the response of each cell type to infection, as THP-1 cells appear to be much more responsive to *T. gondii* and *N. caninum* infection compared with PHTs (Fig. 1A). In both of these cell types, the chemokine CCL22 is one of the genes with the highest transcript abundance after *T. gondii* infection. Furthermore, *T. gondii* infection results in a much greater number of genes that have increased transcript abundance compared with *N. caninum* infection (Fig. 1A, left). In contrast, THP-1 cells showed a much more robust response to *T. gondii* and *N. caninum* infection, with each parasite species inducing changes in its own unique set of genes (Fig. 1A, right). Gene set enrichment analysis (Fig. 1B) provides clear evidence for cell type-specific responses to infection by *T. gondii* and *N. caninum*. For example, the cell cycle-related gene sets (*p53* and *MYC* targets *v1* and *v2*) were enriched in *T. gondii*-infected THP-1 cells but not PHTs, while *N. caninum* induced changes in multiple inflammation-related genes sets (Inflammatory response; Complement, and IL6 JakStat3 signaling; Fig 1B) in THP-1 cells but not PHT cells.

In both THP-1 and PHTs, *T. gondii* induced changes in the abundance of more transcripts compared with *N. caninum*, although this difference was much more pronounced in PHT cells (Fig. 1 A,B). In both cell types, *T. gondii* induced the *CCL22* gene in high abundance. Despite having a small number of genes differentially regulated in PHTs compared with THP-1, gene set enrichment analysis revealed similar enrichment of IFN α (Type 1) responses in PHTs infected with *N. caninum* compared with *T. gondii*-infected PHTs (Fig. 1B,C). These data are consistent with prior work in HFFs showing induction of the type I IFN pathway in *N. caninum*-infected HFFs compared with *T. gondii*; (Beiting et al., 2014). This also suggests that *T. gondii* suppression of Type I (and possibly Type II; Fig. 1B) IFN signaling pathways could be a common adaptation strategy that may be evident in multiple cell types. In contrast, the cell cycle-related gene sets *E2F*targets and *G₂M* checkpoints are more robustly altered by *T. gondii* compared with *N. caninum* in both cell types (Fig. 1B), suggesting that manipulation of the host cell cycle may be a species-specific trait in *T. gondii*. Overall, transcriptome datasets such as these from multiple parasite species and cell types can provide new insights into the evolution of unique traits within one parasite lineage compared with another and identify how cell types respond uniquely to species-specific mechanisms of immune suppression and/or activation.

5. Summary and conclusions

Toxoplasma gondii is an obligate intracellular parasite that infects virtually all mammalian cells (Sibley, 2003). The fact that *T. gondii* is able to naturally infect multiple cell types in multiple species and its near relatives are less able to do so provides a unique opportunity to study the evolution of both host and cell type specificity in tissue-dwelling coccidia, about which very little is known at the molecular level. Throughout its life within a given host, *T. gondii* finds itself in contact with a wide variety of cell and tissue types, and must be able to counteract and/or survive unique aspects of host defenses in each of these replication sites. By comparing closely related species with different degrees of adaptation to a given host species and/or tissue, we can better identify the host barriers to infection that exist, and ultimately how compatible hosts have superseded these restrictions.

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Highlights

- *Toxoplasma gondii* has a very wide host range, and a remarkable ability within those hosts to infect a wide variety of cells
- Here we review the literature as it pertains to the host response of different cell types to *Toxoplasma gondii*
- We also review how relatives of *T. gondii* with more restricted host ranges may induce divergent changes in the host cell

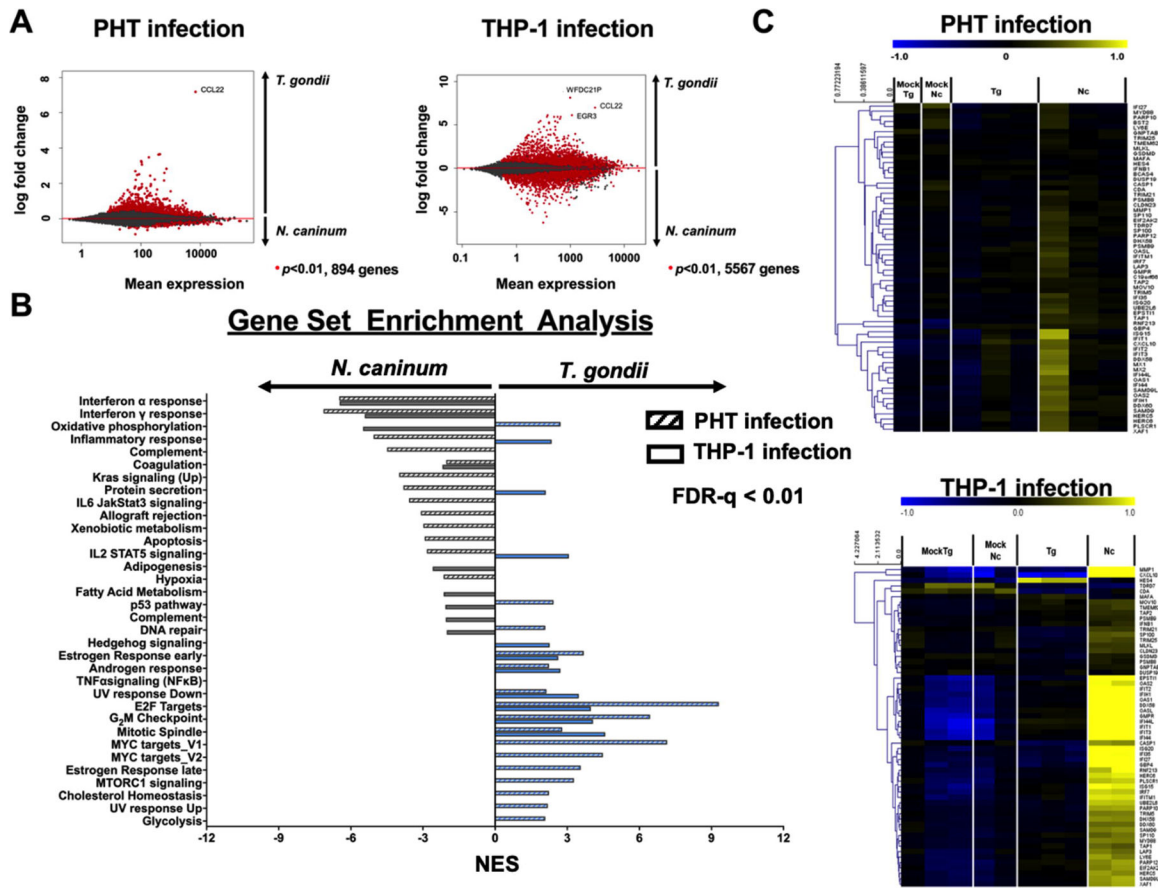


Fig. 1. Differential expression analysis of human trophoblast cells (PHTs) against *Toxoplasma gondii* and *Neospora caninum* tachyzoite infection. (A) Plots showing mean expression against log fold change of the transcriptomic profile of PHTs during *T. gondii* and *N. caninum* infections. Each dot represents a host gene and genes that are significantly different in response to the parasitic infection (in comparison to mock infection) are represented by ● ($P < 0.01$). (B) Gene set enrichment analysis of the PHT (solid bar, either blue or gray) and THP-1 (hatched bar, either blue or gray) transcriptomes in response to *T. gondii* (blue) and *N. caninum* (gray) infection. Shown are Hallmark gene sets that are significantly enriched (false discovery rate (FDR-q) < 0.01 (computed with 1000 Monte-Carlo simulations); positive and negative values show up- and down-regulated gene sets, respectively). (C) Heatmaps showing log₂-transformed expression of gene clusters during *T. gondii* and *N. caninum* infection. Shown are Type I Interferon (IFN) that were specifically induced by *N. caninum* in human foreskin fibroblasts (HFFs) (Beiting et al., 2014). Genes were mean-centered and hierarchically-clustered.