Toxoplasma gondii Development of Its Replicative Niche: in Its Host Cell and Beyond

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Intracellular pathogens can replicate efficiently only after they manipulate and modify their host cells to create an environment conducive to replication. While diverse cellular pathways are targeted by different pathogens, metabolism, membrane and cytoskeletal architecture formation, and cell death are the three primary cellular processes that are modified by infections. Toxoplasma gondii is an obligate intracellular protozoan that infects ~30% of the world’s population and causes severe and life-threatening disease in developing fetuses, in immune-compromised patients, and in certain otherwise healthy individuals who are primarily found in South America. The high prevalence of Toxoplasma in humans is in large part a result of its ability to modulate these three host cell processes. Here, we highlight recent work defining the mechanisms by which Toxoplasma interacts with these processes. In addition, we hypothesize why some processes are modified not only in the infected host cell but also in neighboring uninfected cells.

Toxoplasma gondii is a protozoan, obligate intracellular parasite that is considered one of the world’s most successful pathogens (1). Multiple factors contribute to this success, including a complex life cycle in which the parasite can be transmitted by both vertical and horizontal means, efficient propagation within both its primary (felines) and intermediate hosts, extensive mechanisms to evade and disarm host immunity, an ability to form chronic lifelong infections in intermediate hosts, and a wide host tropism in which the parasite can infect most nucleated cells of warm-blooded animals (2). Central to most of these factors is that Toxoplasma has developed the means to replicate efficiently within the hostile intracellular environment of its host cell. In this review, we highlight recent data that have shed light on how parasite growth is achieved by the parasite interacting with its host cell to manipulate host signaling cascades, transcription, cell survival pathways, and membrane transport. In addition, we discuss how parasites interact with neighboring host cells and propose how this may contribute to establishing a permissive microenvironment to improve its overall success. In particular, we focus on those processes that are essential for the growth of all parasite strains and we refer readers to recent reviews that highlight how polymorphic parasite molecules contribute to Toxoplasma virulence (3–5).

NUTRIENT ACQUISITION

As an obligate intracellular parasite that resides within a nonfusogenic vacuole, Toxoplasma must satisfy its nutritional needs by scavenging essential nutrients from its host cell. These nutrients include carbon sources (glucose and glutamine) to fuel its energy demands, specific amino acids, lipids, and other nutrients. Below, we discuss each of these and highlight pathways and processes that are unique to the parasite that could serve as novel drug targets (Fig. 1).

GLUCOSE AND GLUTAMINE POWER THE PARASITE

Toxoplasma expresses a full complement of glycolytic and tricarboxylic acid (TCA) enzymes, and both metabolic pathways are active in tachyzoites (6). Toxoplasma glycolytic genes function both in glycolysis and in other parasite processes such as parasite motility (7–9). These data led several groups to conclude that glucose was the primary carbon source that was scavenged by Toxoplasma from its host cell. In turn, this conclusion led to questions such as how was the parasite scavenging glucose, what impact did siphoning this nutrient have on the host cell’s physiology, and what was the function of the parasite’s TCA cycle in growth? Toxoplasma expresses a hexose transporter (TgGT1) on its plasma membrane that shows the highest affinity for glucose. Deletion of the TgGT1 gene results in a significant defect in glucose uptake and a defect in parasite motility and replication (10). The requirement for glucose in parasite motility is linked to the observation that during motility, glycolytic enzymes relocalize to the inner membrane complex (a membranous structure that lies directly adjacent to the plasma membrane and serves as an anchor for the actomyosin machinery to propel the parasite into the host cell), suggesting that glucose provides the energy needed for invasion (8, 9). Surprisingly, loss of TgGT1 had no impact on virulence (10), suggesting that Toxoplasma uses other carbon sources to generate ATP.

Identification of this other carbon source came from the observation that motility of the TgGT1 knockout parasites could be restored by the addition of glutamine to the media (10). Together, these data suggested that parasites could generate ATP through either glycolysis or glutaminolysis. This hypothesis was confirmed by isotope labeling and metabolite profiling that showed that Toxoplasma uses host-derived glucose and glutamine to generate ATP via cytosolic glycolysis and mitochondrial oxidative phosphorylation. It is unknown how acetyl-coenzyme A (acetyl-CoA) is generated for the TCA cycle, since the parasite lacks a mitochondrial

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pyruvate dehydrogenase complex. Rather, this complex is localized within the apicoplast, where it generates acetyl-CoA that is used by the fatty acid biosynthetic pathway (11, 12). See Fig. 1 for a current model of glucose and glutamine uptake and use by the parasite.

Whether the parasite’s TCA cycle is essential for growth is unclear. Fleige and coworkers reported that reduced expression of the TCA enzyme succinyl-CoA synthase did not impact parasite growth, whereas MacRae et al. reported that chemical inhibition of aconitase did (11, 13). These seemingly contradictory data were reconciled by the discovery that Toxoplasma synthesizes γ-aminobutyric acid (GABA) from glutamine and that this compound can be shunted into the TCA cycle as succinate via succinic-semialdehyde dehydrogenase and thus bypasses succinyl-CoA synthase (11). However, neither the GABA shunt nor, by extension, the TCA pathway appears to be essential since deletion of the gene encoding glutamate decarboxylase (the enzyme that converts glutamate to GABA) has a relatively minor impact on parasite growth and virulence (11).

LIPIDS: WE ALL NEED FAT
Cholesterol. Sterols are essential components of eukaryotic membranes, and cholesterol is the major sterol in mammalian cells. While parasite membranes contain cholesterol, Toxoplasma lacks cholesterol biosynthetic enzymes and must scavenge it from its host (14). Serum-derived cholesterol is the primary source for cholesterol since parasite growth is not reduced in cell lines unable to synthesize cholesterol (14). Parasites scavenge cholesterol from low-density lipoprotein (LDL) particles and do so by redirecting LDL receptor trafficking to the parasitophorous vacuole (PV) (14, 15). An unexpected player in this process is the P-glycoprotein host multidrug-resistance efflux pump that appears to be required for parasite uptake of cholesterol at some point after cholesterol delivery to the PV (16). But how cholesterol crosses the PV membrane (PVM) and the parasite plasma membrane remains to be determined.

Isoprenoids. The mevalonate and deoxy-D-xylulose-5-phosphate (DOXP) pathways produce isopentyl pyrophosphate, which is the precursor for the biosynthesis of longer isoprenoids. Only the DOXP pathway is expressed by the parasite, and deletion of several genes encoding DOXP enzymes is lethal (17). Toxoplasma expresses a bifunctional farnesyl/geranyl diphosphate synthase (TgFPPS) (18) whose activities are required for isoprenoids to be incorporated into cholesterol, dolichols, or isopentyls. Deletion of the TgFPPS gene results in parasites with growth defects in specific types of host cells (e.g., the knockout can grow in human foreskin fibroblasts but cannot grow in macrophages) (17, 18). TgFPPS knockouts also cannot survive as extracellular parasites for extended periods of time because of a mitochondrial de-
fect that is likely due to a loss of ubiquinone, which is an isoprenylated cofactor of the mitochondrial respiratory chain (18).

Why is the DOXP pathway essential while TgFPPS is not? DOXP isoprenoid biosynthesis occurs within the apicoplast, and presumably its products are transported from the apicoplast to the mitochondria where TgFPPS is localized. TgFPPS, on the other hand, is not essential because the parasite can salvage longer isoprenoids (e.g., farnesyl diposphate and geranylgeranyl diposphate) from the host cell. This requirement for host isoprenoids renders the parasites highly susceptible to statins, which inhibit host isoprenoid synthesis (19). The finding that TgFPPS knockout growth is severely restricted in macrophages suggests that isoprenoid scavenging may be restricted in these cells. The fact that the TgFPPS knockout grows in human foreskin fibroblasts suggests either that there are differences in the basal rates of isoprenoid biosynthesis between fibroblasts and macrophages or that restricting isoprenoid availability is a novel innate immune mechanism in macrophages.

**SPHINGOLIPIDS**

Sphingolipids are a diverse group of lipids that have important functions in cell structure, membrane trafficking, and cell signaling. *Toxoplasma*, which contains at least 20 different sphingolipid species (primarily sphingomyelin and ceramide), expresses sphingolipid biosynthetic enzymes and thus can synthesize these lipids. Many parasite sphingolipids contain saturated and unsaturated long-chain fatty acid moieties (C:20 to C:24), making them structurally distinct from host sphingolipids whose fatty acids are primarily C:16 and C:18.

Addition of fluorescently labeled ceramide to uninfected host cells normally stains the Golgi apparatus as well as punctate cytoplasmic structures. Infected host cells are similarly stained early after labeled ceramide addition, but with time, the ceramide accumulates in intracellular parasites (20, 21). These data suggested that *Toxoplasma* not only synthesizes its own sphingolipids but also scavenges host-derived ones; subsequent radiolabeling assays confirmed this model (22). Since infection induces a redistribution of the host Golgi apparatus to the PV, it was possible that parasites scavenged host sphingolipids through this rerouting of host Golgi membrane trafficking. Rabs are a family of >40 low-molecular-weight GTPases whose primary function is to regulate intracellular membrane trafficking. Consistent with *Toxoplasma* inducing a redistribution of the host Golgi apparatus, infection altered the localization of 3 Rab GTPases (Rab14, Rab30, and Rab43) established as regulators of Golgi assembly and dynamics. Significantly, expression of dominant-negative Rab14 and Rab43, but not Rab30, significantly reduced host-derived sphingolipid accumulation in the parasite (20).

**AMINO ACIDS**

**Tryptophan**. Tryptophan is an essential amino acid that *Toxoplasma* scavenges from the host. The first evidence for this requirement came from studies showing that gamma interferon (IFN-γ), the key cytokine for limiting parasite replication, upregulates indoleamine-dioxygenase (IDO), a gene that encodes the first and rate-limiting enzyme in tryptophan catabolism. In addition, parasite growth cannot be controlled in IFN-γ-treated cells lacking IDO, and the repressive effect of IDO on parasite growth can be reversed by the addition of excess tryptophan to the growth medium (23). These data not only highlighted one manner by which IFN-γ controls *Toxoplasma* growth in human cells but also suggested that *Toxoplasma* is a tryptophan auxotroph that scavenges the amino acid from its host cytosol. Definitive proof that *Toxoplasma* is a tryptophan auxotroph came from the ability to grow parasites in tryptophan-limited medium when they express the *Escherichia coli* trpB gene, which encodes tryptophan synthase (24). Moreover, *in silico* metabolic pathway reconstruction indicates that the parasite lacks tryptophan biosynthetic enzymes (http://www.genome.jp/kegg-bin/show_pathway?org_name=tox&mapname=00400&mapscale=|&show_description=hide).

More recent work, however, has suggested that IDO is not a universally utilized anti-*Toxoplasma* control mechanism in human cells. Niedelman et al. reported that while IDO could restrict parasite growth in IFN-γ-treated HeLa cells, IDO was not involved in killing *Toxoplasma* in the human fibroblasts that they used (25). Since the work by Pfefferkorn discussed above on the function of IDO during *Toxoplasma* infections also used human fibroblasts (23), a likely explanation for the discrepancy between these studies is that genetic and/or epigenetic differences between the different fibroblasts dictate the antiparasitic mechanism used by IFN-γ to kill *Toxoplasma*.

**Arginine**. Arginine plays a unique role in *Toxoplasma* growth and virulence. First, it is an essential amino acid that the parasite scavenges from its host and a decrease in its availability triggers bradyzoite development (26). Besides being an essential amino acid, arginine must also be metabolized by the host cell to generate polyamines (e.g., spermine, spermidine, etc.). These are then transported into the parasite (27, 28). Like tryptophan and other amino acids that the parasite scavenges from its host, arginine and polyamine transporters remain to be identified.

Arginine 1 is a host cell enzyme that catabolizes arginine to ornithine and urea. Its expression in macrophages is the hallmark of alternatively activated macrophages, whereas its lack of expression and the macrophage’s exposure to IFN-γ produce classically activated macrophages (29). Arginase 1 expression is upregulated by *Toxoplasma* type I and III strains due to their expression of the ROP16 polymorphic serine/threonine kinase that is secreted into the host cell, where it phosphorylates and activates the STAT3 and STAT6 (STAT3/6) transcription factors (29–35). In contrast, type II parasites trigger the development of classically activated macrophages by limiting STAT3/6 activity, presumably by expressing a less efficient ROP16 allele as well as by activating other proteins such as the suppressor for cytokine signaling 3 (SOCS3) proteins that act to limit STAT3 activity (36) and also by expressing GRA15, which activates NF-κB, which controls the expression of genes that help skew the macrophages toward becoming classically activated (34). Because arginine availability is rate limiting for parasite growth, upregulation of arginase 1 would be predicted to limit parasite growth. Indeed, arginine limitation reduces growth of wild-type type I parasites but not ROP16 knockouts (35). But arginase 1 expression would also act to reduce polyamine levels in the host cell, and how parasites handle decreased avail-
ability of these nutrients, which also must be scavenged, remains to be determined (27).

THE HOST PLASMA MEMBRANE AS A KEY TARGET FOR TOXOPLASMA TO REGULATE ITS HOST CELL

Being the initial site of contact, the host plasma membrane represents a key interface between Toxoplasma and its host cell both during and after parasite invasion. Yet little is known about how they interact. Before parasites begin to invade a host cell, unknown signals induce rhoptry and microneme secretion. Among the secreted rhoptry proteins, the RONs are a complex of proteins that are injected into the host cell and localize to the host cell surface (37). The RONs then bind AMA1, which is a micronemal protein that is secreted onto the parasite surface. Once AMA1 and the RON complex engage, the parasite can then propel itself into the host cell. Thus, the parasite places its own receptor on the surface of its host cell, which likely explains Toxoplasma’s diverse host cell tropism. But this does not mean that the host cell plasma membrane plays a passive role during invasion. Other microneme proteins (e.g., MIC2) are adhesins that mediate parasite-host cell attachment by binding to as-yet-unidentified host cell surface factors (38).

A primary function of the plasma membrane is to activate cellular responses to extracellular cues by triggering intracellular signaling pathways. Interactions of micronemal and parasite surface antigens with the host plasma membrane suggested that the parasite might regulate host cell signaling by engaging host plasma membrane receptors. This hypothesis was supported by the finding that addition of parasite-secreted factors (mainly composed of microneme-derived factors) to uninfected host cells led to changes in gene expression (39, 40). The fact that many of these host genes encoded chemokines, cytokines, and other immune-response-associated proteins suggested a role for Toll-like receptor (TLR) and/or other pathogen detection receptors in this response. More-recent studies have, however, provided more direct evidence for how Toxoplasma uses host cell plasma membrane receptors to help in establishing its replicative niche.

Concomitant with Toxoplasma penetrating into its host cell, it forms its PV, while avoiding the endolysosomal pathway, since that route would result in autophagy-mediated degradation of the PV and parasite (41–43). Parasite avoidance of the phagolysosome is inhibited by treatment of cells with tyrosine kinase inhibitors, and the epidermal growth factor (EGF) receptor, which is a receptor tyrosine kinase, was identified as at least one target of these inhibitors (44). Significantly, parasite growth was attenuated in cells transfected with small interfering RNAs (siRNAs) targeting the EGF receptor and the PVs in these cells appeared as if they were undergoing autophagic destruction. AKT kinase activation of phosphatidylinositol-3 kinase appears to be the critical downstream target of the EGF receptor signaling. These data support a model where EGF/AKT signaling either prevents the invading parasite from entering the endolysosomal pathway or prevents lysosomal recruitment to the PV. While the parasite ligand(s) that activates the EGF receptor is unknown, the receptor is not activated by parasites with knockout mutations in the MIC1 and MIC3 micronemal proteins (44).

Besides allowing the PV to properly develop, host plasma membrane signaling-dependent reprogramming of host gene expression and intracellular signaling is likely also to be important for Toxoplasma to establish its replicative niche. As an example, initial DNA microarray studies revealed that Toxoplasma infection causes dramatic alterations to the host cell transcriptome (45, 46). While modulation of many of these genes is most likely important for either promoting host resistance or allowing the parasite to evade host defenses, others likely act to modify the host cell’s intracellular environment to make it hospitable for parasite growth. Host genes predicted to promote parasite growth would include those that prevent host cell death and those that function in biosynthetic pathways that provide the parasite with a necessary nutrient.

One clade of upregulated host genes that fulfilled the criteria for possibly being important for parasite replication included those that encoded vascular endothelial growth factor (VEGF), glucose transporter, and glycolytic transcripts (45). These genes are targets for the hypoxia-inducible factor-1 (HIF-1) transcription factor, which is a heterodimer composed of α and β subunits that regulates cellular responses to decreased oxygen availability (47, 48). Toxoplasma activates HIF-1 via a host- or parasite-derived soluble secreted factor that signals through a family of plasma membrane-localized, serine/threonine kinase receptors named the activin-like kinase receptors (ALK4,5,7) (49). Following infection, ALK4,5,7 signaling triggers HIF-1 activation by increasing the stability of the HIF-1α subunit, which is a protein with an inherently short half-life. The mechanism by which HIF-1α is degraded is a well-studied pathway where, immediately after the protein is synthesized, two proline residues are hydroxylated by a prolyl hydroxylase (PHD) (50). Prolyl-hydroxylated HIF-1α is then recognized by and ubiquitylated by the Von Hippel-Lindau (VHL) ubiquitin ligase, which targets HIF-1α to the proteasome. Toxoplasma infection stabilizes HIF-1α by blocking its prolyl hydroxylation, and this correlates to a decreased abundance of PHD2, which is the PHD most critical for controlling HIF-1α protein levels (49). The importance of HIF-1α for parasite growth was demonstrated by the finding that parasite growth is reduced in HIF-1α-deficient cells (48). Interestingly, Toxoplasma dependence on HIF-1α is increasingly important at physiological O2 levels but it is unknown how HIF-1 promotes parasite growth under this condition.

KEEPING THE HOST CELL ALIVE: APOPTOSIS AND THE INFLAMMASOME

Toxoplasma must ensure that its host cell remains alive long enough for the parasite to replicate and then egress and invade the next host cell. Thus, the death of infected host cells is a key weapon in the metazoan host’s defense against infection (51). The two best-characterized forms of host cell death during infection are apoptosis and pyroptosis, and both are modulated during infection. Apoptosis, which is dependent on the activation of a signaling cascade that leads to caspase-3 activation, is typified by membrane blebbing, nuclear condensation, and DNA fragmentation. The membrane blebbing results in the formation of apoptotic bodies that are taken up by phagocytic cells. In contrast, pyroptosis is dependent on the inflammasome activating caspase-1, which leads to the release of interleukin-1β (IL-1β) and IL-18 and membrane permeabilization following cell swelling and osmotic lysis (51, 52).

APOPTOSIS

Apoptosis is a noninflammatory form of programmed cell death that been implicated as an innate defense mechanism to
eliminate intracellular pathogens (51). There are two major apoptotic pathways. The first, the intrinsic pathway, is triggered by cytotoxic stress and DNA damage (e.g., UV irradiation and chemotherapy) and involves increased expression and mitochondrial localization of proapoptotic Bcl-2 family members. This leads to release of cytochrome C, promoting assembly of the apoptosome, which is a multiprotein complex that activates caspase-9. The second is the extrinsic pathway that is triggered by death receptor activation (e.g., FasL and TRAIL) and involves activation of caspase-8 (53–55). Activated caspase-8 or -9 can then cleave caspase 3, which then allows it to trigger apoptosis by cleaving cellular targets.

Toxoplasma infection renders the host cells resistant to stimuli that activate either the intrinsic pathway or the extrinsic pathway (56–58). Consistent with these findings, Toxoplasma-infected cells show decreased levels of cleaved caspase-3, as well as of caspase-8 (extrinsic pathway) and caspase-9 (intrinsic pathway) (57, 59). A variety of potential upstream changes have also been noted, including increases in expression of inhibitor of apoptosis proteins (IAPs) (60) and of antipapptic members of the Bcl-2 family (45), activation of PI 3-kinase signaling (61), an NF-kB-dependent degradation of proapoptotic Bcl-2 family members (BAX, BAD, and BID) (59, 62), and upregulation and expression of STAT6-dependent specific serine protease inhibitors, including SERPIN B3 and B4, which are known to protect against tumor necrosis factor alpha (TNF-α)-induced apoptosis (63).

Although Toxoplasma clearly impacts host cell apoptosis, a key issue that remains to be addressed is whether this effect on the host cell’s physiology provides an advantage for parasite growth and/or immune evasion. Or are these effects on host cell apoptosis an off-target effect of the parasite modulating other host cell pathways? Directly testing these possibilities awaits identification of the parasite factor(s) that impacts host cell apoptosis.

THE INFLAMMASOME

The inflammasome is a multiprotein complex first described in 2002 that assembles in the cytoplasm after a sensor protein within the complex detects a microbial or environmental factor danger signal (52, 64, 65). Inflammasome activation requires two signals. The first is signal 1, which is often initiated by TLR binding of PAMPs (pathogen-associated molecular patterns) or other receptors sensing DAMPs (danger-associated molecular patterns). This signal leads to an upregulation of pro-IL-1β through activation of the transcription factor NF-κB. Signal 2 then triggers the activation of an intracellular sensor that leads to inflammasome assembly and caspase-1 activation, which then leads to the processing and secretion of IL-1β and IL-18. (See Fig. 2 for a schematic of inflammasome activation.) Several inflammasome sensors have been defined, but the ones pertinent to this review belong to the nod-like receptor (NLR) family and are called NLRP1 (NALP1) and NLRP3 (NALP3). Unlike other intracellular sensors that respond only to foreign molecules (e.g., RIG-I binding to double-stranded RNA [dsRNA]), inflammasome sensors respond to both PAMPs and DAMPs (52, 66). In some cells, such as macrophages, inflammasome activation can trigger pyroptosis, a rapid, inflammatory cell death that is caspase-1 dependent (52, 66).

In humans, polymorphisms in the nlrp1 gene have been linked to susceptibility in congenital toxoplasmosis (67). Moreover, parasite growth was enhanced whereas host cell viability and IL-1β and IL-18 expression were reduced in monocytes engineered to express decreased levels of NLRP1 protein (67, 68). The idea of a link between the inflammasome and human toxoplasmosis was further supported by data showing that IL-1β expression in human monocytes was dependent on caspase-1 and ASC, which is an adaptor protein that mediates caspase-1 binding to either the NLRP1/caspase-5 or the NLRP3/CARD8 inflammasomes (64, 68). IL-1β was significantly upregulated by type II strain parasites (i.e., the Pru strain), and this polymorphic effect was dependent on GRA15, which mostly likely upregulates expression of IL-1β by virtue of its ability to activate NF-kB (68). Infection of human monocytes with type I, II, or III Toxoplasma tachyzoites did not result in rapid cell death, as the assays in the studies cited above were carried out at 24 and 36 h (67, 68), suggesting that under those conditions, inflammasome activation was not triggering pyroptosis. Thus, in human monocytes, activation of the inflammasome restricts parasite growth either through IL-1β- and IL-18-dependent mechanisms or through another unknown mechanism. This manner of controlling Toxoplasma is distinct from that seen in macrophages from Toxoplasma-resistant rats, in which NLRP1 inflammasome activation led to rapid cell death (within 3 to 10 h) after infection with Toxoplasma, consistent with pyroptosis (69, 70).

It had previously been noted that diverse rat strains differed in their susceptibility to Toxoplasma and that this difference was linked to a specific 1.7-cM genetic locus named the toxol locus (71). The rat nlrp1 gene lies within this locus, and polymorphisms in this gene determine how macrophages from Toxoplasma-susceptible and -resistant rats differentially respond to Toxoplasma. In susceptible rats, macrophages infected by Toxoplasma do not undergo pyroptosis or secrete IL-1β, while in resistant rats, the macrophages do secrete IL-1β and undergo pyroptosis (72, 73). This difference in triggering the inflammasome then leads to a difference in parasite expansion within macrophages (72). Most Toxoplasma strain types are similarly detected by the resistant-rat inflammasome (72) regardless of whether or not the macrophages were first primed with lipopolysaccharide (LPS) (69). In mice, both NLRP1 and NLRP3 contribute to inflammasome activation by Toxoplasma as evidenced by induction of IL-1β maturation and secretion from macrophages (73, 74). But unlike the results seen with rats and akin to those seen with humans, inflammasome activation did not trigger pyroptosis (73). Interestingly, in mice, few strain-specific differences in inflammasome activation were noted when the macrophages were primed with a substance such as LPS or Pam3CSK4 (73, 74). Finally, in mice, Toxoplasma does not activate NLRP1 by cleaving its N terminus, unlike the Bacillus anthracis lethal factor (69, 72, 73), the only previously known mechanism for activation of NLRP1 (75). Thus, even though Toxoplasma activates the inflammasome through NLRP1 (and NLRP3 in mice), this activation occurs through novel mechanisms.

Collectively, these studies clearly implicate inflammasome activation in playing an important role in innate defenses against Toxoplasma, but their contradictions also raise questions. Why do priming murine macrophages eliminate the strain-specific initiation of the NLRP1 inflammasome seen in unprimed murine macrophages? In mouse macrophages, certain strains can clearly give both signal 1 and signal 2 (e.g., type II Pru- and GRA15-dependent NF-kB activation), but other strains may trigger only signal 2 (73), which means that they do not activate the inflammasome in vitro.
unless the cell has been exogenously primed to activate signal 1 (i.e., LPS or Pam3CSK4). This may also explain differences reported in human monocytic cells in two studies; the cells were not primed in either study, but one study found that type I parasites triggered the inflammasome by 36 h postinfection (hpi) \( (67) \) and the other found strain-specific differences in inflammasome activation at 24 hpi \( (68) \). Thus, without priming, type I (RH) strains may activate signal 1 and signal 2 of the inflammasome only by 36 hpi whereas another strain type (type II) can do so more rapidly. Currently, it is unknown if prestimulation of the human macrophages/monocytes with LPS or Pam3CSK4 would eliminate these strain differences \( (70, 73, 76–78) \).

While these are very exciting developments in the Toxoplasma-inflammasome story, there is much that remains to be understood. Macrophages have been the major focus of the inflammasome work, but it is possible (and likely) that the inflammasome is important in Toxoplasma resistance in other cell types. In addition, definitive proof that macrophage (or other cell) inflammasome activation drives Toxoplasma susceptibility or resistance remains to be developed, possibly through studies using bone marrow-chimeric rats and mice. In addition, understanding how Toxoplasma is sensed by NLRP1 will offer insights into why polymorphisms in this gene impact infection outcomes in humans (congenital disease) and rodents. Clearly, the effect of Toxoplasma on both apoptosis and pyroptosis/inflammasome activation underscores the importance of host cell viability for parasite growth and survival.

**TOXOPLASMA REGULATES HOST CELL NUCLEAR FUNCTIONS**

As described in the preceding sections, modulating host cell gene expression is one important way for the parasite to develop its replicative niche (Fig. 3). This is achieved by the activation of host transcription factors such as STAT3/6, NF-κB, and HIF-1. In contrast to factors that activate NF-κB (GRA15) and HIF-1 (identity currently unknown), STAT3/6 is activated by ROP16, which is a polymorphic protein that translocates to the nucleus after it is injected into a host cell \( (32) \). This is reminiscent of another rhoptry-localized protein phosphatase 2C (PP2c) homolog that also translocates to the host cell nucleus following invasion \( (75) \), although the function of this protein is unknown. Spurred by these findings, Bougdour and colleagues used an in silico approach to identify parasite proteins that, like ROP16 and the rhoptry PP2c homolog, contained both a signal sequence (to facilitate export...
into the host cell) and a nuclear localization signal. This screen led to the identification of GRA16 and GRA24, which are released from the dense granules and are constitutively secreting, apicomplexan-specific organelles. GRA16 binds to a complex composed of a host deubiquitinase (HAUSP) and a host PP2A phosphatase (79). Together, the components of this complex act to maintain levels of the host p53 protein, which may impact host cell growth and cycle progression and/or proinflammatory responses (79, 80).

Importantly, deletion of GRA16 severely attenuates the virulence of type II strain parasites. Thus, GRA16 is the first nonpolymorphic factor to be identified that is secreted into host cells and impacts parasite virulence.

GRA24 was the second protein identified by this screen, and it binds to and promotes and maintains activation of the host p38 MAP kinase. GRA24-dependent activation of p38 MAP kinase results in the activation of several transcription factors, including the early-growth-response proteins (81). GRA24 deletion leads to a significant reduction in the expression of chemokines, including those critically required for recruitment of inflammatory monocytes (81). Since inflammatory monocytes are required for resistance to *Toxoplasma* infection (82, 83), it was surprising that loss of GRA24 had no discernible impact on virulence, and further experiments are required to establish the basis for this (81).

Studies on immune evasion have largely revealed that many (but not all) of the known *Toxoplasma* virulence factors act by disengaging the immune response from properly detecting and responding to the infection. IFN-γ is the key cytokine that mediates the host cell defense and does so by upregulating the expression of IFN-γ effectors that kill *Toxoplasma* by a number of distinct mechanisms. These include limiting nutrient scavenging, degrading the parasitophorous vacuole, and increasing antigen presentation in infected cells so that they can be recognized by *Toxoplasma*-specific T cells. Recent work in both human and murine cells demonstrated that virulent *Toxoplasma* strains can prevent IFN-γ from triggering the degradation of their PVs by inhibiting IFN-γ-stimulated GTPases (the immunity-related GTPase [IRG] proteins and guanylate-binding proteins [GBP]) from becoming activated and associating with the PVM (25, 84–89). However, all known strains escape IFN-γ-dependent killing if they infect the host cell before it becomes activated by IFN-γ. This evasion is due to the parasite dysregulating IFN-γ-induced gene

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**FIG 3** Modulation of host gene expression by nonpolymorphic parasite factors. *Toxoplasma* can modulate gene expression either by modulating host transcriptional regulators (EGRs, p53, or HIF-1) or indirectly by affecting chromatin remodeling.
expression, including blocking upregulation of the IRGs, GBP, nitric oxide synthase, IDO, and major histocompatibility complex (MHC) class I and II (90, 91). STAT1 is the major transcription factor downstream of the IFN-γ receptor, but its activation (as assessed by its phosphorylation) is not affected in parasite-infected cells (90, 92). Rather, Toxoplasma inhibits STAT1 by altering histone acetylation and by other chromatin modifications at STAT1-activated promoters (93). It was also reported that Toxoplasma can also prevent dissociation of STAT1 from DNA, which would limit its recycling between STAT1-responsive genes (94).

**TOXOPLASMA MODULATION OF UNINFECTED CELLS: IS THE PARASITE SETTING UP A MICROENVIRONMENT?**

While the primary focus of this review has been on the interaction between *Toxoplasma* and its infected host cell, the parasite grows in diverse and dynamic settings in vivo. In analogy to the tumor microenvironment, parasite survival in such an environment would likely require that the parasite manipulate not only the cell in which it is currently residing but also neighboring resident tissue cells as well as immune-derived cells recruited to the site of infection. Perhaps the best-described example of this interaction is in the gut, where *Toxoplasma* triggers the expression of dendritic-cell- and monocyte-recruiting chemokines. While these cells are important for host resistance, the parasite also infects and uses them to disseminate in the host via a Trojan horse-like mechanism (95–97). In addition, early microarray studies demonstrated that a subset of the host genes modulated by infection were regulated as a result of secreted parasite- and/or host-derived factors being released into the culture medium following infection (45).

As noted in the previous section, several host cell plasma membrane receptors are activated by *Toxoplasma*. ALK4,5,7 signaling is required to activate HIF-1, and HIF-1 is activated in both infected and neighboring uninfected cells, suggesting that the parasite either secretes an ALK4,5,7-inducing ligand or induces the host cell to release one. The finding that HIF-1 can be induced even when direct contact between the parasite and host cell is prevented supports this prediction (48). Similarly, a secreted host- or parasite-derived low-molecular-weight factor modifies the cell cycle progression of both infected host cells and neighboring uninfected host cells by having them enter the S phase (98). Why *Toxoplasma* induces this bystander effect in the S phase is unclear but could be related to the long-standing observation that parasites prefer to invade cells that are in the S phase (99, 100).

EGF receptor activation is dependent on the expression of at least 2 micronemal proteins, MIC1 and MIC3, and addition of recombinant MIC1 and MIC3 proteins to cells infected with *mic1 mic3* double-knockout parasites restores EGF receptor-dependent inhibition of CD40L-induced autophagy (44). Both of these proteins are shed from the parasite’s plasma membrane during invasion (101), and it is therefore possible that the released ectodomains can interact with and activate EGF receptor signaling in noninfected cells and thus initiate an antiautophagic response in a host cell prior to its infection.

Besides host plasma membrane receptors, intracellular host proteins are also targeted during infection and this is due in part to the secretion of rhoptry effector proteins (102). The previously established model predicted that rhoptry proteins functioned only in the infected host cell since in theory they were injected concomitant with invasion (103). This paradigm has recently been challenged by a system in which *Toxoplasma* parasites were engineered to secrete Cre recombinase (Cre) into host cells. Using these *Toxoplasma*-Cre parasites to infect reporter cells that express only a green fluorescent protein (GFP) after Cre excises a stop codon, GFP could be detected in both infected and uninfected cells (104). In addition, pSTAT6 nuclear translocation, which is dependent on ROP16, can be observed in vitro and in vivo in a percentage of uninfected cells, consistent with the idea that multiple rhoptry proteins are entering these uninfected cells (105). Importantly, rhoptry secretion into uninfected cells appears to be a widespread phenomenon and can be observed in diverse types of immune and nonimmune cells (105).

The host-parasite interaction is a continuous battle, and we propose that *Toxoplasma* modulation of its microenvironment provides it two important advantages for winning this battle. First, activation of host cell processes prior to parasite invasion provides additional time and an opportunity for the parasite to establish its replicative niche. This would include altering host cell metabolism in a way that would help the parasite gain access to necessary nutrients and to activate mechanisms to evade intrinsic immune defenses such as autophagy, pyroptosis, and apoptosis. Second, this would allow the parasite to disarm IFN-γ and other immune effector killing mechanisms before the parasite enters the host cell. Finally, the ability to interact with and regulate immune cells may provide another mechanism for the parasite to evade the immune response. For example, HIF-1 activation can dampen T-cell receptor signaling in effector T cells (106, 107) and can also promote regulatory T-cell development (108, 109). Not only would negatively regulating T cells aid in immune evasion, but it also could limit the collateral immune-mediated tissue damage. Whether the parasite truly modulates its microenvironment is therefore an important issue that needs to be addressed.

**CONCLUSION**

As an obligate intracellular microbe, *Toxoplasma* must contend with a variety of pressures in order to survive in the intracellular environment. In this review, we primarily focus on those processes that predominantly act in a parasite strain-independent manner—nutrient acquisition, keeping the host cell alive, and manipulation of the microenvironment. Each likely represents a critical area in which *Toxoplasma* has coevolved with its host cells in order to ensure that both survive. Studies that define the host cell processes targeted by infection will continue to provide insights into the fundamental biology of these cellular processes. We also believe that the host cell pathways that are rate limiting for *Toxoplasma* growth, the parasite factors that activate them, and the parasite processes dependent on these host cell pathways are potentially important and untapped drug targets.

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Toxoplasma gondii is accompanied by reduced activation of the caspase-1/1pase-1 (1pase-1) family, which mediates inflammatory responses, such as proinflammatory cytokine secretion and host cell apoptosis. The host cell response to Toxoplasma infection is mediated by the adaptive immune system, which recognizes the presence of the parasite and mounts an immune response. This response includes the activation of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ), which are produced by activated T cells and macrophages. These cytokines can induce the cleavage of caspase-1 and -1pase-1, leading to the production of the inflammatory cytokine interleukin-1β (IL-1β) and IL-18, respectively.

In addition to the adaptive immune response, Toxoplasma infection can also induce innate immune responses, which are mediated by the natural killer (NK) cells and other innate immune cells. These responses include the production of type I interferons, which can inhibit the replication of Toxoplasma in infected cells. However, the presence of Toxoplasma can also lead to the activation of the intracellular signaling pathways, such as the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) pathways, which can promote the survival and proliferation of the parasite. These pathways can be regulated by the host cell response to Toxoplasma, which can affect the outcome of the infection.

Furthermore, Toxoplasma infection can also induce chronic inflammation, which can lead to the development of chronic diseases, such as cardiovascular disease and neurodegeneration. The chronic inflammation can be mediated by the production of cytokines, such as TNF-α and IL-1β, which can activate the MAPK and PI3K pathways, leading to the proliferation of immune cells and the production of anti-apoptotic proteins, such as Bcl-2. These pathways can also be regulated by the host cell response to Toxoplasma, which can affect the outcome of the infection.

Overall, the host cell response to Toxoplasma infection can be complex and regulated by multiple signaling pathways, which can affect the outcome of the infection. Further research is needed to understand the mechanisms of the host cell response to Toxoplasma and how these pathways can be targeted to develop new therapeutic strategies for the treatment of toxoplasmosis.


