FEATURE ARTICLE

Toxoplasma gondii Parasites Take Control within Host Cells

This parasite puppet master lives mostly without causing disease for its hosts, establishing lifelong asymptomatic infections

Eric Y. Denkers and Barbara A. Butcher

The extraordinarily successful *Toxoplasma gondii* protozoan parasite is globally distributed, infects a vast range of mammalian and avian hosts, replicates in a variety of host cells, and can bypass its definitive host to be directly transmitted from one intermediate host to the next. Most importantly, *Toxoplasma* lives mainly without causing disease for its hosts, establishing life-long asymptomatic infections. More than 1 billion humans are infected with *T. gondii*.

Toxoplasma was seen as an intracellular microbe that lived within host cells but virtually separated from them via membrane barriers. Now, the situation is viewed as more complex because several *T. gondii* effector proteins directly interact with molecular components of—and leverage survival within—the host cell.

Parasite Replication Is Rapid during the Acute Phase of Infection

Humans become infected with *Toxoplasma* by consuming undercooked, cyst-containing meat, while animals become infected through predation (Fig. 1). Within the small intestine, parasites emerge as tachyzoites, actively invading cells of the intestinal mucosa in a process involving sequential discharge of apically oriented organelles called micronemes and rhoptries. Early after invasion, the parasitophorous vacuole is established, a membrane-bound structure within which tachyzoites replicate until the host cell lyses and infectious organisms are released.

During this acute stage of infection, *Toxoplasma* widely disseminates through host tissues, typically causing mild flulike symptoms. As the host adaptive immune system begins to respond, the parasites differentiate into slow-growing bradyzoites that form cysts in tissues of the muscle and central nervous system. During this long-lived stage of the parasite, cysts are maintained in tissues with little or no host inflammatory reaction.

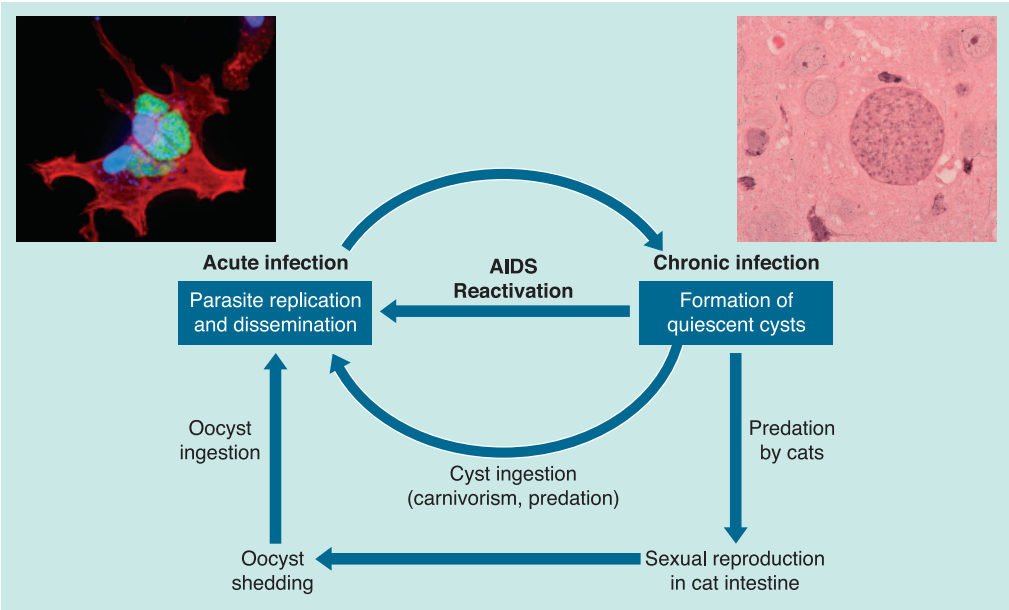
Yet, for individuals with declining immunity such as those with AIDS, toxoplasmosis is a life-threatening infection. Parasite cysts within the brains of such individuals may reactivate, with bradyzoites redifferentiating into tissue-damaging tachyzoites. Ensuing toxoplasmic encephalitis can be lethal in the absence of appropriate drug treatment.

Domestic and wild cats, members of the family *Felidae*, are the definitive hosts of *Toxoplasma*. When cats prey on infected animals, ingested parasites reproduce sexually within the small intestines of their hosts. Subsequent fecal shedding distributes large numbers of highly infectious oocysts, which are readily ingested to continue the life cycle of this parasite in intermediate hosts. However, *Toxoplasma* can also be orally transmitted directly from one intermediate host to the next, meaning passage through cats is not oblig-

SUMMARY

- ***Toxoplasma gondii* protozoan parasites are globally distributed, infect a vast range of mammalian and avian hosts, replicate in a variety of host cells, can bypass cats, their definitive host, and infect more than 1 billion humans.**
- **During the acute stage of infection, *Toxoplasma* widely disseminates through host tissues, typically causing mild flu-like symptoms; later growth slows, and cysts form in muscle and central nervous tissues.**
- ***T. gondii* withstands challenges from both the innate and acquired arms of the host immune system.**
- ***T. gondii* isolates from Europe and North America mainly fall into three clonal lineages with varied degrees of virulence.**
- **The ROP16 protein of this parasite provides the first concrete example of an effector molecule from an intracellular eukaryote that targets its host signal transduction machinery.**

FIGURE 1



Life cycle of *Toxoplasma gondii*. Infection begins with ingestion of oocysts or tissue cysts. Parasites cross the intestinal epithelium and begin replicating and disseminating as intracellular tachyzoites. The image on the left shows a mouse astrocyte (red, actin stain) harboring proliferating tachyzoites (green). Parasites reach the brain and differentiate into long lived bradyzoites contained within cysts. The image on the right shows a cyst within a mouse brain. Latent infection is notable for lack of inflammatory infiltration around tissue cysts. When the infected host is preyed upon by members of the cat family, sexual reproduction in the cat intestine leads to fecal shedding of highly infectious oocysts. The parasite can also transmit itself from one intermediate host to the other through carnivorism and predation. In most scenarios, the life cycle plays out with little ill effect on the host. However, immunosuppression in chronically infected individuals (as in AIDS) can result in brain cyst reactivation resulting in life-threatening toxoplasmic encephalitis.

atory. This feature of *Toxoplasma* might be a recent adaptation that helps to account for the global distribution of this parasite.

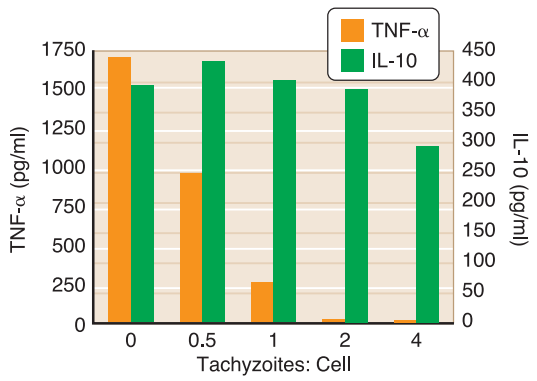
Encounter with the Host Immune System

T. gondii withstands challenges from both the innate and acquired arms of the host immune system. Simply evading the immune system could lead to uncontrolled replication and death for both the parasite and host. For example, when immunodeficient mice that cannot produce interferon-γ (IFN-γ) are infected with *Toxoplasma*, the parasite spreads rampantly, leading the host to die rapidly. Thus, the host needs to mount a Th1 response if the parasite is to develop a latent infection.

At the other extreme, a very robust immune response eliminates the parasite, but may lead to proinflammatory tissue damage that can be lethal for the host. For example, mice lacking the down-regulatory cytokine interleukin-10 (IL-10) succumb to *Toxoplasma* much as do mice that are deficient in IFN-γ. However, in this case, death is attributed to unchecked production of pro-inflammatory cytokines such IFN-γ and tumor necrosis factor-α (TNF-α). Because latent *T. gondii* infection is widespread throughout the vertebrate animal kingdom, this suggests that this parasite is adept at balancing immune activation against evasion.

We discovered a striking example of *Toxoplasma*-directed evasion of immunity several years ago. Macrophages, which along with dendritic cells are targets of in vivo infection, produce cytokines such as TNF-α and IL-12 when activated through Toll-like receptors on the cell surface. Yet, if these cells are first exposed to tachyzoites, they lose their ability to respond and TNF-α production is suppressed. They also lose their ability

FIGURE 2



Cytokine-specific suppressive effects of *Toxoplasma* in infected macrophages. In this experiment, mouse macrophages were infected with *T. gondii* then 2 hr later cells were subjected to stimulation with bacterial lipopolysaccharide. Supernatants were collected after 6 hr for cytokine measurement. The parasite strongly suppresses production of tumor necrosis factor-α (TNF- α), but the same cells continue to produce interleukin-10 (IL-10). (This figure is adapted from C. W. Lee, S. Bennouna, and E. Y. Denkers, Infect. Immun. 17:1916–1923, 2006.)

to respond to IFN-γ. Because other mediators such as anti-inflammatory IL-10 are unaffected, carrying *Toxoplasma* does not make such cells generally dysfunctional (Fig. 2). Moreover, only cells containing intracellular parasites become nonresponsive. In other words, there is no bystander effect on uninfected cells. Instead, *Toxoplasma* directly subverts these signaling pathways within host cells.

Highjacking the JAK/STAT Pathway

T. gondii affects host macrophages much like IL-10, an anti-inflammatory cytokine that strongly down-regulates macrophage production of TNF-α and IL-12. However, it is unlikely that IL-10 is the cause of *Toxoplasma*-mediated suppression, because only infected cells are affected. Indeed, macrophages generated from IL-10 knockout mice retain sensitivity to the inhibitory effects of the parasite.

T. gondii instead bypasses IL-10 and directly activates anti-inflammatory signaling. Like many cytokines, IL-10 depends on a Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling. In the canonical JAK/STAT pathway (Fig. 3), cytokine binding to its receptor leads that receptor to form dimers. In turn, cytoplasmic STAT molecules undergo JAK-dependent tyrosine phosphorylation, leading STAT molecules to form dimers, translocate to the nucleus, and bind to STAT-responsive promoters. In many cases, full transcriptional activity requires STAT serine phosphorylation dependent upon mitogen-activated protein kinase (MAPK) signaling. Specificity in cytokine-mediated signaling comes from differential involvement of multiple JAK molecules (JAK1, JAK2, JAK3, TYK2) and multiple STAT proteins (STAT1, 2, 3, 4, 5a, 5b, 6) that form various cytokine-dependent complexes.

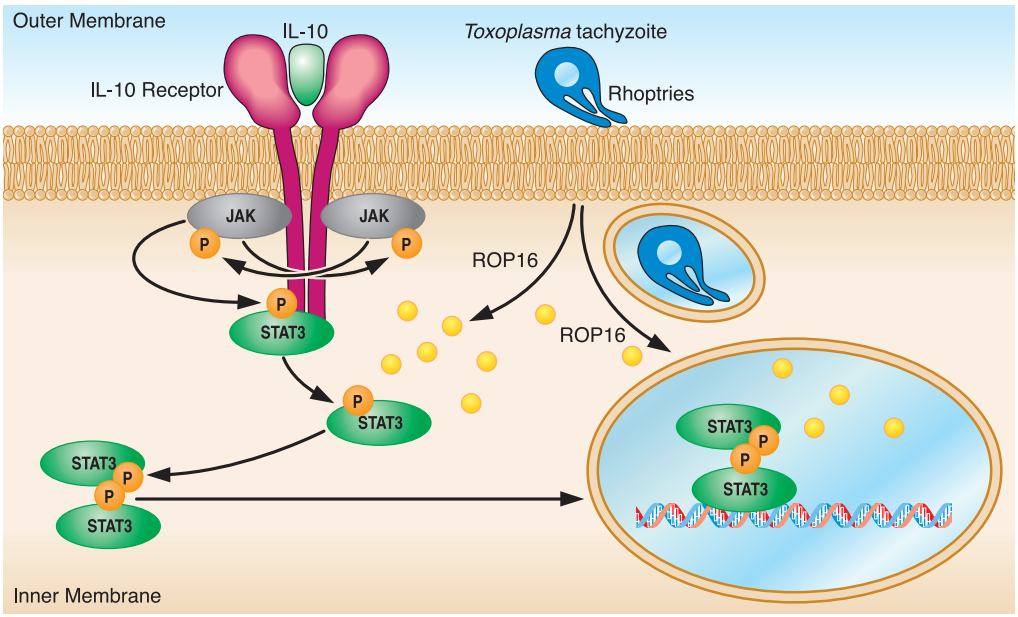
Interleukin-10 receptor-mediated signaling depends upon STAT3. Within minutes, *Toxoplasma* activates STAT3 in infected but not bystander cells. With macrophage-specific STAT3 knockout cells from Peter Murray of St. Jude Children's Research Hospital and Stephanie Watowich of M. D. Anderson Cancer Center, we proved that *Toxoplasma*-activated STAT3 was necessary to interfere with signaling within infected cells.

Parasite Secretory Kinases Interact with Host Cell Signaling

T. gondii isolates from Europe and North America largely fall into three clonal lineages. In mice, Type I is extremely virulent, Type II is intermediate, and Type III has low virulence. These strains emerged from genetic crosses roughly 10,000 years ago, which coincides with when humans began domesticating animals. During that period, animal species such as cats and rodents came into close contact with one another and humans—all of which likely increased the frequency of sexual reproduction among *Toxoplasma* strains.

These three types of *T. gondii* strains also differ in terms of the immune responses they elicit. For example, Type II parasites induce relatively high amounts of IL-12 and activate NFκB, whereas Type I stains induce only low amounts of IL-12 and no activity in the NFκB pathway. By analyzing F_1 progeny from interstrain crosses, John Boothroyd at Stanford and David Sibley at Washington University identified the *rop16* virulence locus and, later, linked it to activation of STAT3 and STAT6. Thus, Type I and Type III strains possess a ROP16 allele that activates STAT3 and

FIGURE 3



The JAK/STAT pathway and its co-option by *T. gondii*. Cytokine binding – in this case interleukin 10 - causes dimerization of the receptor and trans-tyrosine phosphorylation of receptor associated JAK molecules. In turn, STAT3 undergoes JAK-mediated tyrosine phosphorylation. Phosphorylated STAT3 forms dimers, enabling nuclear translocation and binding to promoter responsive elements in target genes. Full STAT3 transcriptional activity requires MAPK-mediated serine phosphorylation. During invasion, *Toxoplasma* injects several molecules, including rhoptry protein ROP16, into the host cell cytoplasm. Based upon in vitro biochemical evidence, ROP16 directly tyrosine phosphorylates STAT3 mediating its activation independently of JAK kinases. For reasons that are unclear, STAT3 possesses a nuclear localization sequence and therefore traffics to the host cell nucleus.

STAT6, whereas in Type II parasites ROP16 fails to activate STAT.

The *rop16* locus encodes the rhoptry protein ROP16, which with other rhoptry proteins, is injected into the host cell cytoplasm as the parasite invades. Some of these proteins are found along the parasitophorous vacuole membrane, others are cytoplasmic.

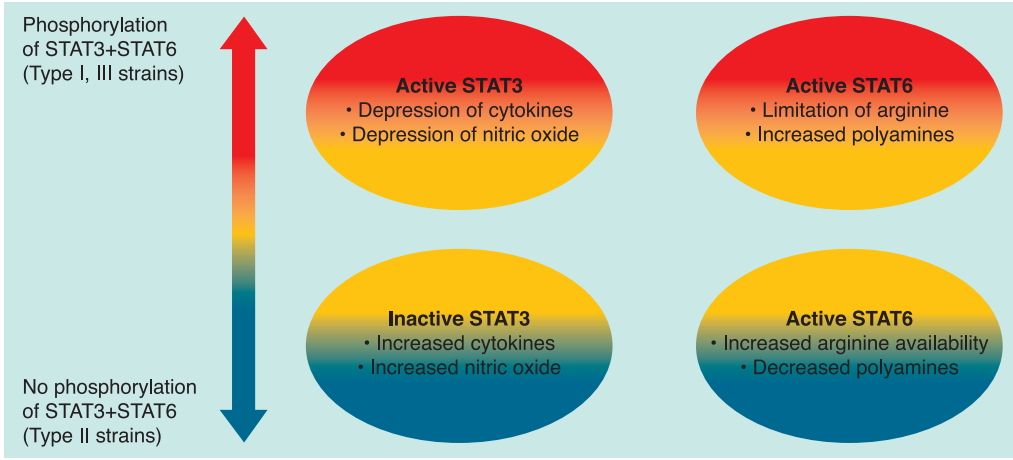
ROP16 provides the first concrete example of an effector molecule from an intracellular eukaryote that targets host signal transduction machinery—in this case, JAK/STAT signaling. Thus, Type I ROP16 is a kinase that directly phosphorylates tyrosine and can activate STAT3 and STAT6, according to John Boothroyd of Stanford University and M. Yamamoto and T. Takeda of Osaka University in Osaka, Japan. Shortly after being injected into the cytosol, at least some of ROP16 traffics to the host cell nucleus, even though STAT is activated in the host cell cytoplasm. Why ROP16 moves to the host cell nucleus remains a mystery.

Biological Function of the ROP16 Secretory Kinase

David Bzik and Barbara Fox at Dartmouth Medical School constructed mutants of Type I parasites in which the ROP16 molecule is deleted. Working with these and other mutants, we showed that tachyzoites survive and replicate in the absence of ROP16. Yet, the extent to which ROP16 alleles contribute to differences between high and low virulence strains of these parasites during in vivo infections is not fully explored. Regardless, because ROP16 activates both STAT3 and STAT6—two distinct transcription factors with distinct activities—the functions of this rhoptry kinase are likely to be multifarious.

The STAT3 transcription factor is complex, capable of activating an IL-6-mediated proinflammatory profile. Opposing this, the anti-inflammatory effects of IL-10 are also mediated through a STAT3 activation pathway. The molecular switch that determines whether STAT3 is

FIGURE 4



Choosing between activating STAT3 and STAT6 versus avoiding activation of STAT3 and STAT3. Type I and Type III *T. gondii* strains activate STAT3 and STAT6. The consequences of triggering these host cell signaling pathways are complex and may act to suppress or promote immunity and parasite growth. Likewise, lack of STAT3 and STAT6 activation in Type II parasite-infected cells is likely to have effects that can positively or negatively enhance infection. Which effects predominate may depend on cell type or stage of infection.

pro-inflammatory or anti-inflammatory seems to be the molecular suppressor of cytokine synthesis (SOCS)-3, because IL-6 signals an anti-inflammatory transcription program in its absence. *Toxoplasma*-activated STAT3 seems to have anti-inflammatory effects similar to those of IL-10 (Fig. 4).

Activating STAT6 in macrophages leads to an alternatively activated (or M2) phenotype. Such M2 macrophages likely help to repair tissues, relying in part on elevated arginase-1 to convert arginine to ornithine, a precursor of polyamines and collagen. By contrast, IFN-γ and lipopolysaccharide induce M1 macrophages, which function as antimicrobial effector cells, producing nitric oxide and other antimicrobial agents. Parasite-mediated STAT3 activation is expected to antagonize M1 macrophage function, while STAT6 activation would directly promote M2 macrophages during Type I infections.

STAT3 and STAT6 phosphorylation by *T. gondii* strains I and III versus lack of activation of these same transcription factors by Type II parasites may all serve the same end, namely persistence and transmission (Fig. 4). Type I parasites activating STAT3 via ROP16 may modulate pro-inflammatory cytokines, thereby evading immunity and establishing an infection.

By activating STAT6 and inducing arginase-1, Type I parasites limit arginine within host cells, possibly avoiding the effects of nitric oxide, whose synthesis depends on arginine. Because *Toxoplasma* is an arginine auxotroph, inducing arginase-1 likely also slows parasite replication, better enabling *T. gondii* to disseminate within its host. Our evidence suggests that deleting ROP16 increases replication of Type I parasites. However, arginase-1 promotes polyamine production, which the parasite requires as a source of amino acids. According to Jeroen Saeij of Massachusetts Institute of Technology, STAT6-dependent arginase-1 promotes parasite growth, speeding cycles of replication and host cell lysis.

For *Toxoplasma* Type II strains, failure to activate STAT3 and increased proinflammatory mediators may stimulate enough of an immune response to allow the host to survive. Absence of STAT6 phosphorylation could increase arginine and facilitate parasite replication. Alternatively, lack of arginase-1 could decrease polyamines, acting to limit parasite replication. What predominates in any particular host cell likely depends on cell type and stage of infection.

Recent phylogenomic studies indicate that ROP16 is a member of a rhoptry kinase family of up to 40 genes whose function is largely unexplored. With the work on ROP16 we have only scratched the surface, and it seems certain that

the years to come will bring deeper insights into how this intracellular protozoan employs its arsenal of rhoptry proteins to leverage survival in host cells.

Eric Y. Denkers is Professor and Barbara A. Butcher is a Senior Research Associate in the Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, N.Y.

Suggested Reading

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