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REVIEW ARTICLE

Intracellular Survival of *Toxoplasma gondii*: Success and Adaptation.

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ABSTRACT
T. gondii was described as the most successful parasite on earth because of its wide range of host agents, intracellular adaptations and its ability to maintain continuous survival for the life span of its host. Its complex movement, penetration and intracellular replication within the host cell are organized in such a way that it invades and evades immune cells. Formation of parasitoporous vacoule within the host cells and strong anti-oxidant system, are factors that add to its ability to maintain itself in a latent stage, evasion of immune cells attack as well as the effects of reactive oxygen species. In immunocompetent individuals, the infection is asymptomatic, and the parasite exists and persists in a slowly replicating bradyzoite stage in skeletal muscle, heart, brain, retina, and placental tissues. Reactivation of the rapidly replicating tachyzoite stage in settings of immune-depression results with severe consequences. This, therefore, prompts the need to understand certain mechanisms through which this organism succeeds and adapts the harsh condition of host cells during infection. The review further portrays the applicability of diagnostics and therapeutics to diagnose, treat and prevent infection with *T. gondii*.

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INTRODUCTION

T. gondii as an obligate intracellular parasite that must survive within a host cell for proper growth, replication, and survival. For this to be possible, the parasite converts the intracellular environment to fit its need for growth and development. The parasite induces expression of several anti-apoptotic pathways (Brunet *et al.,* 2008), which becomes necessary because the parasite cannot survive within a cell that undergoes apoptosis. For *T. gondii* to accomplish this, various mechanisms are involved. Immediately after infection with established parasitoporous vacuole (PV), the parasite modulates some transcription factors that will help to reorganize host cell at the transcription level. This is achieved through the secretion of its rophtry kinase and dense granules protein from the secretory organelles. These proteins were reported to keep certain transcription factors such as signal transducers and activator transcriptors (STATs) and NF-ϰB in control, which were reported to be specie specific (Du *et al.,* 2014). STAT3 was reported to be specifically activated to induce up regulation of the host cell miRNAs with anti-apoptotic activity (Cai *et al.,* 2013).

The parasite stylishly creates its own way to derive nourishment from the host and to evade host immune system as well as the oxidative stress within the host cell. *T. gondii* derives amino acids, cholesterol, choline and polyamines from host cells to support its growth (Coppens *et al.,* 2006). Furthermore, the parasite can derive nourishment by diffusion through the porous parasitophorous vacuole membrane (Laliberté and Carruthers, 2008) and these encourage rapid growth and replication of the tachyzoite intracellularly. In its effort to survive and evade the effect of oxidative stress in intracellular compartments of both hematopoietic and non-hematopoietic cells, the parasite is equipped with strong antioxidant system that detoxifies the reactive oxygen species (ROS) within the cells (Bosch *et al.,* 2015). As mentioned earlier, *T. gondii* is one of such organisms that survive intracellularly, establishing itself in a PV thereby escaping lyses through fusion with endolysosome. Its ability to maintain itself in its hosts in slowly dividing bradyzoite stage is a function of its inherent ability to modulate immune system, use its virulence factors in cell invasion and maintenance of strong antioxidant mechanism to combat cellular oxidative stress.

Summarily, the formation of a cystic stage in host tissue proved to be a challenging phenomenon in that: (a) the currently used drugs have no penetrative power to cross the modified cystic membrane to attack the bradyzoite in a parasitophorous vacuole; (b) within the cellular matrix, metabolic activities that result in the release of free radicals

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that in turn effectively clear the infectious agents are being overcome by the strong antioxidant activities of *T. gondii,* and (c) there is an expression of stage-specific molecules that protect the stage from the effect of the immune system. In this regard, therefore, the host cell penetration, survival mechanism and adaptation of *T. gondii* will be reviewed, highlighted and discussed.

INTRACELLULAR GROWTH, REPLICATION, AND SURVIVAL

Invasion of host cells by the rapidly dividing tachyzoite is a rapid and active process that occurs within seconds (Robert-Gangneux *et al*, 2012). The process involves the effort of both the secreted protein from the parasite and the actin-myosin based motility system between the parasite and the host's cell membrane. The secretory organelles (Figure 1), micronemes, rhoptries, and the dense granules, present a sequential system of protein secretion after a proper attachment to a host's cell membrane and initiation of the gliding movement. This is believed to be due to increase in cytosolic calcium through calcium dependent protein kinase (CDPK), a group of kinases that can be use as drug target against *T. gondii* (Blader, 2009; Muller, 2013; Abdullahi *et al*., 2019; 2023).

Initially a moving junction is formed between the apical end of the parasite and host's cell membrane. This leads to internalization of the parasite into the cell, carrying a special host's cell membrane-derived covering from its apex to its posterior end (Figure 1). The covering, thus form an oval shaped structure containing the parasite, known as the parasitoporous vacoule (PV). This event started by spread of an apical membrane antigen (AMA1) and secretion of RON2, RON4, RON5 on to the surface of the host cell membrane by the micronemes and the rhoptry neck (RON) respectively, helping in formation of the moving junction . This is followed by secretion of rhoptry proteins from the rhoptry bulb, including ROP2, ROP16, ROP18 and ROP5 both of which have been implicated in contributing to continuous parasite growth, maintaining its virulence and influencing interleukin secretion by the host cell (El Hajj, 2007). Another set of proteins that contribute to the maintenance of the PV are the dense granular proteins (GRA) also secreted by the tachyzoite immediately after penetration. These proteins especially GRA1, GRA2, GRA4 and GRA6 were described to be tools, functionally developed to enable communication between the parasite and the surrounding cytoplasm of the host cell in association with membrane tubules that ensure exchange of nutrients. The remaining GRA3 and GRA5 proteins are located on the parasitophourous vacoule membrane (PVM) (Dubey, 2011).

While in the PV, the tachyzoite continue to divide via endodyogeny. After several divisions, accumulation of daughter cells leads to rupture of the cell with subsequent release of the newly formed tachyzoites into the circulation with rapid infection of the neighboring cells.

EGRESS OF THE INTRACELLULAR TACHYZOITES

Egress is a stage that initiates an extracellular form of a cycle after the first infection of the host. It is often considered as the first step of the lytic cycle (Blader *et al.,* 2015) because it initiates the infection of new cells. Egress is triggered by several factors such as immune response and parasite driven factors. The immune system causes damage to the host cell by perforins due to $CD8^+$ T cell response. This activity lowers the level of intracellular K^+ and that triggers egress of the tachyzoites. The second factor that aids egress is an accumulation of intracellular abscisic acid (ABA) produced by the apicoplast that resulted due to intracellular replication. The high concentration of ABA signals the release of calcium from the intracellular store that activates calcium-dependent protein kinase 3 (CDPK3) which stimulates MIC secretion that facilitates egress of the tachyzoites out of the cells (McCoy *et al.,* 2012; Blader *et al.,* 2015). The released extracellular tachyzoites, freely circulate and establish a new infection cycle on new uninfected cells.

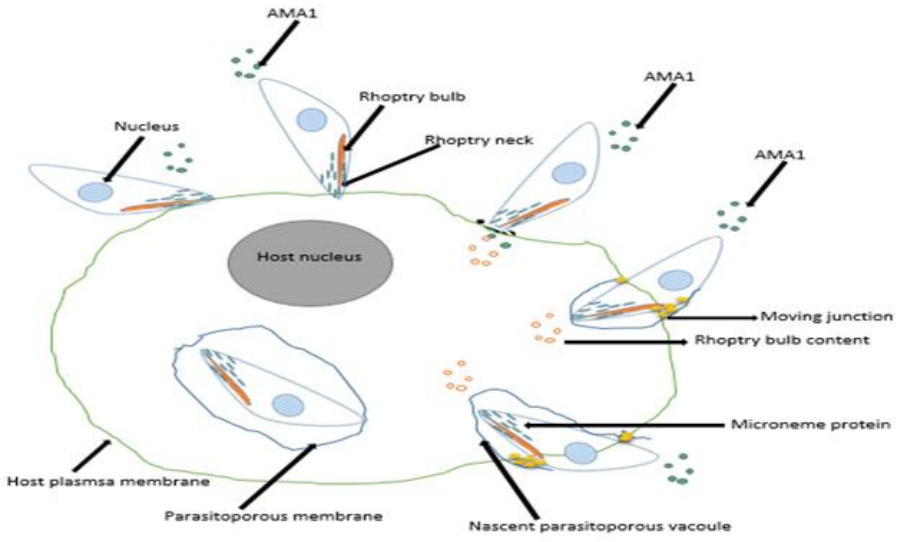

Figure. 1: A schematic diagram demonstrating tachyzoite invasion of the host cell

IMMUNE RESPONSE AGAINST *TOXOPLASMA GONDII*

Both innate and adaptive immune responses play a role in infection with *T. gondii*. This starts from the initial recognition of the parasite to the final localization of its latent slowly dividing bradyzoite stage in tissues such as brain, skeletal muscles, heart and the retina. In this scenario, acquired immunity is established for life and offer protection against further contact with the parasite otherwise reactivation of the latent cyst stage in settings of immunodeficiency state (Dupont, 2012). Immune cells such as the Dendritic cells (DC), macrophages and the lymphocytes are actively involved in cascade of events that lead to production of cytokines which contribute toward parasite control (Blader *et al.,* 2015).

Parasite recognition

Monocytes, neutrophyls, macrophages and DC, play an important role in parasite recognition through their membrane bound Toll-like receptors (TLR) via an adapter molecule myeloid differentiated factor 88 (MyD88) mechanism (Dupont, 2013) (Figure 2). These cells, first encounter parasite in lamina propria of the small intestine. The recognition and binding of *T. gondii* to TLR11 on DC result in secretion of IL-12. More so, TLR2 and TLR4 on both macrophage and Monocytes interact with glycosylphosphatidylinisitol (GPI) on the parasite to produce IL-12 and more particularly TNF-α which serves as a cofactor to IFN-γ to effect its anti-parasitic activity against *T. gondii* in microphages (Blader *et al.,* 2015).

Production of cytokines

During the infection, the natural killer cell (NK) contributes to the production of IFN-γ and also from $CD4^+$ and $CD8^+$ T-cells as its primary sources (Abou-Bacar *et al*., 2004). This is due to their activation by IL-12 produced by tachyzoite activated DC, macrophages and the Monocytes. However overproduction of IFN-γ from T-cells is being checked by IL-10 and IL-27 (Blader *et al.,* 2015). The overall IFN-γ produced via the innate and adaptive immune system is central to the control of tachyzoite within the hematopoietic and non-hematopoietic cells.

Parasite clearance and control

IFN-γ and TNF-α mediate the steps toward parasite clearance in cells in both hematopoietic and non-hematopoietic cells (Fibroblasts, epithelial, endothelial, glial cells) through cascade of events (Yap, 1999; Filisetti, 2004). IFN-γ, through activation of nuclear transcription factor (STAT1), causes up-regulation of gene responsible for production of nitric oxide (NO) and reactive oxygen species (ROS) that play a role in control of intracellular organisms. In addition, TNF-α activates nuclear factor kB (NFkB) which stimulates inducible nitric oxide synthetase (iNOS) that results in NO production. The NO produced through both IFN-γ and TNF-α acts on the tachyzoite and can have several effects on it via non-oxidizing mechanism. Depending on the strain causing the infection, NO can stimulate production of heat shock protein 70 (HSP70) that allows the parasite to proliferate during infection with virulent strain or stimulate inter conversion to bradyzoite stage in case of avirulent strain. However, NO can also effectively kill the tachyzoite via its toxic effect. Furthermore, IFN-γ was also reported to activate immunity related GTPhase and guanylate binding protein (GBP) that have specialized role in developing resistance to microbes. These defense proteins are thought to have direct effect on the PV, causing rupture of the membrane, suppressing parasite growth and its subsequent clearance (Taylor, 2006; Blader *et al.,* 2015) within both hematopoetic and non-hematopoetic cells. Moreover, two amino acids essential for *T. gondii* growth are depleted thereby restricting parasite growth. IFN-γ induces expression of indoleamine2,3-dioxygenase (IDO), which stimulates secretion of iNOS. These two enzymes cause depletion of tryptophan and argentine respectively, and this restrict the growth of *T. gondii* within infected cell (Figure 2).

Resistance immunity is conferred by activities of both $CD4^+$ and $CD8^+$ T-helper cells via production of cytokines. $CD4^+$ exerts its effect by production of IFN-γ and IL-2. The effect of $CD4^+$ cells is via two mechanisms. It divides in to two subpopulations following activation to Th1 which produces IFN-γ and IL-2 to serve as pro-inflammatory cytokines. The IL-2 activates NK cells so that resistance to infection is established at early stage of the infection. In an effort to checkmate the activities of the pro-inflammatory cytokines to avoid immunopathology on the host, the Th2 subpopulation of $CD4^+$ produces anti-inflammatory cytokines such as IL-4, IL-5, IL-6 and IL-10. They inactivate anti-parasitic activities of macrophages, DC, T-cells and NK cells thereby promoting parasite growth.

Humoral immune response in toxoplasmosis occurs and aids in serological diagnosis of the infection. The immunoglobulins (Ig) so produce due to exposure to the parasite play important role in opsoniation and subsequent phagocytosis of the parasite. These immunoglobulins include the IgM, IgG, IgA and IgE.

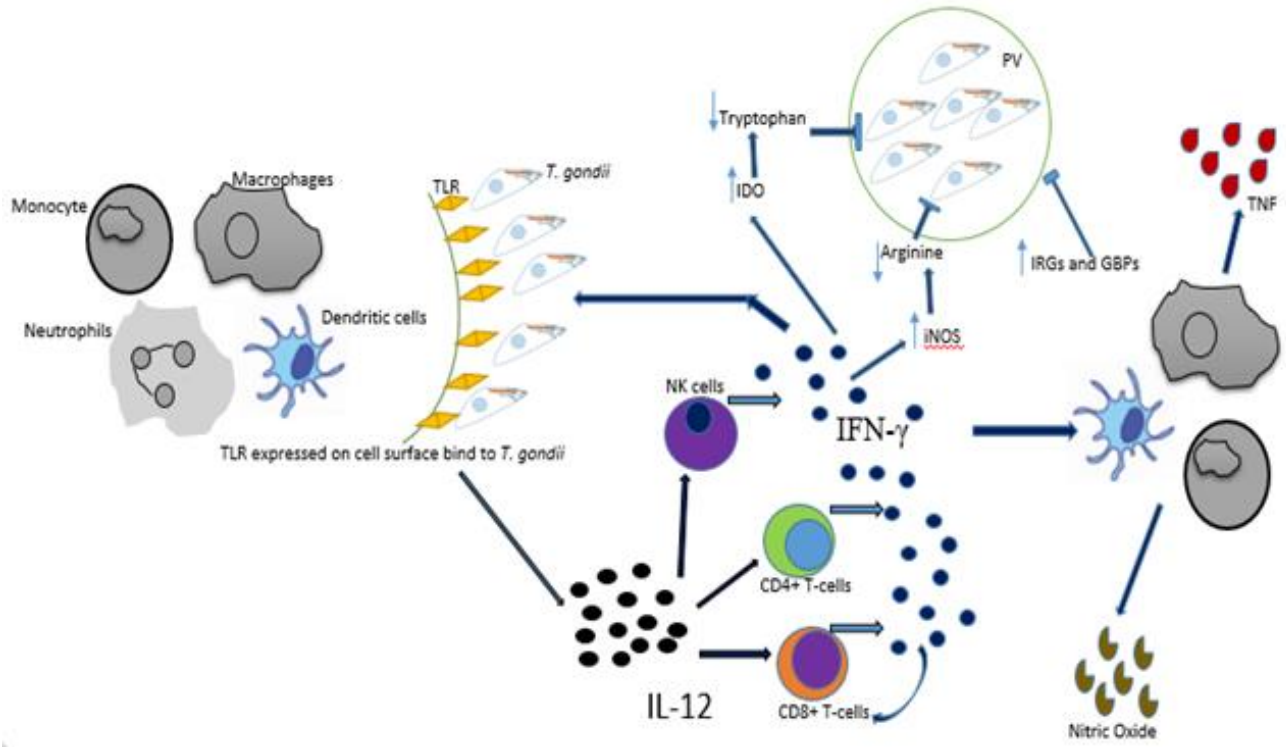

Figure.2: A schematic diagram demonstrating parasite recognition, recruitment of phagocytic cells and secretion of cytokine for innate and adaptive immune response respectively and of IFN-γ on parasite growth through activation of iNOS, IDO, IRGs and GBPs.

OXIDATIVE STRESS AND CONTROL BY ANTIOXIDANT SYSTEM OF *T. gondii*

Oxidative stress results from an imbalance between the oxidative states dictated by intracellular accumulation of reactive oxygen species (ROS) due to aerobic metabolic processes and antioxidant systems of the cell. In oxidative stress, if the effects of the reactive oxygen species outweigh the effect of anti-oxidants intracellularly, it results in irreversible lipid peroxidation, oxidative damage of the DNA and protein or positively involves in regulation of intracellular signal transduction (Kwok, 2004). The ROS are produced in a cell as byproducts of metabolisms in the cytosol, mitochondria and in the peroxisomes of all aerobic cells with increase in the rate of production in settings of infection by some parasites (Pino *et al.* 2007; Sautel *et al.* 2009; Bosch *et al.* 2015; Bahrami *et al.* 2016). They include superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxide radical ($OH^{\bullet}$). More importantly however, the cells involved in innate and adaptive immune response to microbial infections such as the macrophage and the neutrophils use the ROS through oxidative burst to kill the invading infectious agent (Kwok *et al.* 2004). In order to limit the harmful effect of the ROS or the oxidative stress within the cell against the infectious agents, the defensive gene in microorganisms are activated through alteration of transcription factors. This will lead to increase in expression of enzymes and molecules involved in protecting the organism from the deleterious effects of ROS (Sautel *et al.* 2009; Bosch *et al.* 2015).

Antioxidant system in T. gondii

T. gondii is well equipped with defense mechanisms which enable it to survive intracellularly within aerobic compartment (Pino, 2007). In its typical characteristics, this protozoan has a broad range of intermediate hosts (Dubey, 2011), invades within the hosts, variety of nucleated cells including immune cells (Sautel *et al.* 2009: Bosch *et al.* 2015) and shows more affinity to brain cells, the skeletal muscle cells, the cardiac muscle cells and the retina (Dubey, 2011). In its effort to survive and evade the effect of oxidative stress within the intracellular compartments of both hematopoetic and non-heamatopoetic cells, the parasite is equipped with a strong antioxidant system that detoxify the ROS within the cells . In this regard therefore, antioxidant system in *T. gondii* can be a drug target pathway (Bosch *et al.* 2015) to curb the challenging need of a potent drug against the endemic pathogen globally.

Like other apicomplexan parasites such as *Plasmodium spp, T. gondii* has variety of antioxidant systems but differ in its possession of cytosolic catalase (Kwok *et al.* 2004; Pino *et al.* 2007; Bosch *et al.* 2015). In *T. gondii*, there are two major antioxidant defense systems: the enzymatic antioxidants and the classical glutathione/thioredoxine system (Turens, 2004; Bosch *et al.* 2015). While some are found within the cytosol (Catalase), others are purely mitochondrial enzymes.

Enzymatic antioxidants

Catalase

This antioxidant enzyme is essential in defense against oxidative stress in cell infected with *T. gondii.* The parasite contains a gene coding for the enzyme which is present in the cytosol of all the parasite stages and has a high substrate turnover (Kwok *et al.*, 2004; *Bosch et al.*, 2015). It detoxifies H_2O_2 in to water (H_2O) and oxygen molecule (O_2). Furthermore, catalase has been considered and proved to be a virulent factor with important roles in aiding parasite invasion and partakes in replication of the parasite while in PV (Kwok *et al.* 2004). It was shown that the strain containing mutant gene of the parasite becomes more susceptible when exposed to H_2O_2, unable to replicate and displayed low virulence in infected mice (Kwok *et al.*, 2004). More so, for efficiency in its activity the enzyme catalase, requires a reducing agent NADPH and Fe+ to serve as cofactors (*Bosch et al.* 2015).

Superoxide dismutase (SOD)

These are also essential for defense by *T. gondii* within the host cell. Three SODs are present: 2 are located in the mitochondria; SOD2 and SOD3 while the SOD 1 is located in the cytosol (Kwok *et al.* 2004; Pino *et al.* 2007; Bosch *et al.* 2015). The three enzymes are both reported to catalyse the conversion of the $O_2^{\bullet-}$ in to O_2 and H_2O_2 . Despite closed similarities in function and location, they are not expressed in all parasitic stages of the parasite. The SODB1 is seen in the cytosol of both tachyzoite and bradyzoite, SOD3 is seen only in the sporulated oocyst and SOD2 is seen in both stages (Kwok *et al.* 2004). The cytosolic SOD1 utilizes iron as a cofactor (FeSOD1) and this occurs in other protozoans such as *Plasmodium, trypanosome* and *leishmania* that is different from that obtained in mammal (Turrens, 2004). The genes coding for the three SODs (*TgSOD1, TgSOD2, TgSOD3*) are contained in *T. gondii* genome (Kwok, 2004; Bosch, 2015).

Peroxiredoxins (Prx)

These are also found in *T. gondii* and play an important role in detoxification of H_2O_2 to H_2O and O_2. Three of the Prx were shown to differ in location within the parasite and shown to have differences in metabolism because of their cystein residue base on which they were classified as 1-cys and 2-cys Prx (Bosch *et al.* 2015; Mercel *et al.* 2020). They are named as Prx1, Prx2 and Prx3 based on cysteine residue. Those with 2 cysteine residues are Prx1 and Prx3 while Prx2 contain only 1 cysteine residue. However, localization within the parasite differs. Prx1 and Prx2 are located in the cytoplasm whereas Prx3 are found in the mitochondrion. More so, Prx1 and Prx3 are found in all the developmental stages of the parasite whereas Prx2 are found only in tachyzoites and bradyzoite.The genes (*TgPrx1, TgPrx2 and TgPrx3*) coding for the Prx are located in *T. gondii* genome (Kwok *et al.* 2004; Bosch *et al.* 2015).

Glutathione and thioredoxine Systems

Gluthathion(GSH/GSSG) and Thioredoxins (Trx) are classical antioxidant systems that also found to be important in defense against oxidative stress by *T. gondii.* These systems are believed to be involved in antioxidant activities in apicomplexan parasites. They act as thiol or disulfide pairs taking part in many biological processes of the cell. In gluthathione system, the reduced glutathione (GSH) converts H_2O_2 to H_2O in the presence of GSH peroxidase and in the process the GSH becomes oxidized glutathione disulfide (GSSG). The GSSG is also essential for the recycling of the reduced GSH, it is then converted by the NADPH dependent GSH reductase (Turrens, 2004).

CONCLUSION

Strong defense mechanisms in *T. gondii* greatly influenced its ability to perpetuate itself from initial replication at the point of infection to latent survival for the life span of its host. Its opportunistic nature favors reactivation in settings of immunosupression. It thus successfully adapted itself to continuous survival among its hosts. Immune mediated activities against *T. gondii* and anti-oxidant system are subject of concern in search of new drugs, drug repurposing, application of herbal remedy that can be used against the current regiment, have poor placental penetration, adverse effect on the host and poorly effective on the parasite.

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