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Review Article

Host Defence and *Toxoplasma gondii*: A Mini Review

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Abstract

Toxoplasma gondii is a parasite intracellular in nature that affects pregnant animals and humans especially immunocompromised persons. Infected hosts show a robust inherent response followed by an adaptive response to contain the parasitic infection. More focus has recently been placed on innate lymphocytes, inflammatory monocytes, and inherent immunological processes. The operation of MyD88 independent pathways is necessary for these processes to function. Despite the host's immense immune reaction, the parasite has evolved to overcome the host's immune response either by down-regulation of signaling pathways or altering host gene expression. Also, the parasite continues to thrive as a lifelong infection in the infected individuals and may revive to its lethal form in stress conditions.

Keywords: Toxoplasma gondii, innate immunity, adaptive immunity, immune evasion

1. Introduction

Food-borne infections are one of the major issues around the globe and toxoplasmosis is among the leading pathogens(Rani and Pradhan 2021). This pathogen can be transmitted through the oral route via cyst ingestion from improperly cooked meat, and raw fruits and from mother to fetus through placental route. It is commonly thought that 1/3rd of the population on earth has a chronic infection but disease symptoms are manifested in immunocompromised individuals only(Almeria et al. 2021). However, a majority of the parasite population is eliminated in immunocompetent individuals due to aggressive response by the Innate and acquired immune system (Sasai, Pradipta, and Yamamoto 2018). Although a sufficient immune response is generated in toxoplasma infection, the parasite has developed tactics to mimic this response and cause prolonged infection (Lima and Lodoen 2019). The initial response in toxoplasma infection is provided by innate immunity which has been broadly explored. Apart from the crucial role of Innate response acquired immunity is also necessary for host survival. CD8+ and CD4+ T cell-mediated immunity is essential to keep parasites in chronic infection and to inhibit the reactivation of dormant parasites (Khan, Hwang, and Moretto 2019). This review aims at the discussion about the interaction of parasite with innate and acquired immune systems, immune evasion, and persistence of *Toxoplasma gondii* in the host.

2. Interaction with Innate Immune System

The inherent immune response shows an initial response against invading pathogens(Yarovinsky 2014). This immune response is moderated by cell surface receptors such as NLRs (Nod-like receptors) and TLRs (Toll-like receptors) (Sasai and Yamamoto 2019). An adapter protein of TLRs MyD88 has a crucial role in inhibiting parasitic infection and it has been accepted through different studies(Scanga et al. 2002). TLR11 and TLR12 among Toll-Like Receptors are activated by parasitic protein profiling and are involved in host protection, while TLR2, TLR4, and TLR7 have a supportive role in parasitic infection (Andrade et al. 2013, Mercer et al. 2020). Although MyD88 and TLRs mediated immune response is important for innate immunity against *Toxoplasma gondii* infection but MyD88 pathway produces protection against *T. gondii* through IFNγ production (Snyder et al. 2021). Toxoplasma gondi infection and TLR response are well studied in mice as compared to the human species which lacks TLR-11 and TLR-12 receptors (Gazzinelli et al. 2014). In human infection alarmin released from infected cells is detected by dendritic cells (DCs) and in response CCL2 is secreted which causes enhanced monocyte response (Safronova et al. 2019). Macrophages and Dendritic cells are the important agents that detect microbes mediated by TLRs and initiate biological mechanisms such as antigen detection, cytokine production, and germicidal functions in these cells(Poncet, Blanchard, and Marion 2019). Macrophages, DCs, and Neutrophils produce cytokine and IL-12 which have the primary role of host defense against *T. gondii* invasion (Mukhopadhyay, Arranz-Solís, and Saeij 2020). Mouse experiments showed conventional DC1 was a major source of IL-12 in *T. gondii* invasion (Aliberti et al. 2000). However, in humans, conventional DC1 is non-stimulant and IL-12 production is mainly mediated by conventional DC2 cells(Tosh et al. 2016). In addition to Dendritic Cells, macrophages also show supportive function in *T. gondii* infection. Latest studies show that murine macrophages synthesize inflammasomes which may act as a secondary inhibition mechanism against *T. gondii* invasion (López-Yglesias et al. 2019). This process includes caspase-intervened modification of IL1 and IL12 which causes elimination of pathogen invaded cells. Despite the function of macrophages in the inhibition of *Toxoplasma gondii* multiplication, this pathogen can target these cells and change their functional ability by inhibiting IRGs (Immune-Related GTPases) to the parasitophorous vacuole membrane. This activity is carried out by rhoptry proteins (RP5, RP17, and RP18) secreted during the formation of moving junction by the pathogen (Park and Hunter 2020) and escapes the action of IFNγ an IRG-induced mechanism for parasitic elimination (Frickel and Hunter 2021). The most prominent result of T.gondii infection in immunocompromised ones is encephalitis(Marra 2018). Interestingly, the inherent immune system has an important part in limiting pathogen replication in the brain through inflammatory monocytes (Schneider et al. 2019). These cells physically develop Gr1 and Ly6C when stimulated by CCR2 and their protective efficiency is dependent on the ability to secrete Nitric oxide, IL1, IL6, and TNFα(Biswas et al. 2015). Moreover, inflammatory monocytes change to monocyte-derived macrophage and monocyte-derived dendritic cells that have an important role in limiting *T.gondi*i-induced encephalitis(Matta et al. 2021). Microglia cells which are part of the innate immune system have a role in antigen detection and parasite elimination from nervous tissues in toxoplasmosis(Batista et al. 2020). However, further research is needed to study the involvement of microglial cells in *T.gondii*-induced encephalitis. IFNγ produced by Natural Killer cells has an important part in immunity against acute Toxoplasma gondii invasion (Mahmoudzadeh et al. 2021). Although Natural Killer cells show resistance in experiments against Toxoplasma gondii outside the body of the

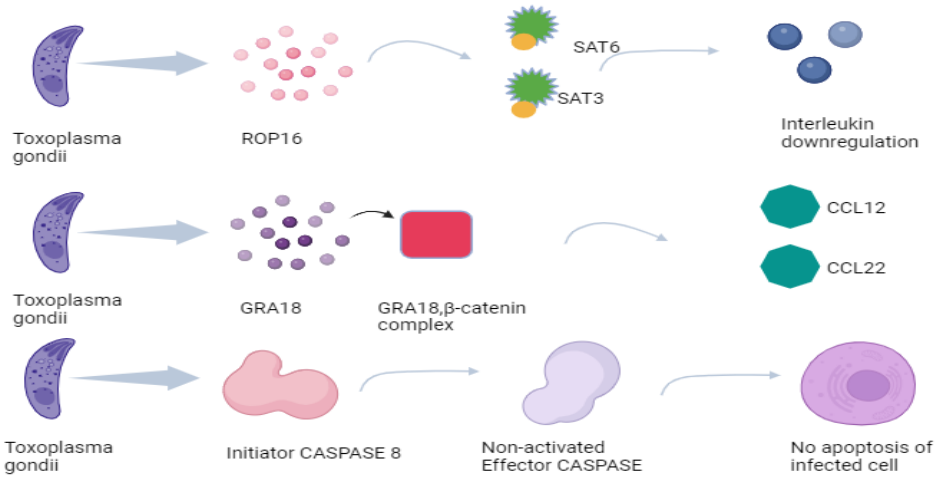

Figure 1: Immune evasion mechanisms of T. gondii.

organism but its protective efficacy inside the organism is still ambiguous(Ivanova, Fatima, and Gigley 2016). Overall, it has been concluded that the defensive role of NK cells during Toxoplasmosis is dependent on their capability to yield IFNγ (Sasai and Yamamoto 2019). Curiously, during T.gondii infection an altered Natural Killer cells population is produced which hinders CD8+ T cells functioning (Ivanova et al. 2020). In contrast to acute infection in which during the absence of CD4+ T cells, Natural Killer cells have an important part in the immunity against *T. gondii* (Combe et al. 2005). Furthermore, it has also been seen that NK cells increase CD8+ T cell-mediated immunity during the early stages of T.gondii invasion (Guan et al. 2007). Besides Natural Killer cells, (Innate Lymphoid cells) are alternative equivalents that inhibit pathogens by cytokines secretion (Panda and Colonna 2019). Both Natural Killer cells and ILCs function against T.gondii infection and excitingly during parasitic infection Natural Killer cells convert to ILC1-like cells (Park et al. 2019). In general, the immunity provided by Dendritic Cells, ILCs, and inflammatory monocytes plays an important part in parasite eradication during acute infection of T.gondii.

3. Adaptive Immunity

Acquired immunity in T.gondii is mainly provided by CD8+ cells aided by CD4++ cells in long-standing infections (Khan, Hwang, and Moretto 2019).

a. Role of CD4+ T Cells

CD4+ T cells play a pivotal role in limiting *T. gondii* infection among immunocompromised patients like HIV patients. In an advanced study on mice without CD8+ T cell-mediated immunity, CD4+ T cell immunity plays a main controlling agent against *T. gondii (Tussiwand et al. 2020)*.t is understood that NK cells show an immune response against *T. gondii* infection even in the absences of helper T cells but it cannot be maintained for a long time sa(Combe et al. 2005). CD-4+ T cells are essential for the sustenance of CD8+ immune response (Hwang et al. 2016). These studies show that persistent infection leads to BLIMP1-induced CD4+ depletion causing CD8+ functional impairment. Cancellation of BLIMP1 in CD4+ T cells restored their ability to induce CD8+ T cell functionality which is necessary for limiting parasite revival. Furthermore, cytokines that regulate the TFH (T Follicular Helper cells) subset also control antibody reactions against Toxoplasmosis(Olatunde, Hale, and Lamb 2021). Even though the response of B cells to *T. gondii*

invasion is not completely explained, studies show that mice lacking B cells are more susceptible to Toxoplasmosis (Kang, Remington, and Suzuki 2000). Treg (T regulatory cells) which are another subset of CD-4+ T cells have a protective role against *T. gondii* but still, it is not completely defined. It has been recognized that during deadly *T. gondii* infection, these cells develop an effector behavior (Oldenhove et al. 2009). It's interesting to note that regulatory T cells by expressing Tbox (transcription factor) contribute a vital part in regulating the immunopathology brought on by *T. gondii* infection (Warunek et al. 2021). Similar to this, a decline in Treg counts in the pregnant host has been linked to fetal abortion caused by *T. gondii* infection (Gao et al. 2021). In general, further research is needed to determine the specific function and interaction of CD4+ T cell subsets in acute and chronic Toxoplasmosis.

b. Role of CD8+ T Cells

CD8+ T cells have a pivotal role in the defense against parasites of intracellular nature including *T. gondii* (Khan, Hwang, and Moretto 2019). IFNγ, a cytokine that is produced by CD4+, CD8+, and Natural Killer cells has a crucial role in assisting host immunity against *Toxoplasma gondii*. Along with the production of IFNγ, CD8+ cells have the ability to lyse the infected cells (Khan, Hwang, and Moretto 2019). This cytolytic activity plays a critical role in limiting chronic toxoplasmosis (Lutshumba et al. 2020). It is considered that IFNγ produced by the host cells plays the main role in limiting the acute infection of T.gondii, whereas cytolytic activity of CD8+ T cells has a vital role in limiting the persistent *T. gondii* infection (Suzuki 2020). The presentation of neuronal antigen has a critical role in provoking CD8+ T cells mediated response for limiting persistent brain infection of T.gondii (Salvioni et al. 2019). Even though an elevated CD8+ T cell immune response is produced in infection, the increased expression of PD-1 checkpoint inhibitors limits the functioning of these cells (Bhadra et al. 2011). The basic mechanism behind this effect is a reduction in memory response development (Bhadra, Gigley, and Khan 2012), which limits the host response in reducing chronic toxoplasmosis. Even though CD8+ T cell subsets that react to checkpoint inhibitor inhibition during viral infections and malignancies have been observed (Collier et al. 2021), but similar response is not observed in the case of toxoplasmosis. The innate immune response is not only crucial for limiting parasite replication in the initial stages but also moderates the acquired immune response. However, despite that, the long-lived response is dependent on the acquired immune response. One important aspect is that due to the depletion of CD8+ T cells, a moderate revival of parasites may take place in immune-competent individuals having chronic infection. In that scene, recruiting functional CD-8 T cells may limit the spread of parasitic revival.

4. Immune Evasion

Toxoplasma gondi has evolved mechanisms to evade the host immune response by controlling the transcription process of host genes and controlling the activity of signaling mechanisms that lead to altered host signaling pathways, arresting infected cell apoptosis and preventing intracellular death as indicated in Figure 2 (Bedard and Krause 2007, Friedrich et al. 2017, Saeij et al. 2007).

Production of cytokines by signaling pathways is an efficient means to control the pathogens but *T. gondii* alters these pathways by activation of STAT 3 and STAT 6 leading to down-regulation of IL12(Butcher et al. 2005). IFN-γ mediated immune response provides effective control of *T. gondii* infection but the parasite stops the increased activity of all the 127 genes that were upregulated by treatment with IFN-γ (Kim, Fouts, and Boothroyd 2007). GRA-18 a granular protein secreted by the apical secretory organelles binds with β-catenin destruction complex leading to its nuclear translocation. GRA-18 induced β-catenin gene expression produces CCL17 and CCL22 which have anti-inflammatory activity thus

countering the immune reaction of the host (He et al. 2018).

Programmed cell death (apoptosis) is an important pathway to get rid of pathogens but these organisms have adapted ways to counter this process to ensure their survival in the host species (Friedrich et al. 2017). T.gondi inhibits the release of Cytochrome C which n turn reduces the cleavage of caspase 9 and 3 leading to the inhibition of apoptosis. Also, the parasite induces an anti-apoptotic factor Mcl1 which further inhibits apoptosis(Goebel, Gross, and Lüder 2001, Goebel, Lüder, and Gross 1999). T.gondi interferes with initiator caspase 8 leading to low levels of pro-caspases 8 which in turn affects effector caspases and inhibits cell death by apoptosis (Vutova et al. 2007).

ROS production also provides protection against pathogens but in the case of *T. gondii* infection ROS production in infected cells is equal to those of normal cells as the parasite targets NADPH oxidase which is necessary for increased ROS production in infected cells (Bedard and Krause 2007, Shrestha et al. 2006).

5. Latency and Persistence

Toxoplasma gondi life cycle involves the transfer of infection from the final host (felines) to the intermediate host (warm-blooded animals), where asexual reproduction takes place (Dubey 2020). This parasite, which affects 1/3rd of the world population, has a broad spectrum of intermediate hosts, including humans.

Transfer of infection from final hosts to intermediate host occurs through oocyst shed by the prior host. These parasites enter the body through an intermediate host, where they mature into the asexual tachyzoite stage, which multiplies and spreads throughout the body to cause toxoplasmosis (Montoya and Liesenfeld 2004). In immune-competent people, the immune system may easily limit this acute infection stage; however, in immunocompromised people, the illness can have serious side effects including encephalitis (McAuley 2014).

However, in immunocompetent individuals, these tachyzoites are not cleared from the body of the infected individual rather they convert into a bradyzoite form which slowly develops within tissues of the muscular and nervous systems (Remington and Cavanaugh 1965). This prolonged form of infection may persist within the individual for the rest of his life and may revive to acute form leading to severe pathology in case of immune suppression (Rougier, Montoya, and Peyron 2017).

The transformation from the tachyzoite phase into the bradyzoite phase of the parasite occurs due to stress factors caused by the immune system of the host (Bohne, Heesemann, and Gross 1994, Radke et al. 2006). Different changes occur when the parasite form changes from tachyzoite to bradyzoite stage such as thickening of the cyst wall (Ferguson and Hutchison 1987, Lemgruber et al. 2011), metabolic shift to anaerobic glycolysis for energy requirements (Denton et al. 1996, Shukla et al. 2018) and massive buildup of starch granules in cytoplasmic space (Dubey, Lindsay, and Speer 1998). The transition between the tachyzoite and bradyzoite stage occurs through changes in gene expression along with epigenetic changes in histone protein also plays a part (Bougdour et al. 2009, Kim 2018). ApiAP2 transcription factor in toxoplasma plays an important role in tachyzoite-bradyzoite transformation. Notably, the discovery of the BFD1 transcription factor seems to play a key role in bradyzoite formation (Waldman et al. 2020).

Parasites in bradyzoite form multiply at a slow rate to escape the immune system of the host but this form also converts to lytic (tachyzoite) form on the arrival of favorable conditions such as a decrease in the host immunity due to stress. The increase of the host's stress decreases stress on the parasite and bradyzoites again change to tachyzoites and another acute phase begins after a

chronic infection and this cycle continues till the death of an individual.

Interestingly some research approaches are under consideration that involve either blocking of parasite transcription or translation programs which leads to inhibition of tachyzoite-bradyzoite interplay or epigenetic inhibition of bradyzoite gene expression by acetylation of histone proteins(Bougdour et al. 2009, Maubon et al. 2010, Naguleswaran et al. 2010).

BFD1 gene knockout completely inhibits the ability of the parasite to change into the bradyzoite stage (Waldman et al. 2020). In addition to these several other approaches including metabolic alterations, disruption of cyst wall, and genetic engineering-induced mutations in bradyzoites are being explored (Buchholz et al. 2011, Sidik et al. 2014, Nolan et al. 2018). Bradyzoites have a pivotal role in the persistent infection of toxoplasma and new treatment approaches are essentially required to contain this global issue.

Conflict of Interest

The authors declare that they have no competing interests.

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Consent Forms

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Authors Contribution

KN conceptualized the study and wrote the final manuscript, SH and MM critically reviewed and edited the manuscript, AN critically reviewed the manuscript and made editing and language improvements. MN and RB helped in the analysis and figures, SUD and MAS were responsible for supervision, critical review and finalized the manuscript writing.

Data Availability

Data are available upon reasonable request from the corresponding author.

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