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Mechanisms of Escape from Host Immune Defenses by Apicomplexan Parasites

Review Article by Reannan Barlow

Overview:

A successful pathogen manipulates its host from the moment infection occurs. Apicomplexan parasites are no different. *Plasmodium* spp., the causative agents of malaria, evade the human immune system so well that they kill hundreds of thousands of people per year. *Toxoplasma*, though not particularly dangerous to a healthy adult, is a widespread pathogen due to how easily it spreads between host and how it modulates host functions (27). This review discusses how *Toxoplasma* and *Plasmodium* avoid clearance by host defenses, highlighting their similarities and differences.

Background:

Malaria:

Malaria is an arthropod-borne disease resulting from an infection with a protozoan parasite of the genus *Plasmodium* belonging to the phylum Apicomplexa (14). This parasite is arguably, one of the deadliest parasites across the globe. According to the World Health Organization, there were roughly 228 million cases of malaria worldwide and 405,000 deaths in 2018 (10). Most of these cases were in sub-Saharan Africa, infecting mainly children (14). Two hosts are vital for the lifecycle of *Plasmodium*, the mosquito vector, and a vertebrate host (9). In this review, the vertebrate host of focus will be humans.

In humans, the parasite will undergo two distinct stages. The first is called the liver stage; during this period, individuals are asymptomatic. The second stage is the blood stage, during which people experience symptoms (fever, chills, nausea, etc.) (17). The lifecycle of malaria begins when a malaria-infected female mosquito takes a blood meal from a human. During the blood meal, thousands of sporozoites are deposited into the skin of the individual. The sporozoites travel to the liver, where they mature into schizonts. The schizonts eventually rupture, releasing merozoites into the bloodstream. The merozoites infect red blood cells. In the red blood cell, the parasite will differentiate into gametocytes or mature trophozoites. The mature trophozoites develop into schizonts, which will all be released when the red blood cell ruptures. The gametocytes can then be taken up by another mosquito during a blood meal, and the schizonts will infect more red blood cells, continuing the cycle (7).

Toxoplasmosis:

Toxoplasmosis is a disease due to infection with another protozoan parasite in the phylum Apicomplexa called *Toxoplasma gondii*. Infections with *T. gondii* in a healthy adult are usually mild; however, there are serious complications that result from infection in utero or an immunocompromised individual (5). Due to this parasite's relevance in veterinary and medical fields, it is one of the most well-studied parasites. A standard route of human infection with *T. gondii* is via domestic cats, the definitive host (33). In intermediate hosts, like humans, *T. gondii* undergoes two phases. In the first phase, Tachyzoites multiply quickly by repeated endodyogeny in various host cells. In the second phase of development, some tachyzoites convert to bradyzoites that form tissue cysts. In the cysts, bradyzoites replicate slowly by endodyogeny. These cysts have a high affinity for the central nervous system, eyes, skeletal muscle, and cardiac muscle (31). The tissue cysts formed are infectious. An individual can get toxoplasmosis via horizontal transmission due to the ingestion of oocysts from the environment or eating undercooked meat containing tissue cysts, or by vertical transfer across the placenta. Definitive hosts can infect intermediate hosts and vice versa (31).

The success of *Toxoplasma gondii* stems from how easily transmission between the definitive and intermediate hosts occurs. This parasite can modulate the host cell to develop a chronic infection while evading the immune system and anti-toxoplasmosis drugs. There is a delicate balance between stimulating and avoiding the host's immune system to induce chronic toxoplasmosis (27).

Discussion:

MALARIA PRE-ERYTHROCYTIC STAGE IMMUNE EVASION

1. Outsmarting the Mechanical Barrier

The skin has a multitude of immune functions. It acts as a barrier, and when a pathogen crosses the epithelium, the complement system is activated (3). For the *Plasmodium* parasite to successfully establish infection, it must evade this first defense system. The mosquito vector assists with this process; when the mosquito bites, it injects vasodilators, anticoagulants, and immunomodulators from its salivary glands. This promotes the survival and injection of the sporozoites (35).

Another innate immune defense present in the skin are phagocytes; these are cells that can phagocytose or ingest invading pathogens. The freshly injected sporozoites must avoid these cells to make it to the liver. They do this by their ability to migrate. One-way sporozoites migrate through the host dermis is via cell traversal. A protein called sporozoite microneme protein essential for cell traversal (SPECT) allows for the penetration of host cells for cell traversal. It was found that sporozoites deficient in SPECT were impaired in the skin and eradicated by phagocytes. Therefore, SPECT is one protein necessary for the parasite to evade the skin's immune functions and successfully travel to the liver to continue its lifecycle (14).

2. Silencing Kupffer Cells

Plasmodium uses cell traversal to travel through Kupffer cells (KCs) as well. Kupffer cells are macrophages in the liver that help prevent pathogens from invading hepatocytes. KCs prevent infection through various mechanisms, a few that malaria takes advantage of are the release of pro-inflammatory cytokines and reactive oxygen species. It was found that the circumsporozoite protein (CSP) binds to low-density lipoprotein receptor-related protein (LRP-1) and proteoglycans on the KCs, which prevents the formation of reactive oxygen species due to an increase in cAMP/EPAC (34,35). This mechanism disarms the KCs, allowing for the spread of the *Plasmodium* parasite.

Another way the KCs protects hepatocytes is by releasing inflammatory cytokines when a pathogen invades. However, when sporozoites encounter KCs, they downregulate the release of inflammatory cytokines like TNF- α , IL-6, and MCP-1 while upregulating the release of IL-10, an anti-inflammatory cytokine (19,35). The infection of sporozoites decreases the expression of MHC I on Kupffer cells and causes KCs death via apoptosis (19). How the *Plasmodium* parasite affects the KCs is illustrated in Figure 1.

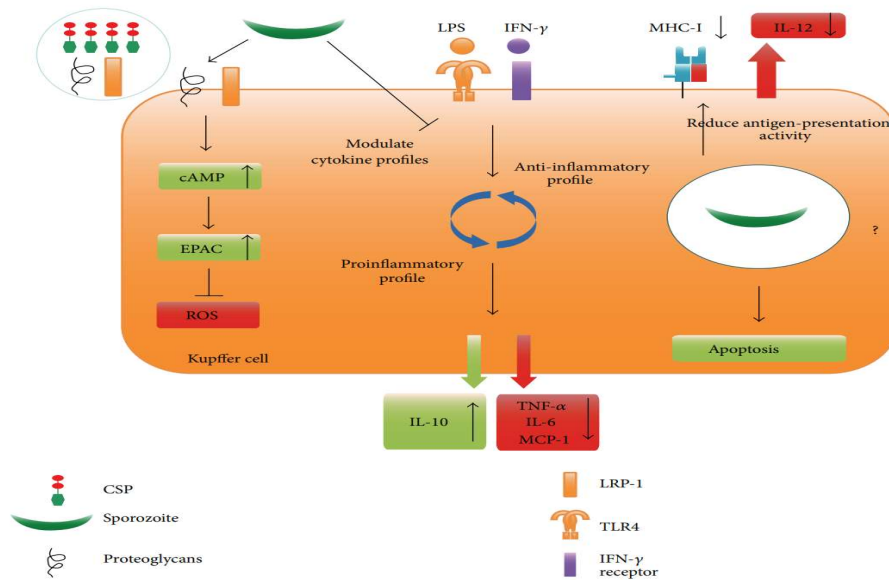


Figure 1: Sporozoites inhibit the function of KCs. Binding of the sporozoite CSP to proteoglycans and LRP-1 on the KC causes an increase in cAMP/EPAC levels, ultimately preventing the formation of reactive oxygen species (left). Sporozoites also cause a decrease in the release of inflammatory cytokines and an increase in anti-inflammatory cytokines and induce KC apoptosis (right) (19).

3. Controlling Hepatocytes

Once in the liver, the *Plasmodium* parasite must invade hepatocytes and mature into exoerythrocytic forms (EEFs). Sporozoites travel through many hepatocytes before they reach the final liver cell, in which they will differentiate into merozoites (23,35). This parasite has refined a few ways to repress the function of hepatocytes while preventing cell death. One-way *Plasmodium* does this is by transporting CSP into the cytoplasm of the hepatocyte. Once CSP is in the cytoplasm, it binds ribosomes overall, inhibiting host cell protein synthesis (11,29,35). Also, the release of CSP into the cytoplasm results in a suppression of nuclear factor- κ B (NF κ B), which is an important transcription factor centrally involved in stimulating inflammation (29,35).

Once the sporozoite differentiates into a merozoite within the hepatocyte, it must successfully leave the cell to proceed with its lifecycle in the blood. Again, the *Plasmodium* parasite must evade the host's immune system to do so. During the process of exiting the hepatocyte, the merozoite will be vulnerable to host immune cells like KCs and dendritic cells (DCs). To bypass the KCs and DCs, merozoites will exit the hepatocytes via merozoites, which are packets of merozoites coated in host cell membranes (30). Traveling in a vessel made of host cell material allows the parasite to exit the hepatocyte undetected (8,19). During the budding process, the hepatocyte infected with malaria dies. However, the merozoites uptake calcium and sustain low levels of calcium to prevent phosphatidylserine (PS) exposure on the outer membrane. Without the PS tag, the infected, dying hepatocyte is not recognized by phagocytes. Therefore, the merozoite can continue to the next stage of its lifecycle (8,30).

MALARIA ERYTHROCYTIC STAGE IMMUNE EVASION

1. Lacking MHC I

MHC 1 is major histocompatibility complex 1, which is expressed in all cells that contain a nucleus. It functions to present segments of pathogens to cytotoxic T cells to trigger an immune response. Once merozoites exit

hepatocytes, they infect red blood cells (RBCs) and mature into trophozoites. The RBC will eventually burst, releasing many trophozoites that can continue contaminating more RBCs (12). Infecting RBCs leans in favor of the parasite due to RBCs lacking a nucleus and, therefore, not having MHC I. In addition to lacking MHC I, the terminally differentiated RBC, is also unable to initiate a cell-autonomous immune response upon infection. The malaria parasite thereby goes undetected and escaping clearance by immune cells, like cytotoxic T cells, etc. (6)

2. Forming Rosettes and Sequestering

The *Plasmodium* parasite induces the formation of rosettes in order to avoid immune defenses of the host. Another benefit to forming rosettes is infected RBCs are clustered with uninfected RBCs, allowing for more efficient spreading of the parasite. The *Plasmodium* parasite can quickly invade new RBCs when they are aggregated around a bursting RBC, assisting in avoidance of immune recognition (22).

Blood type can also affect how well RBCs form rosettes. An individual with blood type A forms stronger rosettes than an individual with blood type O. Therefore, there is a slight advantage for the parasite when it is inoculated into a person with blood type A (22).

Proteins from genes like *Var* and *Rifin* allow infected RBCs to attach to vascular endothelium sequestering in the microvasculature of organs, helping the parasite to avoid clearance (18,24). The spleen is important for filtering the blood for pathogens and ridding the blood of old erythrocytes (21). Proteins encoded by *Var* genes are known as PfEMP1 and localized to the RBC surface. They are expressed one at a time and the parasite periodically switches from the expression of one *Var* gene to another, allowing the parasite to evade the immune response the host mounts against each successive PfEMP1 variant (26). Along with this, these proteins also affect host platelets and inflammation, which leads to agglutination of uninfected RBCs with the infected ones, again assisting with the spread of infection (18). Sequestration in these microenvironments prevents infected RBC clearance by the spleen and helps the spread of the parasite.

3. Avoiding Recognition by Antibodies

Antibodies are essential to host protection during the blood stage of malaria. Antibodies can recognize antigens on the parasite or the parasite-infected RBC (32). Antibodies can block processes like adhesion and invasion of the parasite. Therefore, *Plasmodium* as successful as it is must-have ways to avoid these defenses. IgG subclass of antibodies, specifically IgG3, have shown protection against malaria parasites in the blood stage and target antigens like merozoite surface protein 1 (32). However, merozoite surface proteins (MSPs) are used by the parasite to avoid immune attack and show strong homology to host proteins, making it difficult for antibodies to recognize MSPs (5).

TOXOPLASMOSIS IMMUNE EVASION:

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1. Revamping Host Transcription Factors

Toxoplasma modulates more than 1000 host genes that regulate inflammation, apoptosis, metabolism, and differentiation to avoid clearance (27). Similar to *Plasmodium*, NFκB is one of the transcription factors manipulated to ensure successful infection (27). There are a few ways *Toxoplasma* affects NFκB and evades the immune defenses of the host. First, during infection, NFκB may lose subunits, overall increasing the

susceptibility during acute and chronic infections (27). By blocking the translocation of NFκB, the parasite can prevent the stimulation of pro-inflammatory genes like IL-12p40 and TNF-α (27). Lastly, NFκB can be manipulated to avoid apoptosis in cells infected with *Toxoplasma* by stimulating anti-apoptotic genes (24).

2. Inhibiting Immune Signaling Pathways

Toxoplasma suppresses critical signaling pathways like the JAK/STAT pathway, which is essential for activating vital cells responsible for fighting infections. The parasite does this by the delivery of proteins to the cell via specialized organelles called rhoptry and dense granule (16). During the invasion of the host cell, rhoptry proteins are deposited into the cell. These proteins will either localize in the cytoplasm or be trafficked into the nucleus. ROP16 is a Rhoptry protein that is translocated to the nucleus of the host cell and changes gene expression. Consequent to secretion, ROP16-I prevents activation of the JAK/STAT pathway by phosphorylating tyrosine residues on host STAT3 and STAT6 proteins (16). The phosphorylation causes a decrease in Th1 cytokine release due to the prolonged activation of the IL-4 signaling pathway. Th1 is necessary for activating immune cells like lymphocytes, neutrophils, and macrophages. Without these cells, the host is vulnerable to infection, promoting *Toxoplasma* survival (16).

IFN-γ stimulation also initiates the JAK/STAT pathway, and *Toxoplasma* interferes with this process in two ways. First, *Toxoplasma* can indirectly affect the response of IFN-γ by upregulating suppressor of cytokine signaling proteins (SOCS) (27). These proteins can prevent IFN-γ action by inhibiting the catalytic activity of the JAKs or impeding the recruitment of STATs. Both of which benefit the parasite by inhibiting the action of STAT, allowing for widespread infection (27). Secondly, the parasite also inhibits the expression of IFN-γ responsive genes. *Toxoplasma* blocks the activation of these genes by hindering the dissociation of STAT1 from the DNA. Overall, impeding STAT1 recycling and further cycles of STAT1-mediated transcription (2).

Toxoplasma inhibitor of STAT1-dependent transcription (TgIST) is a dense granule protein that is released into the host cell and trafficked into the nucleus. TgIST binds to phosphorylated STAT1 dimers, which bind to gamma-activated sequences in the promoters of IFN-stimulated genes (20). STAT1 is bound in the correct location to be activated; however, transcription is inhibited. This block caused by TgIST is due to the recruitment of nucleosome remodeling and repressive (NuRD) complex (20). TgIST also binds to the MI-2/NuRD complex, which recruits it to activated STAT1 dimers (25). TgIST recruits the MI-2/NuRD to STAT1 on gamma-activated factor (GAF) sites of its cognate-regulated genes in IFN-γ activated cells. TgIST remodels the local environment by creating a nonpermissive chromatin state that shuts down RNA polymerase II transcription. Preventing the transcription of IFN-stimulated genes promotes persistent *Toxoplasma* infection (25,13). Overall, inhibition of STAT1 aids parasite survival because it downregulates MHC, inducible NOS2, and transcription of IFN-stimulated genes (25,13).

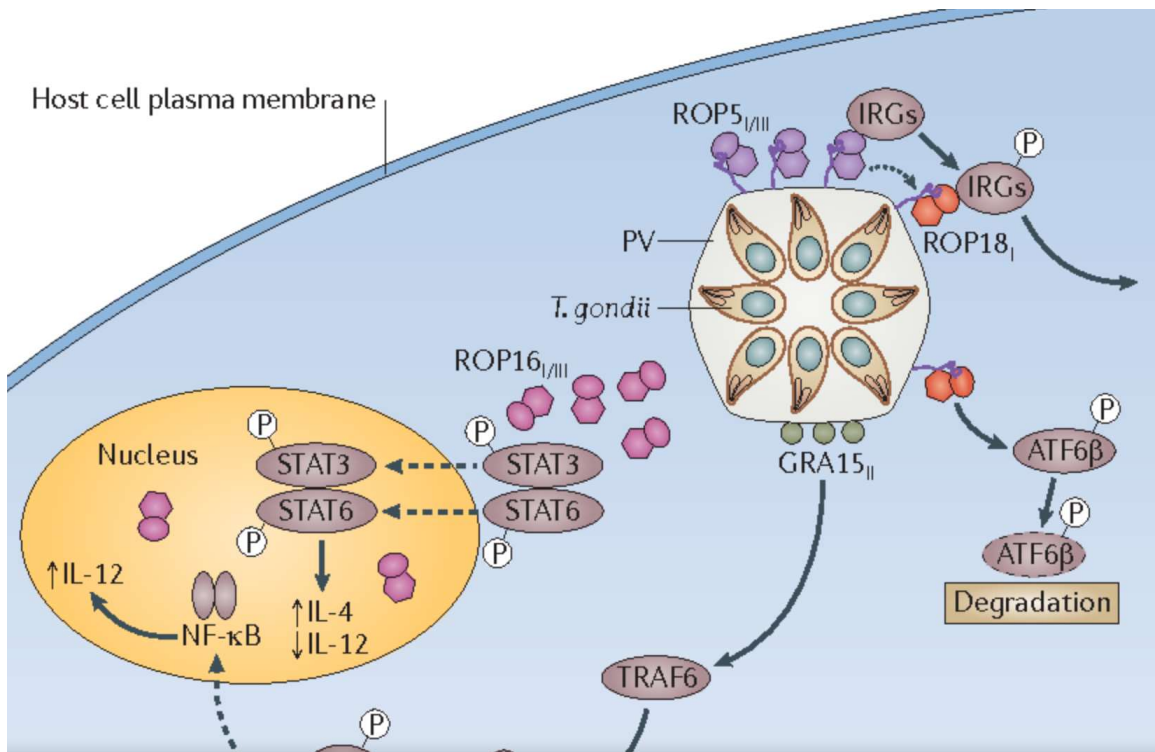


Figure 2: ROP16 phosphorylating STAT3 and STAT6 in host cell causing an upregulation of IL-4 thereby inhibiting the JAK/STAT pathway (28).

3. Inhibiting Apoptosis

Apoptosis is a useful protection mechanism performed by the host cell to prevent the spread of infection. *Toxoplasma* must, therefore, stop this process to persist. This parasite can arrest apoptosis via the extrinsic and intrinsic pathways (2). The intrinsic pathway of apoptosis is blocked due to the parasite causing a reduction in the secretion of cytochrome c from the mitochondria. Decreasing cytochrome c levels results in diminished cleavage of caspase 9 and caspase 3. Caspase 3 is the executioner caspase, meaning it is what initiates destruction of the cell. *Toxoplasma* affecting this protein is vital to the survival and spread of toxoplasmosis (2).

The extrinsic pathway of apoptosis is blocked due to *Toxoplasma* interfering with caspase 8, which is a protein vital for starting apoptosis. Decreased levels of pro-caspase-8 lower its association with the death-inducing signaling complex. This diminishes activation of the effector caspases, thereby inhibiting apoptosis (2).

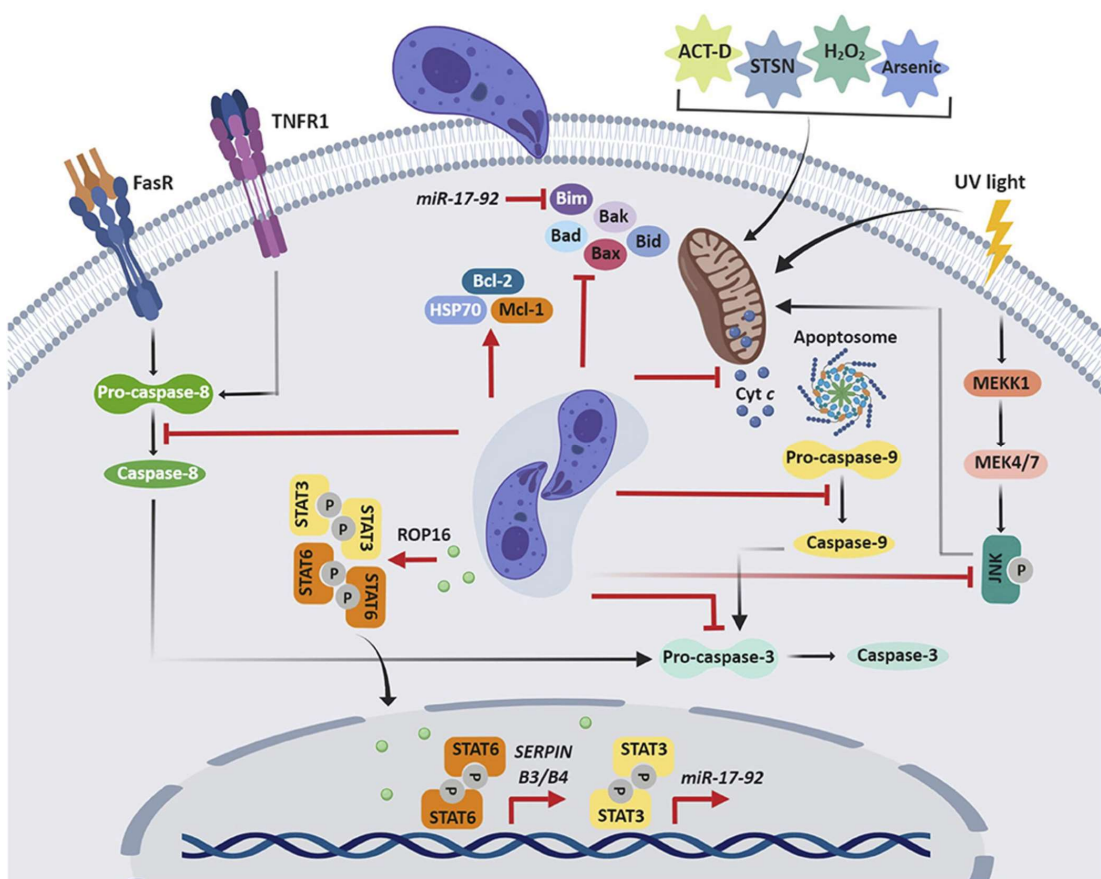


Figure 3: *Toxoplasma* inhibits the extrinsic apoptotic pathway by reducing levels of pro-caspase-8 overall, lowering its interaction with the death receptor (Left). *Toxoplasma* prevents the intrinsic apoptotic pathway by decreasing cytochrome c levels affecting activation of caspase-3 (2).

Conclusion:

Apicomplexan Parasites evade their host's immune system in several ways, often controlling and inhibiting host function. *Plasmodium* and *Toxoplasma* both manipulate NFκB. *Plasmodium* releases CSP into the cytoplasm of hepatocytes to suppress NFκB and prevent the stimulation of inflammatory cytokines. *Toxoplasma* also can prevent activation of inflammation by causing NFκB to lose subunits, inhibit the transportation of the transcription factor, and govern it to release anti-apoptotic genes instead of pro-apoptotic genes.

Evasion of the human immune system for a *Plasmodium* parasite involves inhibiting many processes that cause inflammation and apoptosis. This is a trait shared by *Toxoplasma* as well. A critical difference between the two parasites is that one area of focus to avoid immune defenses for malaria is the sequestration of infected RBCs and healthy RBCs as *Toxoplasma* does not do this.

Another difference between these parasites is how they manipulate apoptotic pathways. *Plasmodium*, as it has two stages in the lifecycle, induces apoptosis at the end of their development in the liver in order to enter blood circulation to proceed to the blood stage. *Toxoplasma*, however, manipulates both the extrinsic and intrinsic pathway of apoptosis to avoid cell death and continue to spread.

Understanding *Toxoplasma* infection is essential for two reasons. One, *Toxoplasma* can cause severe illness in growing fetuses, as well as immunocompromised individuals. The second reason is that *Toxoplasma* can be used as a model to study a more deadly relative, *Plasmodium* (27). More insight from research on malaria is vital for the development of successful treatment—awareness about how these parasites avoid clearance further benefits the advancement of a cure.

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