Chapter

Close Encounters: Pathogenic Protists-Host Cell Interactions

María Cristina Vanrell and Patricia Silvia Romano

Abstract

In this chapter, we summarize the highlights of the early events in the interaction of parasitic protists and the host cell. Pathogenic protists are a group of eukaryotic organisms, responsible for causing different human diseases, such as malaria, Chagas disease, leishmaniasis, and toxoplasmosis. These pathogens display complex life cycles and go through different cellular transformations to adapt to the different hosts in which they live. Part of these life cycles takes place in mammals, inside the host cell. Host cell entry ends with the formation of phagosomes or parasitophorous vacuoles, which differ from each parasite and each type of host cell. While canonical phagocytosis involves the fusion of phagosomes with compartments of the endocytic pathway to produce normal maturation through the phagocytic route, pathogenic microorganisms have developed Different evasion mechanisms to resist the intracellular defense systems. These strategies, including phagosome maturation arrest, resistance to the harsh lysosomal environment, or exit to the host cell cytoplasm, will be also presented in this work.

Keywords: phagocytosis, parasitophorous vacuoles, phagosomes, pathogenic protists, and parasites

1. Introduction

With the exception of *Trypanosoma brucei*, the etiological agent of African trypanosomiasis (sleeping sickness), pathogenic protists of Kinetoplastida and Apicomplexa lineages are intracellular pathogens causing broadly disseminated diseases: malaria (caused by species of the genus *Plasmodium spp*.), Chagas disease (caused by *Trypanosoma cruzi*), leishmaniasis (caused by species of *Leishmania spp*.), and toxoplasmosis (caused by *Toxoplasma gondii*). These illnesses kill millions of people worldwide; have a significant economic impact, and cause public health issues everywhere.

In the following paragraphs, we present the diseases caused by these pathogens as well as the life cycles they go through in order to adapt to the hosts in which they live.

1.1 Malaria *(Plasmodium spp)*

Different species of *Plasmodium spp*. can infect humans causing malaria disease; the most common, *Plasmodium falciparum*, is responsible for the majority of deaths.

In contrast, *Plasmodium vivax* is responsible for the majority of cases. The symptoms of malaria range from asymptomatic parasitemia to severe disease, including cerebral malaria and death. Pregnant women and children under the age of five are particularly vulnerable to the disease. A combination of infected red blood cell sequestration in the microvasculature, endothelial activation, procoagulant action, and, most importantly, pro-inflammatory responses are thought to be the cause of the pathology. This disease is a huge public health burden, with an estimated 241 million cases reported in 2020 in 85 malaria-endemic countries (including the territory of French Guiana), resulting in 405,000 deaths [1–3].

Female Anopheles mosquitoes transmit the parasites, which have a complex life cycle that alternates between sexual and asexual phases. The infection begins with the bite of the mosquito, which injects parasites into the host in the form of sporozoites, which then travel to the liver. After replicating in liver cells, they mature into merozoites and are released into the bloodstream to invade host erythrocytes. Although the high parasite burden (up to 30,000 merozoites) stresses the host cell, infected hepatocytes do not undergo stress-mediated apoptosis, implying that the parasite interferes with this process in the host cell, In the erythrocyte, the parasites develop into immature gametocytes or ring-stage trophozoites, which are followed by mature trophozoites, schizonts, and merozoites. The immune system attacks these parasites; sporozoites in the liver find hepatic macrophages known as Kupffer cells, while parasites in the blood can find circulating monocytes and neutrophils [4].

1.2 Chagas disease *(Trypanosoma cruzi)*

Trypanosoma cruzi is the causative agent of Chagas disease, also known as American trypanosomiasis. This is a public health problem in Latin America where it affects approximately 7 million people worldwide, and 100 million people are at risk of contracting it. Furthermore, it has become increasingly common in the United States of America, Canada, and many European and Western Pacific countries in recent decades [5, 6].

The life cycle of the protozoan parasite *Trypanosoma cruzi* involves both vertebrate and invertebrate hosts. Vectorial transmission to vertebrate hosts occurs via the bite of insect triatomine vectors (from the Reduviidae subfamily known as "kissing bugs"), which shed metacyclic trypomastigotes in their feces after feeding allowing the entry of trypomastigotes through skin wounds and mucosal membranes. Other infection routes are the oral ingestion of food contaminated with triatomine feces, such as fruit juices, blood transfusion or organ transplant, laboratory accidents, and congenital transmission from the mother to child during pregnancy. The last form became the most important nowadays and explains the presence of new cases in non-endemic countries as mentioned above.

Trypomastigotes can infect a wide range of nucleated cells, including macrophages, cardiac muscle cells, and nervous system glial cells, exploiting phagocytic or non-phagocytic mechanisms depending on the class of cell involved. After a brief residence in a parasitophorous vacuole, parasites go through the cytoplasm and differentiate into amastigotes. After several divisions (binary fission), amastigotes transform back into trypomastigotes, which are released from the host cell and can infect neighboring cells or reach the bloodstream and infect different organs, particularly the heart.

Trigonoscuta cruzi infection in humans is characterized by a brief acute phase with nonspecific symptoms and a long chronic phase in which most individuals do not exhibit pathology. In contrast, some infected people (around 10 to 30% of cases)

develop specific pathology cardiomyopathy and mega syndromes of the digestive system, which cause significant morbidity and may lead to mortality [5–7]. The development of Chagas pathology is complex and multifactorial, involving parasite immune evasion strategies, genetically programmed deficiencies in host immunological homeostasis, and autoreactive events marked by the presence of autoantibodies. *T. cruzi* genetic material has been identified in tissues destroyed during chronic infection, showing that the parasite plays an active role in pathogenesis [8, 9]. In fact, despite a vigorous immune response, the host fails to clear the parasites from the tissues, allowing the infection to remain indefinitely.

1.3 Leishmaniasis *(Leishmania spp)*

To cause leishmaniasis, *Leishmania* parasites infect and develop into phagocytic cells [10]. Clinical symptoms of the disease range from skin or mucocutaneous disorders to visceral infections, which are caused by different parasite strains and the delicate balance of parasite proliferation, the patient's immune response, and the consequent degenerative alterations. Consequently, *L. major*, *L. tropica*, and *L. mexicana* produce mainly the cutaneous forms, *L. braziliensis* causes the mucocutaneous illness, and *L. donovani* causes the most severe visceral disease (called kala-azar which means black fever in Hindi language). Infections caused by *Leishmania spp.* are a major public health concern across the world. This illness is seen in 88 different nations. More than 350 million individuals worldwide are at risk of leishmaniasis [11, 12], with 12 million already infected.

The life cycle of *Leishmania* is rather straightforward, with two basic stages: motile flagellate promastigotes residing in the stomach of the sandfly vector and immotile amastigotes within the phagolysosomal vesicles of vertebrate host macrophages.

A variety of sandfly species from two primary genera, *Phlebotomus* and *Lutzomyia*, transmit the illness to the host. Female infected sandflies spread the illness by injecting the promastigote form into the skin during a blood meal. After being inoculated into the upper dermis, metacyclic promastigotes are phagocytosed by skin-resident macrophages and dendritic cells and largely localized to phagolysosomes [10]. The internal development of Leishmania metacyclic promastigotes into amastigotes devoid of exterior flagella takes 12 to 24 hours. Amastigotes reproduce and survive intracellularly inside the phagolysosomal compartment, acting as a reservoir for transmission [13]. Moreover, polymorphonuclear neutrophils are attracted to the site of infection to clear promastigotes [14]. Explaining the significant inflammatory response produced after roughly 3 weeks [15]. As a sandfly feeds on the blood of an infected vertebrate host, it consumes amastigotes-containing monocytes and macrophages. Amastigotes are discharged into the sandfly's midgut, where they evolve into flagellated promastigotes through a process known as metacyclogenesis. Metacyclic promastigotes enter the throat and oral cavity, where they will be transmitted during the next blood meal.

1.4 Toxoplasmosis *(Toxoplasma gondii)*

Toxoplasma gondii is an obligate intracellular parasite of the order *Coccidia* with felines as the unique definitive hosts. It is a zoonotic illness that regularly affects a range of wild and domestic animals, with humans serving as unwitting hosts.

The protozoan parasite *T. gondii* infects 25 to 30% of the world's human population, with significant prevalences in South America and tropical African countries.

As of 2020, the World Health Organization reported around 240 million illnesses and 600,000 deaths [16] (World malaria report 2021). Infected fetuses (congenital toxoplasmosis) and immunocompromised people are the most vulnerable to this illness. More than 80% of cases of primary acquired infection in immunocompetent people in Europe or North America are asymptomatic. In other instances, patients may develop fever or cervical lymphadenopathy, which may be accompanied by myalgia, asthenia, or other nonspecific clinical symptoms. Toxoplasmosis is extremely dangerous in immunocompromised patients, and toxoplasmic encephalitis, the most common manifestation of the disease in these patients can cause a variety of symptoms ranging from headache, lethargy, lack of coordination, or ataxia to hemiparesis, loss of memory, dementia, or focal major motor seizures, usually associated with fever. The lungs, eyes, and heart are also often damaged, leading to myocarditis, while *Toxoplasma* has been isolated from other organs such as the liver, pancreas, bone marrow, bladder, lymph nodes, kidney, spleen, and skin. Toxoplasmic retinochoroiditis is a less prevalent complication.

Congenital infection is typically the outcome of a primary infection acquired by the mother during pregnancy. The incidence of vertical transmission and the severity of fetal harm is determined by the stage of pregnancy at which the mother becomes infected. It is more dangerous when the infection develops in the early trimester of pregnancy, resulting in significant abnormalities or termination. The parasite's replication causes necrosis and severe inflammation, resulting in serious abnormalities in the brain and eye organs. Mental retardation, convulsions, microcephaly, hydrocephalus, hearing, and psychomotor impairment are all serious consequences. Microphthalmia, cataracts, increased intraocular pressure, strabismus, optic neuritis, and retinal necrosis can also be detected, as can uveitis and retinochoroiditis, which can lead to blindness. Retinochoroiditis is a typical characteristic that can be present regardless of the period of maternal infection [17].

Intermediate hosts become infected by the consumption of sporulated oocytes present in contaminated meat. In the intestinal epithelial cells, *T. gondii* develops in rapidly growing tachyzoites which travel throughout the body. In the infected cells, parasites proliferate in parasitophorous vacuoles. In response to immunological pressure, the parasites encyst as bradyzoites, a slow-growing form. Tissue cysts are most commonly found in long-lived cells like muscular, endothelium, or neural cells.

When members of the cat family consume bradyzoites, they undergo sexual development within intestinal epithelial cells, ending in the discharge of oocysts that undergo meiosis in the environment to generate eight haploid sporozoites. The consumption of oocysts by a wide range of hosts results in acute infection. Humans become infected by consuming oocysts that can contaminate food or drink, or by eating undercooked meat with tissue cysts [7].

To survive in the host cell, *T. gondii* typically resides in a vacuole, which inhibits lysosomal degradation and promotes parasite reproduction.

2. Phagocytosis

The first person to describe the absorption of particles by cells was Élie Metchnikoff (1845–1916), who also highlighted the significance of this process for the host's reaction to damage and infection. Phagocytosis is a sophisticated mechanism for ingesting and eliminating infections that also plays a crucial role in the elimination of apoptotic cells, which is essential for maintaining tissue homeostasis.

Target particle identification, signaling to start the internalization machinery, phagosome formation, and phagolysosome maturation are the four key stages of phagocytosis [18].

The key aspects of the early events of phagocytosis of protist parasites under study will be discussed in the following section.

2.1 Recognition and phagocytosis of *Plasmodium spp*

Microorganisms express molecules known as pathogen-associated molecular patterns (PAMPs), which are only expressed by pathogens and not by host cells. Glycosylphosphatidylinositol (GPI) anchors, nucleic acids, and Hemozoin are all Plasmodium PAMPs [2]. Pattern recognition receptors (PRRs) such as CD36, toll-like receptors (TLRs), and complement receptor 3 identify these PAMPs and trigger the parasite uptake.

Phagocytes, particularly monocytes, and macrophages, may also perform opsonic phagocytosis of *Plasmodium spp*. Certain opsonins, notably antibodies, have been found in functional investigations to increase successful phagocytosis. Protective immunity in malaria has been linked to the IgG1 and IgG3 subclasses. MSP (the merozoite surface proteins) 2 and 3, MSP-Duffy binding-like proteins 1 and 2, and glutamate-rich proteins have been discovered as targets of these opsonizing antibodies in merozoites [19].

Immune system cells have immunoglobulin (Ig) binding receptors, FcgR I receptors, FcgRII and FcgRIIII, and complement receptors CR1 and CR3. These factors, when combined, can aid in the phagocytic absorption of antigens opsonized with components such as IgG or C3b [1].

The complement receptor CR1 recognizes and phagocytoses ring-parasitized red blood cells opsonized by IgG and complement. Parasites cause changes in the membrane proteins of hosts' erythrocytes, exposing antigenic regions identified by autoantibodies. For example, band 3 protein is clustered and oxidized, and it is also underglycosylated [20]. Protein 1 (PfEMP1), which is expressed on the membrane of *Plasmodium falciparum*-infected erythrocytes, is also a significant target of opsonizing antibodies, with antibodies recognizing distinct domains of this protein [20].

When activated, neutrophils can produce reactive oxygen species (ROS), which are highly poisonous chemicals that can kill parasites by inflicting oxidative damage.

2.2 Enfermedad de Chagas-Phagocytosis of *Trypanosoma cruzi*

Tissue-resident macrophages are the first host cells invaded by *T. cruzi* during in vivo infection. Trypomastigotes and epimastigotes are both readily absorbed by macrophages and detected within phagolysosomes. Only the trypomastigotes may escape the phagolysosome and grow in the cytosol, while the epimastigotes are killed. The plasma membrane of macrophages has been demonstrated to envelop the parasite by producing a tubular structure, also known as a coiled phagosome. Although this mechanism appears to be comparable to phagocytosis, data shows that, unlike non-infectious epimastigotes, trypomastigotes actively strive to route their own infection to macrophages. The escape of trypomastigotes to the cytosol is important because nitric oxide (NO) produced in the parasitophorous vacuole is the most potent agent in activated macrophages [5].

The parasite's primary target organ is the heart. Tissue damage in the heart is associated with severe parasitism of the myocardium during acute illness. To regulate

parasite proliferation, monocytes migrate and extravasate from the circulation to the heart, where they develop into macrophages [6].

The surface receptor for sialodhesin can be expressed by macrophages (Sn). This receptor detects sialic acid, which is abundant on the parasite's surface and appears to play a significant role in the adhesion process during *T. cruzi* phagocytosis. TLR2 and TLR9 on the surface of macrophages have also been implicated in the identification of *T. cruzi* antigens: GPI (glycosylphosphatidylinositol) anchors, a dominating glycolipid dispersed on the surface of the *T. cruzi* membrane, and parasite DNA, respectively. Classical activation causes profound metabolic changes in macrophages, such as increased inducible nitric oxide synthase (iNOS or NOS2) activity and respiratory burst, as well as secretory responses, such as the production of proinflammatory cytokines and chemokines that lead to phagocytosis, intracellular pathogen destruction, antigen presentation, and costimulation. During experimental mouse infection, NO released by activated macrophages was thought to be a significant chemical for host defense against the parasite. The infection has also been demonstrated to enhance splenic but not peritoneal macrophage production of hydrogen peroxide (H_2O_2), indicating that in vivo production of antimicrobial compounds appears to be connected to certain kinds of macrophages and/or the parasite's capacity to activate these cells [6, 7].

T. cruzi amastigotes engage in phagocytic processes to invade both professional and non-professional phagocytic cells, depending significantly on the actin cytoskeleton of the host cell [21]. The GTPases of the Rho family of the host cell and their effector proteins were involved in the actin-dependent invasion [22].

2.3 *Leishmania spp*

Leishmania promastigotes access macrophages after opsonization mainly through complement receptor 1 (CR1) or 3 (CR3), Other receptors have also been implicated such as the Toll-like receptor (TLR) family, the receptors for the Fc domain of immunoglobulins (FcR), mannose-fucose receptor (MR), and fibronectin receptors. In this regard, an important molecule is complement component 3 (C3), which mainly binds to gp63 and LPG (glycolipid lipophosphoglycan) *in vitro* after complement activation [23]. This is a RhoA-dependent phagocytosis process. RhoA is a small GTPase protein of the Rho family of GTPases that is primarily involved in the regulation of the cytoskeleton, specifically the formation of actin stress fibers and actomyosin contractility. Phagocytosis has been proposed to be the main mode of invasion of promastigotes since infection by macrophages is reduced in the absence of actin polymerization of the host cell [24]. Phagocytosis of promastigotes by macrophages appears to begin within 2 minutes of contact with the parasites *in vitro* [25]. It should be noted that, during the first few minutes of contact, 90% of promastigotes connect to macrophages with low affinity through their flagellar tip [25], implying a role for this structure in the formation of phagosome Caveolae-dependent phagocytosis is also activated by *Leishmania.* The entry of pathogenic metacyclic promastigotes into murine macrophages has been linked to caveolae, and this route is critical to prevent early lysosome fusion.

During the differentiation process, promastigotes arrest phagosome maturation and exhibit delayed or decreased recruitment of late endosomal lysosome markers such as rab7 and LAMP1. Arrested phagosomes are further distinguished by the presence of host actin coating, related polymerization factors, such as Arp 2, 3, Nck, and WASP, and the recruitment of a variety of host GTPases involved in actin

polymerization. Further phagosome remodeling is related to the breakdown of the lipid raft and reduced formation of the NADPH oxidase complex.

Amastigotes, like promastigotes, are taken up by a conventional phagocytic process that may be opsonic or non-opsonic. Uncoated parasites are taken up by Rho and Cdc42, but IgG-coated parasites are phagocytosed by a Rac1-dependent mechanism. The FcR and CR receptors are mostly involved in amastigotes invading macrophages. Vacuoles containing amastigotes are fusogenic and acquire markers associated with phagosome development into phagolysosomes. The vacuole contains hydrolytic enzymes and is positive for H+ ATPase. It also includes markers such as Rab7, LAMP1, and LAMP2. Amastigotes are resistant to hydrolysis and multiplying the acidic environment (pH 4.5–5.5) of the phagolysosome. The ability of *Leishmania* to control phagosome maturation depends on a surface-abundant glycolipid called lipophosphoglucan (LGP), which is a member of the phosphoglycan family. In addition, the parasite membrane contains a proton translocating ATPase, which presumably helps maintain pH homeostasis inside the parasite and contributes to lysosomal acidification. The proton gradient thus established drives the active transport of nutrients necessary for the growth of the parasite [26].

It has also been described that *Leishmania mexicana* induces an autophagy-like pathway in infected cells, redirecting cytosolic proteins for destruction and making them accessible to parasites within the phagolysosome for nutrition [27, 28].

2.4 *Toxoplasma gondii*

Unlike *Leishmania*, *Toxoplasma gondii* infects by both phagocytic and non-phagocytic cells. The infection and subsequent demise of these cells following the parasite's rapid proliferation is a crucial event in the pathogenic course of this organism. The parasite may enter a cell as a macrophage using the well-known phagocytosis process without causing its own death within the cell.

Trophozoites may actively escape cells after phagocytosis, by reversion of the process of invasion. At the moment, it is considered that entrance into the host cell includes a complicated process that combines phagocytosis with aggressive invasion.

Macrophages can swallow the parasite, opsonized or not. *T. gondii* inhibits phagosome-lysosome fusion after phagocytosis [29, 30]. Toxoplasma phagocytosis occurs primarily via opsonins such as C3b and C3a, which are recognized by their corresponding receptors on macrophages [31].

3. The evasion mechanisms

3.1 *Plamodium* can control the phagocytosis process through a variety of methods

Plasmodium spp. can prevent phagocytosis by changing its interaction with host phagocytic receptors and controlling downstream signaling cascades.

Plasmodium yoelii parasites, for example, preferentially infect erythrocytes expressing large amounts of CD47, allowing them to evade phagocytosis by the red-pulp macrophages in the spleen. CD47 is a marker that inhibits phagocytosis; Therefore, CD47 depletion may enhance phagocytic clearance. Red cells infected with *Plasmodium falciparum* and *Plasmodium vivax* have been shown to display higher amounts of CD47 than uninfected red cells; however, the mechanism behind this increased expression remains unclear. Furthermore, parasites can avoid phagocytosis

by modifying complement regulatory proteins, which protect infected host cells from complement-mediated damage. They can, for example, inactivate C3b on the surface of infected erythrocytes, preventing complement-mediated phagocytic clearance of parasites. Moreover, monocytes and macrophages express less complement receptor 1 (CR1) during infection. Surprisingly, infected red blood cells preferentially bind CR1 produced by uninfected red blood cells to form rosettes, presumably isolating them from phagocyte detection.

Also, by removing superoxide and inhibiting ROS from neutrophils, mosquito salivary proteins can influence neutrophil activity. Ex vivo data demonstrate that neutrophils have a decreased ability to create ROS during malaria (**Figure 1**, Plasmodium spp.). In vitro evidence suggests that neutrophil phagocytosis of parasite products reduces their ability to engulf bacteria [1].

It was similarly shown that ex vivo monocytes from children with acute malaria had lower opsonic phagocytosis than their own monocytes 6 weeks later [2].

Finally, parasites in Kupffer cells during rodent malaria have been shown to directly trigger phagocyte death [4].

Humans are infected by parasite sporozoites, which enter hepatocytes and grow rapidly. *Plasmodium spp.* requires nutritional input to the parasitophorous vacuole to reproduce successfully, which implies the existence of host cell manipulation mechanisms. It has been shown that there are membrane connections of the parasitophorous vacuole to the Golgi membranes that were maintained throughout the growth stage in hepatocytes, which are believed to enhance the nutritional supply of hepatocytes. RAB11, a small GTPase, is important for organelle morphological changes during *Plasmodium berghei* infections, and functional alterations of this protein reduced this impact.

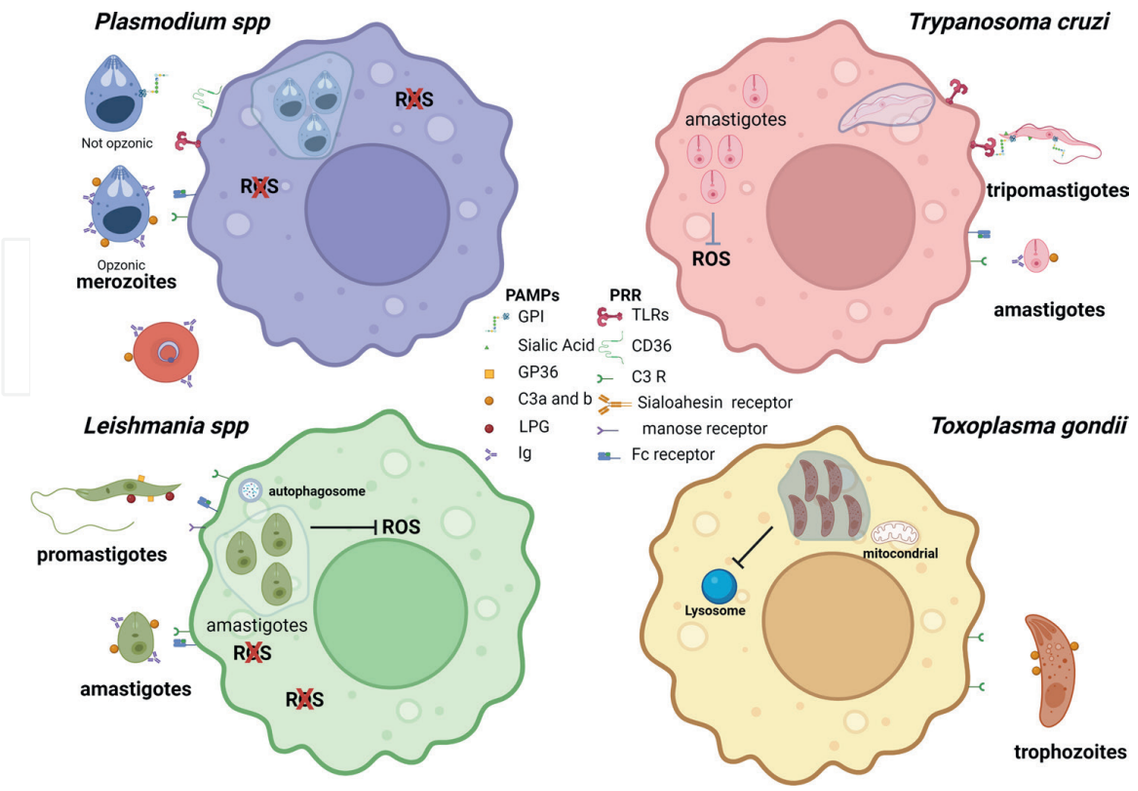

Figure 1.
The image shows the molecules involved in the phagocytosis of pathogenic protists and the evasion mechanisms that evolve to resist in the host cell. Created with BioRender.com.

Mature trophozoites within infected red blood cells can circulate to organs such as the brain, spleen, placenta, and lungs, where they can be sequestered as part of an immune evasion strategy [4].

3.2 Resist the oxidative response, the smart strategy of *T. cruzi*

T. cruzi, in vertebrate hosts, develops a variety of immune evasion strategies. Protection against direct cytotoxic effects of O2•/H_2O_2• on parasite mitochondria within the macrophage phagosome (**Figure 1**, *T. cruzi*); suppression of ONOO production in NO-exposed parasites, and regulation of NO-exposed parasites are among these methods. To resist host-derived oxidants, *T. cruzi* has an arsenal of detoxifying antioxidant defenses, as well as redox metabolism. Trypanothiol (T[SH]2), the main thiol used by the antioxidant system of trypanosomatids, is one of the most important. This system is considered an interesting target route for drug development.

Fe-dependent superoxide dismutases (Fe-SODs) from *T. cruzi* readily remove O2 • and may help to survive intracellularly [32].

TcAPxCcP, a type A hybrid peroxidase that employs ascorbate and cytochrome C as reducing substrates for H_2O_2 detoxification, has also been reported in *T. cruzi* [33]. TcAPxCcP is a membrane-bound peroxidase found in the endoplasmic reticulum and mitochondria throughout the parasite's life cycle, as well as in the plasma membrane during the infective stages of the *T. cruzi* life cycle [34]. Lastly, *T. cruzi* has two GSH-like peroxidases (GPX) that can metabolize fatty acids and phospholipid hydroperoxides despite the absence of selenium in the active site [35]. In the non-infectious epimastigote, GPX-I is found in the cytosol while GPX-II is found in the endoplasmic reticulum. In general, *T. cruzi*'s antioxidant arsenal works as a virulence factor by detoxifying reactive species in the phagosomal compartment.

Furthermore, it has been demonstrated in *T. cruzi* that peroxiredoxins, a family of proteins with antioxidant and redox signaling functions, were upregulated in the infective metacyclic trypomastigote stage and that their expression levels correlated with parasitemia in mice, implying that peroxiredoxin levels mediate *T. cruzi* virulence.

Another pathogen-encoded virulence strategy depends on repair mechanisms that restrict the potentially damaging oxidation of proteins and DNA. Methionine oxidation is mediated by a variety of reactive species such as H_2O_2, peroxynitrite, HOCl, and metal-catalyzed oxidation systems, yielding methionine-(S) and methionine-(R)-sulfoxide (Met-SO) epimers. Enzymatic pathways for methionine oxidation have also been identified. Methionine sulfoxide reductases (Msr) have been identified in a variety of pathogenic organisms, and these enzymes reduce Met-SO by using the reducing equivalents of Trx/TrxR and NADPH [36]. MsrA and MsrB, two distinct enzymes, catalyze the reduction of oxidized methionine diastereomers. MsrA action in proteins is confined to Met(S)-SO residues, whereas MsrB decreases Met(R)-SO. Another essential component for *T. cruzi* pathogenicity is the sanitization of oxidized bases in DNA. Guanine is highly oxidizable, and its most frequent oxidation product is 7,8-dihydro-8-oxo-29-deoxyguanosine (8-oxoG), which has the potential to be mutagenic owing to its structural similarities to thymine [37]. Trypanosomes have effective DNA repair mechanisms as well [38].

3.3 Leishmania subversion of phagocytosis favors the infection

After inoculation, *Leishmania* promastigotes are swiftly phagocytosed, but they can survive and change into immobile amastigote forms that can remain as

intracellular parasites. The parasitophorous vacuole is an acidic intracellular compartment where *Leishmania* amastigotes proliferate. Although the amastigote cytoplasm is controlled to near-neutral pH by an active process of proton extrusion, pH plays an important role in the developmental changeover between the promastigote and amastigote phases. Amastigotes are metabolically more active when their environment is acidic. Endosomes, phagosomes, and autophagosomes can all fuse with the parasitophorous vacuole. *Leishmania* amastigotes have evolved to survive in the particular ecological niche of mammalian macrophage phagolysosomes. The parasitophorous vacuole contains a highly hydrolytic and acidic environment, which the parasite does not appear to mitigate. While the parasite's cytoplasm is deliberately kept at a neutral pH, the amastigote's surface membrane adapts to operate efficiently in an acidic mileu, allowing the parasite to collect nutrition while being exposed to extraordinarily high external proton concentrations [39].

It is remarkable how the parasite avoids this harmful surge of ROS generation: it may counteract endogenous ROS production via antioxidant systems or by actively lowering ROS production (**Figure 1**, Leishmania spp) [40].

Although promastigotes and amastigotes enter macrophages by phagocytosis, the oxidative burst that occurs is very different. After infection, both stages show a rise in O2• production of macrophages, although the reaction is significantly stronger in promastigotes than in amastigotes. The discrepancy can be attributed to a decrease in NADPH oxidase activity following amastigote infection. Only once the gp91phox precursor has matured to its full-length molecule, the NADPH oxidase complex can be successfully assembled. This stage of development is dependent on the availability of heme. Infection with *L. pifanoi* amastigotes causes the production of heme oxygenase-1, the rate-limiting enzyme for heme degradation, which inhibits the development of gp91phox and precludes the assembly of NADPH oxidase. *L. donovani* amastigotes also affected another component of the NADPH oxidase complex. Amastigotes caused barely detectable amounts of p47phox phosphorylation, which resulted in p67phox and p47phox phagosomal recruitment defects. Interestingly, protein kinase C (PKC) mediates p47phox phosphorylation, which is suppressed by *Leishmania* promastigotes and amastigotes. This action has been linked to the lipophosphoglycan (LPG) present in promastigotes; in amastigotes, the mechanism responsible for PKC inhibition is uncertain. Moreover, *L. donovani* amastigotes affect the phagosomal lipid raft integrity, which may lead to defective NADPH oxidase assembly [41].

Lastly, infection with *Leishmania* amastigotes can result in reduced O2• generation by inhibiting inositol phosphate buildup and calcium release in infected macrophages. While promastigotes have little effect on overall O2• generation in macrophages, they have been shown to locally impede the assembly of NADPH oxidase at the phagosomal membrane, a defensive system reliant on the presence of LPG repeat units. Moreover, LPG glycoconjugates can influence macrophage iNOS expression. When LPG is administered before IFN-ɣ, NO generation is decreased compared to control cells. LPG suppresses the production of NO in macrophages in a time and dose-dependent manner. It clearly shows that LPG may regulate iNOS expression in macrophages [42].

Leishmania has an antioxidant defense mechanism as well. Trypanothione/trypanothione reductase has been described in *L. major*, which is crucial for its antioxidant ability against H_2O_2, ONOO, and •NO. $T(SH)_2$ was also discovered to be required for H2O2 elimination in trypanosomatids. $T(SH)_2$ requires the proteins triperedoxin (TXN) and peroxiredoxin (PRX) (which has triperedoxin peroxidase activity) to

decrease H_2O_2. The presence of the enzyme ascorbate peroxidase has also been shown to reduce H_2O_2, this is also present in *T. cruzi*. Trypanothione S-transferase and 5,6,7,8-tetrahydrobiopterin superoxide dismutase are among the main antioxidant mechanisms [40].

In summary, the parasite protects itself from the macrophage's oxidative burst by expressing antioxidant enzymes and proteins and inhibiting the synthesis of O2• and •NO in the macrophage. Surprisingly, promastigotes and amastigotes have opposing inhibitory effects. Amastigotes produce a widespread drop in O2• levels in the macrophage, whereas promastigotes lower O2• production just locally in the phagosome. Amastigotes decrease the synthesis of IL-12, O2•, and •NO in addition to their impact on macrophage redox biology. Unlike promastigotes, where LPG was identified as a parasite effector, no chemical associated with amastigotes has been identified as being responsible for the drop in O2• levels. Finally, parasites of *Leishmania* have evolved to live and multiply within ROS-producing macrophages. They do this not just through the use of antioxidant mechanisms, but also by decreasing ROS generation in macrophages [43, 44].

L. donovani infection also activates nuclear translocation and (Nuclear factor erythroid 2-related factor 2) Nrf2 activity, which reduces oxidative stress, but there is no evidence of which molecular partners are required to trigger this signaling yet. What is known in particular is that Nrf2 expression and activation occur upon initial contact with the host cell by increasing the number of gene products related to an antioxidant profile and turning macrophages into an anti-inflammatory spectrum. Knockdown or inhibition of Nrf2 is also known to decrease parasitic infection. But despite the antioxidant effect on cells, continued Nrf2 activation can greatly decrease ROS levels, which is also essential for cellular homeostasis. One of Nrf2's targets is the ferritin gene, which sequesters Fe^{2+}, reducing iron metabolism for parasite growth [41].

An acid phosphatase found in *Leishmania* has been shown to inhibit superoxide anion generation in chemoattractant-stimulated neutrophils. The parasite's LPG was also found to suppress protein kinase C (a regulator of macrophage oxidative metabolism). It has been proposed that *Leishmania* parasites could block lysosomal hydrolases by producing polyanionic compounds capable of forming complexes with positively modified hydrolases or binding to calcium ions.

3.4 *T. gondii* established a unique vacuole to avoid host cell defenses

As previously observed, microorganisms avoid important host defense processes such as phagocytosis, allowing them to establish themselves in the host cell and growth. In mouse macrophages (where this parasite survives), the organelle containing *T. gondii* appears to be arrested, unable to fuse with lysosomes, unless the organism has been coated with antibodies prior to phagocytosis, in which case it is easily destroyed [29]. *T. gondii* also uses tiny Rab-family GTPases for nutrient delivery, demonstrating that intracellular pathogens use host pathways components to promote proliferation. In *T. gondii*-infected cells, for example, mitochondria are organelles that interact with the membrane of the parasitophorous vacuole. The parasites have a mitochondrial association factor 1 (MAF1) locus, which encodes numerous proteins involved in host cell mitochondrial association and immune evasion, with the MAF1b protein serving as the primary mediator. *T. gondii*'s interaction with host cell organelles is most likely due to a requirement for nutritional input, which allows the parasitophorous vacuole to spread. Pernas et al. discovered that *T. gondii* infection had an indirect effect on mitochondrial morphology (**Table 1**) [45].

	Parasite molecules involved in phagocytosis	**Pathogen recognition receptors on phagocytic cells**	**Evasion mechanisms**
Plasmodium spp	Merozoites Not opsonic: • GPI • Nucleic acids • Hemozoin Opsonic: • IgG1 and IgG3 that recognize the MSP protein	Not opsonic: • CD36 • TLRs • CR3 Opsonic: • FCg • CR1 y CR3	Can prevent phagocytosis. Removing superoxide and inhibiting ROS.
Trypanosoma cruzi	Tripomastigotes Sialic acid GPI DNA Amastigotes C3a and b Ig G	Sialoadhesin receptor TLR 2 TLR4	*Exit* from the parasitophore vacuole. detoxifying antioxidant defense and redox metabolism. Repair mechanisms that restrict the oxidation of proteins and DNA.
Leismania spp	Tripomastigotes Gp36 LPG Amastigotes C3a and b Ig G	CR1 CR3 TLRs Manose receptor Fc Receptor	Reduced formation of the NADPH oxidase complex. Resistance to hydrolysis and multiply within the phagolysosome, proliferating in the acidic environment. reduced the O2 • generation. antioxidant systems.
Toxoplasma gondii	C3a and b	CR	Inhibition of phagosome-lysosome fusion

Table 1.
Summary of the phagocytosis of pathogenic protists and the evasion mechanisms that evolve to resist in the host cell.

Author details

María Cristina Vanrell* and Patricia Silvia Romano*
Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Instituto de Histología y Embriología de Mendoza IHEM-CONICET, Argentina

*Address all correspondence to: vanrellcristina@gmail.com and promano@fcm.uncu.edu.ar

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