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PARASITE DISSEMINATION AND THE PATHOGENESIS OF TOXOPLASMOSIS

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Introduction

Many pathogens are restricted to the site of colonization or have a distribution restricted to specific tissues. For others, the ability to disseminate from the initial point of infection and invade different niches is an integral part of their biology. For example, various protozoan and helminth parasites need to migrate through distinct host tissues to complete their life cycles. Thus, the success of *Plasmodium* is dependent on the ability of different developmental stages to migrate from the skin to the liver, and finally to the blood for transmission. Similarly, *Schistosoma mansoni* undergoes a protracted migration that starts in the skin and proceeds through multiple tissues, including the lungs, before adults pair in the mesenteric venules, allowing eggs to exit through the intestine. For these parasites, the clinical features and tissues affected are a consequence of the natural progression of the infection. For other parasites, inappropriate migration or dissemination forms the basis for disease and this is illustrated by the ability of *Entamoeba histolytica* to cross from the intestine and cause the development of liver abscesses. Similarly, there are several helminthes and nematodes that, when in inappropriate hosts, fail to develop fully and continuously migrate through tissues such as the brain where they can cause extensive tissue damage.

For all of these cases, the ability of the parasites to cross biological barriers at either the point of entry or subsequently within the host is key to reproductive success or to the pathology that can accompany these diseases. Similar principles apply to *Toxoplasma gondii*, a major pathogen of public health importance that causes a chronic infection in approximately one third of the world population [1, 2]. The ability of *T. gondii* to invade many tissues is an important element that contributes to the spectrum of diseases that can be associated with this organism. Thus, following the ingestion of oocysts or tissue cysts of *T. gondii*, this organism infects its host in the small intestine and converts to the tachyzoite form, which then disseminates rapidly to almost all tissues, including muscle, brain, eyes, liver, placenta and lungs. Typically, this infection leads to a robust innate and adaptive response that is characterized by the production of IFN-γ by NK and T cells, which leads to the control of the acute phase (for review see [3]). These events are associated with immune pressure that drives the parasite to develop into a chronic, usually asymptomatic stage, where this organism persists as bradyzoites in cyst form within multiple tissues, most notably the brain, eye and muscle.

Associated with this process of dissemination are a wide variety of clinical manifestations. The first recognized example involved the identification of *T. gondii* in a human fetus [4]. Subsequently, it was recognized that, in the case of primary *T. gondii* infection during pregnancy, the invasion of the placenta allows the parasites to infect the fetus and can lead to abortion, malformation of the fetus and congenital toxoplasmosis. Despite the fact that *Toxoplasma* affects multiple tissues, the most common clinical manifestations of toxoplasmosis involve the brain and eye. Thus, even in adults, primary infection can present as chorioretinitis and, in chronically infected individuals who develop defects in T cell function, such as during HIV infection or following chemotherapy, the reactivation of cysts in the brain can lead to Toxoplasmic encephalitis (TE). The basis for this tropism is uncertain – but could be a consequence of the immune privileged nature of these sites. Alternatively, there are multiple instances of parasites that affect the nervous system of their hosts to alter behaviour and promote predation of intermediate hosts [5–7]. For example, *Dicrocoelium dendriticum,* a flatworm, alters the behaviour of its intermediate host, the ant, to improve the chances of parasite transmission to herbivores (for an entertaining review on parasites and behaviour see [8]). Indeed, behavioural studies using mice and rats indicated that chronic *T. gondii* infection results in a specific switch from an aversion to cat urine to an attraction, presumably a change in behaviour that would lead to increased predation of infected rodents by cats, the definitive host, allowing sexual reproduction in the cat intestine [9].

Regardless of the biological consequences of the dissemination of *T. gondii*, many questions remain about the cellular basis for these events. For many pathogens, it is clear that an effective localized immune response can limit replication and the ability of a micro-organism to disseminate out of these focal areas would decrease the likelihood of the infection being completely eliminated. While the motility of some extracellular pathogens may allow these organisms to avoid the cellular components of the immune system, there

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are also examples where immune populations are hijacked for the success of the pathogen. Unlike other organ systems, the immune system is largely motile: cells detect foreign agents and travel to lymphoid organs for the activation and generation of cell-mediated and humoral responses, primed effector cells can traffic into and out of sites of inflammation. The aim of this review is provide an overview of our current understanding the role of the immune system in these events that lead to the dissemination of *T. gondii* and how this impacts the pathogenesis of this infection.

Mechanisms of spread

In general, dissemination of pathogens through the host may occur either extracellularly, with active or passive movement of the infectious agent via the blood or lymph, or host cell-dependent mechanisms whereby infected cells transport the micro-organism to distant sites. For *T. gondii,* there is evidence (reviewed below) that dissemination can occur via the migration of free parasites in blood and tissues, intracellular dissemination by hijacking migrating leukocytes, or extracellular migration with the parasite attached to the outside of the migrating leukocyte *(Fig. 1)*.

Extracellular migration

Toxoplasma tachyzoites are released from infected cells by either the mechanical rupture of the host cell due to the growing number of parasites within the host cell, or due to the induction of parasite egress in response to changes within the host cell [10, 11]. Free *T. gondii* tachyzoites are detected in the blood of infected mice [12]. Like other members of the *Phylum apicomplexa, T. gondii* tachyzoites employ an actin- and myosin-dependent gliding motility that is required for the invasion of host cells, parasite egress and virulence [13–15] (for review see [16]). The gliding motion in *Toxoplasma* tachyzoites has been largely studied across substrates *in vitro* and it is not known whether free parasites *in vivo* use this process for extended movement across biological barriers or within a tissue [17–19]. However, *in vivo* imaging studies on another apicomplexan have shown that *Plasmodium* sporozoites move with gliding motility in the skin to reach a blood or a lymphatic vessel [20–22] (for review see [23]). Moreover, *Plasmodium* sporozoites can also migrate through cells, without the formation of a parasitophorous vacuole or rupturing these cells, in order to migrate from the skin, into the blood circulation and within the liver before eventually infecting Kupffer cells [20, 24–26]. It remains an open question whether *T. gondii* utilizes a similar mechanism to invade other tissues or cross biological barriers.

Host cell-dependent migration

The concept of a "Trojan horse" in microbiology refers broadly to any infectious agent that hides within a cell to gain access to a target tissue that would normally be difficult to enter. For *Listeria monocytogenes*, a subset of infected monocytes are thought to help this bacterium to enter the central nervous system [27]. The tracking of transferred *T. gondii*-infected dendritic cells and monocytes to the brain in mice provided experimental evidence that similar events may occur during toxoplasmosis [12, 28]. In addition, following colonization of the small intestine, the lamina propria (LP) and the mesenteric lymph nodes (MLN), a population of dendritic cells ($CD11c^{+}CD11b^{+/-}$) and monocytes ($CD11b^{+}CD11c$) are preferentially infected and these can be detected in the blood and then in the brain, suggesting that these infected populations may allow *T. gondii* access to the central nervous system (CNS) [12]. Additional cells types and subsets, such as plasmacytoid dendritic cells, have also been implicated in dissemination of *T. gondii* to specific locations [29]. Interestingly, there is a population of cells that have tachyzoites attached to the external surface of the cell, suggesting that *T. gondii* can bind to surface receptors on the host cell or to the host cell's plasma membrane itself and piggy-back it's way to distant sites [12]. The mechanism involved in this attachment is unknown but since tachyzoites attached to the outside of the cell would be exposed to parasite-specific antibodies, this form of transport may only be relevant early in infection before production of parasite specific antibodies.

Crossing barriers

As highlighted earlier *Toxoplasma* must cross multiple biological barriers in order to infect, escape clearance and persist within the host. Here, we will focus on the main sites of pathology following primary *T. gondii* infection or reactivation, namely the gut, placenta, brain and eye. Each site has unique characteristics, and it should be noted that the latter three sites actively restrict access of immune cells. Regardless, *T. gondii* must cross a polarized cell layer made up of epithelial cells, endothelial cells or trophoblasts in order to access these sites and major questions remain about how these events occur.

The gut

Following ingestion, *T. gondii* must first cross a layer of mucus to access the gastrointestinal epithelium, below which lies the basement membrane, the lamina propria, populated by the host's immune cells, and a thin layer of smooth muscle. Mucosal trapping has been linked to preventing invasion by helminth parasites but whether this represents a serious impediment to *T. gondii* is unknown [30]. In current models the infection of epithelial cells leads to an early amplification step and the subsequent release of tachyzoites into the lamina propria and the blood stream. However, *T. gondii* tachyzoites may also transmigrate across the gut epithelial layer by moving between epithelial cells [31]. Studies using polarized cell monolayers *in vitro*

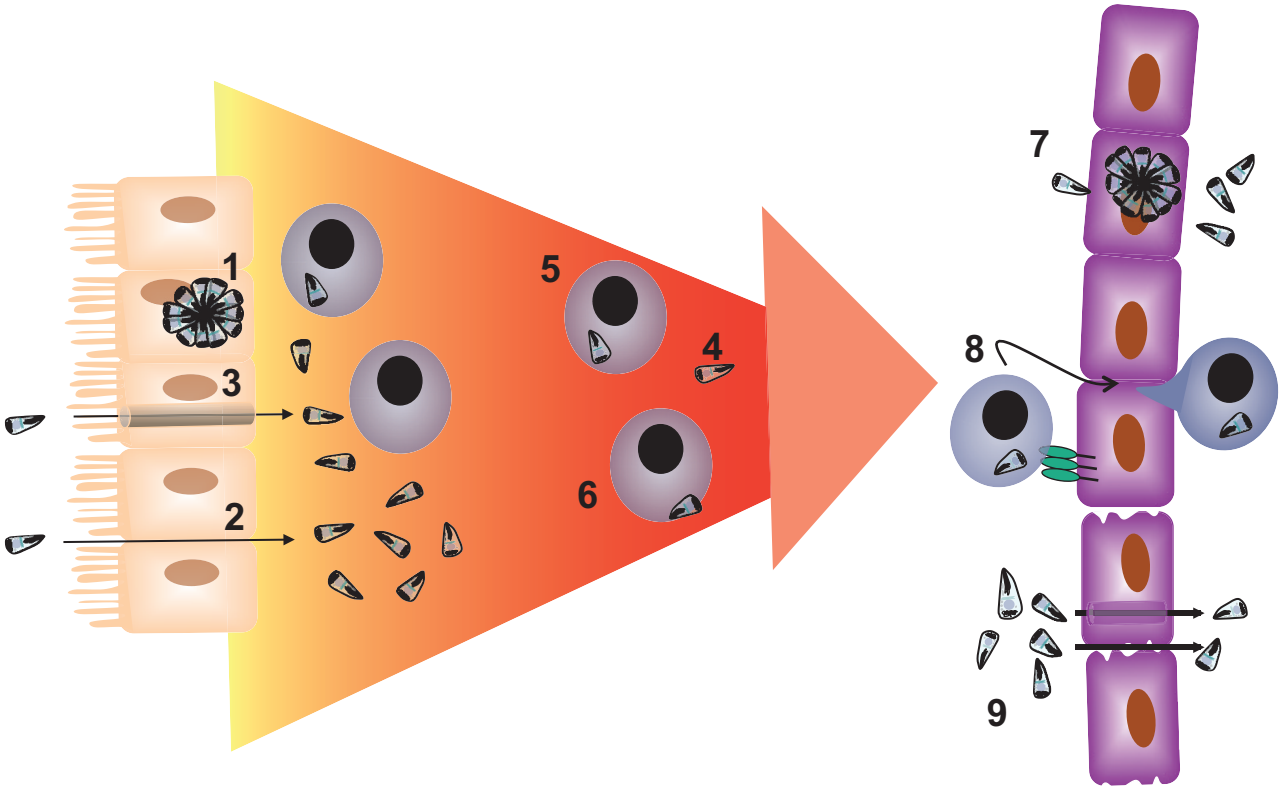

Fig. 1. Dissemination of *Toxoplasma gondii* in the intermediate host. Following ingestion *T. gondii* crosses the gastrointestinal tract via [1] infection of and replication within the gastrointestinal epithelial cells, [2] a paracellular route, and/or [3] by traversal of the gastrointestinal epithelial cell. Dissemination of *T. gondii* throughout the intermediate host may involve [4] the extracellular migration of the tachyzoite in blood or lymph, [5] intracellular migration, and/or [6] cell-dependent migration with adhesion to the host cell membrane. In order to penetrate tissues sites, such as the placenta, brain or eye, *T. gondii* may [7] infect the epithelial/endothelial or trophoblast cells that make up the tissue barrier, [8] employ a Trojan horse, and/or [9] enter the tissue via a paracellular or transcellular means. The parasite may also enter the tissue following increase permeability of the barrier in response to inflammatory signals

and mouse intestines *ex vivo* show parasites concentrated around intercellular junctions, suggesting that *T. gondii* uses a paracellular pathway that does not appear to disrupt the integrity of the cells or the gap junctions between the cells [32]. Interestingly, the parasite adhesin, MIC2, has an extracellular domain with two adhesive modules that can bind to intercellular adhesion molecule (ICAM)-1 on host cells and this binding is required for this migration [32]. Moreover, Barragan and Sybley [31] found that virulent type 1 strains of *T. gondii* have a greater capacity for transmigration across the gut epithelial layer, as well as penetration of lamina propria, submucosa and vascular endothelium, than the less virulent type II or type III strains. These observations suggest that the ability of different strains of *T. gondii* to cause fundamentally different courses of infection may be apparent at the very earliest points in the host–pathogen interaction.

Placental transmission

Once *Toxoplasma* has established infection, the process of dissemination occurs and, for vertical transmission, the parasite must cross the placenta. Materno-fetal transmission is generally restricted to cases where the mother experiences primary *T. gondii* infection during gestation [33]. Within the placenta, a single layer of fetal trophoblast cells is in contact with maternal blood for the metabolic exchange of gas and nutrients. This layer can also act as a barrier, protecting the fetus from infections occurring in the mother. However, there are parasitic, bacterial and viral pathogens, such as *T. gondii*, *Neospora cananium, Plasmodium falciparum, L. monocytogenes* and cytomegalovirus (CMV), that can harm the fetus by direct infection or by disrupting the placenta or fetal environment. Perhaps the best example of the latter process involves the maternal and fetal morbidity during malaria [34–36]. In this case, *Plasmodium falciparum* isolates expressing the variant surface antigen *var2csa* gene on the surface of the red blood cell specifically adhere to the receptor chondroitin sulfate A on the placenta [37–39]. The accumulation of infected red blood cells at the placenta allows the transport of infected red blood cells or *Plasmodium* antigens across the placenta, leading to severe complications that affect fetal development, as reviewed elsewhere (see [40]). For congenital CMV infection, which is considered the most frequent congenital infection in humans, transmission to the fetus is thought to occur by the direct infection of placental tissue,

leading to the release of CMV into the amniotic fluid and ingestion by the fetus, as well as by trans-placental migration of infected leukocytes when the mother has an active infection (reviewed by [41]).

In the case of congenital *Toxoplasma*, although early infection of the fetus results in severe manifestations, transmission across the placenta is more common in the later stages of infection and long-term effects can include retinochoroiditis during childhood and adolescence [42]. Whether parasite strain influences tropism for the placenta has not been directly assessed but, in Europe, greater than 84% of congenital toxoplasmosis cases were found to be due to the avirulent type II strain [43]. *In vitro* studies have shown that *T. gondii* infects human trophoblasts, suggesting that natural egress of parasites would allow the parasites to cross the placenta [44] but whether *T. gondii* tachyzoites make a paracellular transit across this barrier is unknown. *In vitro* studies show that human trophoblast cells up-regulate ICAM-1 and other adhesion molecules in the presence of *T. gondii*-infected cells and that ICAM-1 is required for the binding of these cells to the trophoblasts [46]. Given that *T. gondii*-infected cells may allow this parasite to cross biological barriers at other sites, intracellular traversal of the placenta may also take place. Further studies are required to determine whether *Toxoplasma*-infected maternal cells are found in cord blood, which would provide support for the Trojan horse model during congenital toxoplasmosis.

One other aspect of congenital toxoplasmosis that is poorly understood is how exposure of the fetus to parasite antigens impacts on the developing immune system. For Plasmodium*, in utero* exposure to *malaria* antigens may result in a tolerant phenotype in a subset of pre-exposed children, leading to increased susceptibility to malaria [34, 47]. T cell anergy to *T. gondii* antigen has been reported in some children with congenital Toxoplasmosis, suggesting that *in utero* exposure to *T. gondii* may also result in a tolerance to *T. gondii* [48–50]. This topic requires further investigation to determine whether congenital infection impacts on the long-term control of this persistent infection.

Blood-brain barrier

While the brain and eye are perhaps the two most clinically important sites, there is still remarkably little known about how *T. gondii* can access these unique anatomical locations. There are two main entry sites for motile cells or infectious agents into the brain, namely across the blood-brain barrier (BBB) or indirectly through the choroid plexus into the cerebrospinal fluid (CSF). The ependymal cells of the choroid plexus form a barrier between the blood capillaries of the choroid plexus and the CSF (reviewed in [51]). The endothelial cells of these capillaries are fenestrated, allowing easy leakage of blood components, while there are tight junctions between the ependymal cells, limiting blood-CSF mixing. Infection of the choroid plexus is observed amongst AIDS patients with acute cerebral toxoplasmosis, suggesting that the CSF may also be involved in the dissemination of *Toxoplasma* [52]. Indeed, *Toxoplasma* cysts and tachyzoites have been found in the CSF of mice and patients with Toxoplasmic encephalitis but there is little evidence for the dissemination of tachyzoites in the CSF during the acute stage of a primary infection [53–55].

The BBB separates the luminal contents of the blood vessel from the brain parenchyma, and consists of microvascular endothelial cells and pericytes surrounded by a layer by basement membrane, and astrocytic end feet (reviewed in [51]). The tight junctions between endothelial cells on the lumen side, as well as transporters, control the movement of ions, proteins and cells across the BBB into the brain parenchyma. This physical barrier limits direct access of many systemic pathogens to the CNS. However, many bacteria, viruses, fungi and parasites are thought to employ transcellular, paracellular and/or Trojan horse mechanisms to penetrate the BBB [27, 56–61]. It is not known whether the first tachyzoite to enter the brain does so in free tachyzoite form or within a host cell. Although brain endothelial cells can be infected by *T. gondii in vitro*, it is not known whether brain endothelial cells are infected during the acute stages of infection and whether this would facilitate the migration of tachyzoites into the brain [62]. Certainly, during toxoplasmosis, cell adhesion molecules ICAM-1 and VCAM-1 are up-regulated on the brain microvascular endothelial cells [63, 64] and these adhesion molecules are involved in the ability of T cells to crawl along the brain endothelium towards sites permissible for diapedesis [65, 66]. Chemokines, such as RANTES, monocyte chemoattractant protein-1 and CXCL10, which attract leukocytes into tissues, are expressed in the brain during chronic stage *T. gondii* infection and, together with adhesion molecule expression, may be involved in the recruitment of effector leukocytes to the brain [67, 68]. Similar events may aid in the movement of *Toxoplasma,* residing in leukocytes, over the BBB.

Blood retina barrier

T. gondii infection is responsible for considerable ocular diseases and *T. gondii* is the leading cause of infectious posterior uveitis [69, 70]. Ocular lesions are frequently a consequence of congenital infection; however, there are instances where postnatally acquired *T. gondii* can also cause ocular disease [71]. Indeed, like the brain, the eye is considered immune privileged – and the reactivation of this infection in this site, even in immune compromised patients, can cause severe tissue destruction that is the hallmark of this clinical disease. Human retinal epithelial cells are more susceptible to infection by *T. gondii* than human dermal epithelial cells *in vitro*, perhaps indicating that *T. gondii* may be specialized to infect retinal epithelial cells [72]. It has been proposed that tachyzoites can reach the retina by migration from the brain via the optic nerve, through passage of infected monocytes or dendritic cells across the retinal blood barrier, and by subsequent infection of the retinal vascular endothelium (summarized by [72]).

Future directions

Toxoplasma is a successful parasite due to its ability to navigate the host's barriers, to disseminate widely throughout the host, and to persist in immune-privileged tissues, poised for further transmission. The dissemination of this organism, as well as the traversal of tissue barriers, could involve a combination of direct migration or utilization of host cells. These mechanisms, however, do not appear to be unique to *T. gondii* and similar mechanisms are exploited by other infectious agents. For example, the bacterium *L. monocytogenes* also crosses the intestinal, placental and blood-brain barriers, and it is at these sites that the pathology associated with this infectious agent occurs (reviewed in [73]). Like *Toxoplasma*, host adhesion molecules on the gastrointestinal epithelia are used by *L. monocytogenes* for entry into the host, and this pathogen employs monocytes as Trojan horses to penetrate the CNS [27, 74–76].

Despite similarities to other pathogens, there remain major questions about the precise mechanisms that allow *Toxoplasma* to access multiple immune privileged sites, but progress in understanding these events has been limited by technology. Bioluminescence imaging of mice infected with luciferase-expressing *T. gondii* has provided a more quantitative approach to follow dissemination and insights into the timing and the location of parasite spread in the same animal [77, 78]. However, bioluminescence imaging in its present state is not sufficiently sensitive to distinguish individual invasion events or track single infected cells over time. In recent years, multiphoton imaging of host immune cells has led to a better understanding of the behaviour of these cells within *Toxoplasma* infected mice, particularly within the brain during the chronic stage of infection [79–81]. Intravital imaging of single fluorescent *Toxoplasma* tachyzoites at barriers within the model system will allow many of the finer details of *Toxoplasma* dissemination to be elucidated. Given the genetic tractability of this parasite, understanding the cellular and molecular basis for these types of studies could be broadly applicable to other related parasites, like *Neospora* and *Plasmodium,* which also face similar challenges within the intermediate host.

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