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From the blood to the brain: avenues of eukaryotic pathogen dissemination to the central nervous system

Norikiyo Ueno and Melissa B Lodoen

Infection of the central nervous system (CNS) is a significant cause of morbidity and mortality, and treatments available to combat the highly debilitating symptoms of CNS infection are limited. The mechanisms by which pathogens in the circulation overcome host immunity and breach the blood–brain barrier are active areas of investigation. In this review, we discuss recent work that has significantly advanced our understanding of the avenues of pathogen dissemination to the CNS for four eukaryotic pathogens of global health importance: *Toxoplasma gondii*, *Plasmodium falciparum*, *Trypanosoma brucei*, and *Cryptococcus neoformans*. These studies highlight the remarkable diversity of pathogen strategies for trafficking to the brain and will ultimately contribute to an improved ability to combat life-threatening CNS disease.

Address
Department of Molecular Biology and Biochemistry and the Institute for Immunology, University of California, Irvine, CA, USA

Corresponding author: Lodoen, Melissa B (mlodoen@uci.edu)

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Introduction

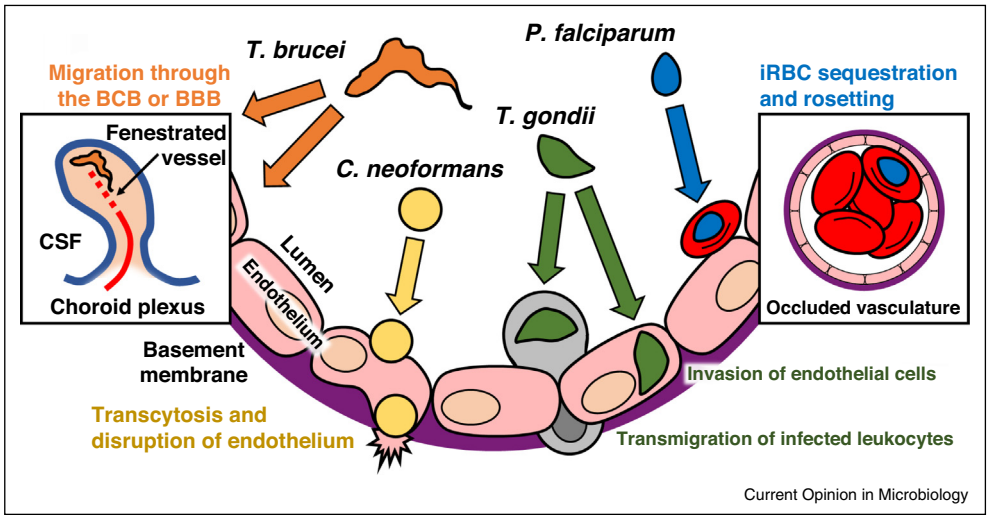
The central nervous system (CNS) is protected by a formidable and unique barrier system. Remarkably, several pathogens have evolved mechanisms to breach this barrier and cause disease. Pathogens circulating in the bloodstream may access the brain parenchyma by crossing the blood–brain barrier (BBB) or through other portals of entry, such as the peripheral nerve root ganglia or the choroid plexus, which generates the cerebrospinal fluid (CSF). The blood vessels of the BBB are comprised of densely packed endothelial cells that are linked by tight junctions and surrounded by pericytes and astrocyte endfeet [1]. Collectively, these cells function to restrict the passage of molecules, pathogens, and leukocytes into the parenchyma. In contrast, endothelial cells of the choroid plexus are fenestrated, permitting molecules from the bloodstream into the choroid plexus stroma. Tight junctions interconnect plexus epithelial cells, which line the choroid plexus and form the blood–CSF barrier (BCB) [2]. These barriers physically protect the brain from microbial invasion and toxins and mediate the selective permeability of nutrients and ions. As discussed below, the pathogens that can overcome this unique barrier and enter the CNS often cause severe, life-threatening disease.

In this review, we will highlight recent findings on four eukaryotic pathogens of global health relevance. Although many researchers have made valuable contributions to the field of CNS infection, the focus for this review will be on research published in the past three years. Interestingly, the pathogens discussed here appear to use distinct routes for trafficking to the CNS (Figure 1) and cause a diverse range of disease symptoms. *Toxoplasma gondii* establishes a chronic infection in the parenchyma, and parasite reactivation can cause encephalitis in immune compromised individuals. *Trypanosoma brucei* invasion of the brain leads to the neurological disorders and sleep disturbances that characterize African sleeping sickness. In contrast, red blood cells infected with *Plasmodium falciparum* do not enter brain tissue, but instead become sequestered in the cerebral microvessels, resulting in vascular obstruction, a defining feature of cerebral malaria (CM). The fungal pathogen *Cryptococcus neoformans* causes a respiratory infection that can undergo hematogenous spread to the brain and is a major cause of CNS disease in HIV/AIDS patients. The outcomes of these CNS infections vary depending on the pathogen, the immune status of the host, and the stage of infection that occurs in the brain. Recent work in these fields and the use of intravital imaging technologies have significantly expanded our understanding of pathogen entry into the brain as well as revealed areas that require further investigation.

T. gondii entry into the brain: free parasites or 'Trojan horse'?

Infection by *T. gondii* typically occurs via the oral route through the ingestion of parasite cysts. Evidence suggests that the circulation is a major avenue for *T. gondii* dissemination to distal organs, including the brain. The replicative tachyzoite form of the parasite multiplies in the small intestine [3] and encounters an influx of neutrophils and inflammatory monocytes [4]. Within days after oral infection of mice with tissue cysts, *T. gondii* can be detected both inside monocytes and as extracellular parasites in the blood [5]. Systemic inflammation is

Figure 1



Eukaryotic pathogens utilize a diverse range of strategies to migrate from the blood to the CNS. A schematic shows the cross section of a blood vessel of the BBB and potential pathways of CNS entry for four global pathogens. *T. gondii* can invade and replicate in brain endothelial cells and may undergo transendothelial migration either as a free tachyzoite or inside an infected leukocyte. Cerebral malaria is associated with sequestration of *P. falciparum*-infected red blood cells (iRBCs) in the brain microvasculature and binding of iRBCs to uninfected RBCs in a process known as rosetting. This leads to the obstruction of blood flow and may contribute to breakdown of the BBB and vascular leakage. *T. brucei* likely crosses the BCB via fenestrated vessels inside the choroid plexus, followed by trafficking to the meninges. Some evidence suggests that enhanced vascular permeability induced by host inflammatory responses may allow the parasite to directly cross the BBB. *C. neoformans* can cytoadhere to endothelium, often at narrow points in the blood vessels and undergo transcytosis. Endothelial cells that internalize yeast lose their structural integrity, resulting in cell stress and injury.

characteristic of *in vivo T. gondii* infection, and increased BBB permeability has been associated with the development of toxoplasmic encephalitis [6]. Ultimately, the parasites enter the brain parenchyma and establish a chronic infection as bradyzoite-containing tissue cysts.

The precise mechanism by which *T. gondii* breaches the BBB remains unknown; however, several possibilities have been investigated. Extracellular *T. gondii* tachyzoites can adhere to human vascular endothelium in conditions of shear stress by using the parasite adhesin MIC2 [7]. After adhesion, tachyzoites may either transmigrate across the endothelial barrier [8] or invade endothelial cells [9], and invasion appears to predominate [7]. Interestingly, human brain endothelial cells are more permissive to *T. gondii* replication than neurons or microglia [10]. The extracellular parasite can also migrate through multiple tissue layers of the human retina [11]. Infection enhances the expression of the host adhesion molecules ICAM-1, VCAM-1, and ALCAM in the CNS [6,12]. In particular, ICAM-1 interacts with MIC2 to facilitate the migration of extracellular tachyzoites across epithelium [13] and retinal endothelium [8] without disrupting monolayer integrity.

Recent studies have also expanded our understanding of the 'Trojan horse' mechanism of dissemination, by which the extravasation of parasitized leukocytes facilitates tachyzoite translocation across barriers. Following oral infection of mice, *T. gondii* are found in the brain in $CD11b^{+}/CD11c^{-}$ monocytes [5], and a direct comparison of GFP^{+} intracellular and $DsRed^{+}$ extracellular parasites injected into mice resulted in a significantly greater number of GFP^{+} tachyzoites in the brain [14]. More recently, infected human dendritic cells (DCs) and murine monocytes were shown to efficiently transmigrate across retinal and brain endothelium, respectively [12,15], supporting the idea that infected cells may facilitate *T. gondii* entry into the CNS.

T. gondii infection induces hypermotility in migratory cells, and this phenotype correlates with cytoskeletal rearrangement in infected DCs [16] and monocytes [17]. Interestingly, the DC hypermotility is linked to signaling by the neurotransmitter GABA [18••]. The degree to which hypermotility plays a role in crossing the BBB is still not well understood. Under fluidic shear stress, *T. gondii* delays the firm adhesion of infected human monocytes on vascular endothelium [17] and enhances their subsequent crawling [19]. This could be due in part to changes in adhesion molecules, since infection impairs integrin clustering [16,17]. *T. gondii*-infected monocytes appear to undergo TEM (transendothelial migration) via the paracellular route *in vitro* by a process involving the monocyte surface integrin Mac-1 (CD11b/CD18) and its binding partner ICAM-1 [19].