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Invited Review

Molecular mechanisms of host cell traversal by malaria sporozoites

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ABSTRACT

Malaria is a pernicious infectious disease caused by apicomplexan parasites of the genus *Plasmodium*. Each year, malaria afflicts over 200 million people, causing considerable morbidity, loss to gross domestic product of endemic countries, and more than 420,000 deaths. A central feature of the virulence of malaria parasites is the ability of sporozoite forms injected by a mosquito to navigate from the inoculation site in the skin through host tissues to infect the liver. The ability for sporozoites to traverse through different host cell types is very important for the successful development of parasites within the mammalian host. Over the past decade, our understanding of the role of host cell traversal has become clearer through important studies with rodent models of malaria. However, we still do not understand the stepwise process of host cell entry and exit or know the molecular mechanisms governing each step. We know even less about cell traversal by malaria parasite species that infect humans. Here, we review current knowledge regarding the role and molecular mechanisms of sporozoite cell traversal and highlight recent advances that prompt new ways of thinking about this important process.

1. Introduction

Malaria has been a scourge of humanity and is still responsible for enormous loss of productivity and human life. In 2015, over 200 million infections occurred, which caused severe morbidity and the loss of over 420,000 human lives (World Health Organization, 2015). Malaria is caused by *Plasmodium* parasites that are transmitted to humans via the bite of an infected female *Anopheles* mosquito. Once inoculated into the host, the sporozoite form of the parasite migrates from the dermis to the circulating blood and is carried to liver sinusoidal capillaries (Amino et al., 2006). Here, it penetrates the sinusoids to reach the liver parenchyma and then to infect a hepatocyte (Pradel and Frevert, 2001; Frevert et al., 2005). Within the hepatocyte, a sporozoite differentiates inside a parasitophorous vacuole membrane into exoerythrocytic forms (EEFs) that eventually become thousands of merozoites within merosomes (Sturm et al., 2006). Following completion of development, merosomes exit the liver parenchyma and enter the bloodstream. Rupture of merosomes releases the large number of merozoites, which subsequently infect erythrocytes and cause the clinical pathogenesis of malaria.

The path from the dermis to the liver is complex and consists of various physical host cellular barriers that must be navigated and overcome. These barriers include dermal fibroblasts, endothelial cells of blood vessels, phagocytes in the dermal and liver sinusoidal layers including Kupffer cells and hepatocytes. Sporozoites have evolved the remarkable ability to transmigrate through these host cells by a process known as cell traversal. Cell traversal involves active sporozoite entry into a host cell, rapid migration through the cytosol, and then exit through the host cell plasma membrane (Vanderberg et al., 1990; Mota et al., 2001). Although the entry step of traversal has historically been considered to involve lysis of the host cell membrane and entry without a parasitophorous vacuole, very recent evidence has demonstrated that traversing sporozoites can enter the hepatocytes through a transient vacuole, so called due to the rapid escape of the sporozoite from this compartment before exiting the cell (Risco-Castillo et al., 2015). This important advance raises a variety of mechanistic questions such as what are the proteins involved in the entry and exit steps of traversal, what factors determine the formation of the transient vacuole as opposed to the longer-lasting parasitophorous vacuole, and do sporozoites form these structures when traversing cells other than hepatocytes? Furthermore, since the majority of studies informing this fascinating area have been conducted with malaria parasites that infect rodents, there is a large gap in knowledge regarding the *Plasmodium* spp. responsible for the full human toll of malaria. It is clear that while extensive understanding has been

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gained over the past decade, our understanding of traversal is continually evolving and that there are many more fundamental questions still to be answered.

2. Early observations of sporozoite traversal

Sporozoite traversal of host cells was first observed by the rodent-infecting malaria parasite *Plasmodium berghei* using rodent macrophages (Vanderberg et al., 1990). Sporozoites were seen to actively enter and exit host cells rapidly, sometimes leading to cell death. This process is highly dependent on motility of the parasite, as traversal was not observed when sporozoites were immobilized using anti-sporozoite antibodies. Mota and colleagues (2001) first showed that sporozoite traversal was not limited to macrophages but could also be observed in hepatocytes and epithelial cells (Mota et al., 2001). It was shown that the host cell membrane is damaged during this process, using a cell wounding and membrane repair assay involving entry of fluorescent dextran; this remains an important tool to study traversal to this day. In the wounding assay, the normally cell-impermeant fluorescent tracer could be observed inside host cells by microscopy following incubation with sporozoites, demonstrating that the host plasma membrane had been breached, although the timing of rupture, i.e. upon parasite entry or exit from the host cell, was unclear from this assay alone. Electron microscopic analysis identified some hepatocytes with intracellular sporozoites lacking a parasitophorous vacuole membrane and, in rare cases, an apparent entry wound in the host cell membrane of infected cells (Mota et al., 2001). This evidence suggested that sporozoites traverse hepatocytes by actively wounding the host plasma membrane during the entry step, without the formation of a parasitophorous vacuole membrane, and during exit from the host cell.

3. Role of cell traversal during infection of the rodent host

3.1. Sporozoite traversal at the dermis

The skin is the largest organ of the body and provides the first line of protection from external pathogens. Before taking a bloodmeal, a parasite-infected female *Anopheles* mosquito will penetrate the skin and dermal layer of a mammalian host with her proboscis to find a blood vessel. During this time, she secretes saliva that anesthetizes the dermal area and inadvertently injects a small inoculum of sporozoites from the salivary glands into the dermis (Rosenberg et al., 1990; Amino et al., 2006) (Fig. 1). This location presents various physical cellular barriers such as fibroblasts and leukocytes that the sporozoite must surmount in order to enter a blood vessel for transport to the liver. Following an infectious mosquito bite, sporozoites migrate within the skin of mice for extended time periods of up to 3 h (Vanderberg and Frevert, 2004; Yamauchi et al., 2007). Sporozoite migration out of the dermis requires gliding motility, and mutation of the thrombospondin related anonymous protein (TRAP) dramatically impairs motility and sporozoite infectivity (Ejigiri et al., 2012). Navigation from the dermis involves traversal through host cells (Coppi et al., 2007; Amino et al., 2008) and has been imaged and quantified in rodents. One study observed that approximately 50% of the sporozoite inoculum leaves the site after 30 or more minutes, either by invading the blood (70%) or the lymphatic (30%) vessels (Amino et al., 2006). In contrast, traversal-deficient sporozoites (see Section 5.1 for a detailed description of these transgenic parasites) were often found trapped in dermal fibroblasts or leukocytes and were destroyed; thus, they were unable to reach the blood vessels and liver infection did not ensue (Amino et al., 2008). Parasite entry to blood vessels can be blocked by anti-sporozoite antibodies

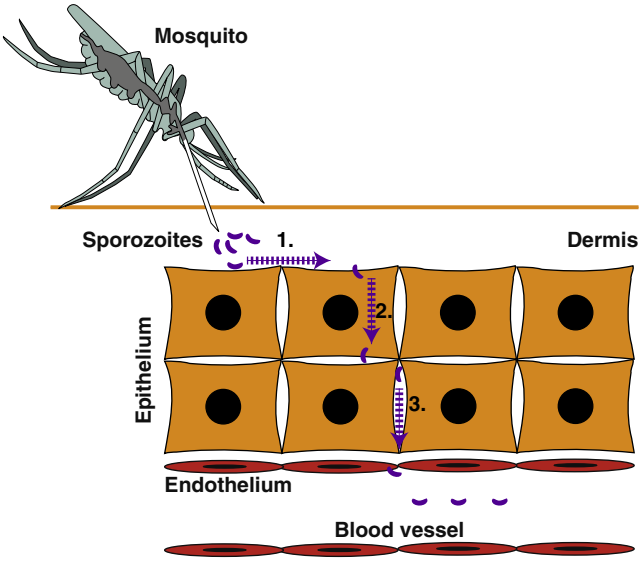

Fig. 1. Malaria sporozoite cell traversal at the dermis. During a blood meal by an infected *Anopheles* mosquito, a small number of sporozoites (purple) are deposited into the host's skin. The sporozoite must navigate the host cellular barriers present at this site in order to enter the blood circulation and be transported to the liver. To do so, they display a range of motility including gliding (1), host cell traversal (2) and transmigration between cells (3).

(Vanderberg and Frevert, 2004) and parasite exit from blood vessels involves passage through endothelial cells (Tavares et al., 2013). This provided important evidence that cell traversal is required to breach dermal epithelial cells as well as to escape phagocytosis to ensure successful access to a blood vessel for subsequent infection of a hepatocyte (Fig. 1).

3.2. Sporozoite traversal at the liver sinusoids

Once the sporozoite enters a blood vessel near the dermis, it is swiftly transported to liver lobules via the hepatic artery and arterioles, and then into sinusoidal capillaries. Here, the sporozoite intimately attaches to the endothelium before crossing the blood vessel by one of several possible routes. Sporozoites can either traverse sinusoidal resident Kupffer cells (Pradel and Frevert, 2001; Ishino et al., 2004; Frevert et al., 2005; Tavares et al., 2013), or migrate through endothelial cells (Tavares et al., 2013). They may also migrate between endothelial cells, as the liver sinusoids are comprised of a discontinuous, fenestrated endothelium. Exit from the sinusoidal layer allows sporozoites to access the parenchyma and infect hepatocytes (Fig. 2). Different experimental approaches provided evidence that Kupffer cells are targeted by sporozoites as the main gateway out of the liver sinusoids. Various microscopy techniques showed sporozoite passage through Kupffer cells by *P. berghei* and *Plasmodium yoelii* sporozoites (Pradel and Frevert, 2001; Frevert et al., 2005). Furthermore, infection with *P. yoelii* sporozoites of *op/op* mice, which contain a point mutation in macrophage colony stimulating factor (M-CSF) causing loss of the majority of Kupffer cells, caused a five-fold reduction in liver infection relative to wild type mice, suggesting that Kupffer cells are an important barrier and target for sporozoites (Baer et al., 2007). Using differential fluorescent labeling of Kupffer and endothelial cells in mouse liver sinusoids, it was shown by intravital microscopy that while the majority of the traversal events involve Kupffer cells (approximately 60%), approximately 17% of events occurred exclusively through endothelial cells (Tavares et al., 2013) (Fig. 2). This provided the first evidence of another gateway used by sporozoites to exit the sinusoids. This study also showed

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