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Invited Review

A model for the progression of receptor–ligand interactions during erythrocyte invasion by *Plasmodium falciparum*

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ABSTRACT

Multiple and seemingly sequential interactions between parasite ligands and their receptors on host erythrocytes are an essential precursor to invasion by the obligate intracellular pathogen, *Plasmodium falciparum*. Consequently, identification and characterisation of the specific effectors that facilitate these recognition events are of special interest for the development of novel therapeutic and prophylactic solutions to malaria. There have been many recent advances regarding the identification of host–parasite receptor–ligand pairs, however the precise function and temporal aspects of these interactions are far from resolved. This review provides an update on the current details of these interactions to place them in sequence and super impose them upon the known kinetic events of invasion.

1. Introduction

Malaria has plagued humankind for over 50,000 years and remains one of the world's most devastating diseases (Joy et al., 2003). It is estimated that several hundred million clinical cases of malaria arise each year resulting in 1 million deaths largely, but not exclusively, among children (WHO World Malaria Report, 2010; Murray et al., 2012).

Malaria is caused by parasites of the *Plasmodium* genus within the phylum Apicomplexa, which includes several other important human and animal pathogens such as the causative agents of toxoplasmosis (*Toxoplasma gondii*) and cryptosporidiosis (*Cryptosporidium parvum*). Of five species that infect humans, *Plasmodium falciparum* causes the greatest morbidity and mortality. Central to malaria pathogenesis is the asexual proliferation within erythrocytes where parasites undergo tremendous amplification at each replicative cycle. On lysis of the infected host cell, mature parasites can release up to 32 daughter merozoites that are capable of invading new red blood cells with remarkable co-ordination and speed (reviewed in Cowman and Crabb, 2006). The brief extracellular nature of the progeny merozoites, however, renders them fleetingly vulnerable to host defenses. Over time, naturally-exposed individuals mount antibody responses to various merozoite antigens and this gamma globulin has been harvested from malaria-immune individuals to treat non- and partially immune children (Cohen et al., 1961). It has become increasingly clear that a better understanding of the molecular aspects of invasion will pave the way for the development of novel targets against malaria. Here, the latest discoveries concerning host–parasite interactions within the pre-invasion stage of *P. falciparum* are reviewed. We propose a sequence in which these interactions occur within a 10 s window of distinct morphological stages (Fig. 1).

2. Kinetics and morphology of red blood cell invasion

Invasion of red blood cells by the extracellular merozoite encompasses a highly orchestrated sequence of molecular interactions and signal transduction events between the parasite and host erythrocyte that are likely similar for all *Plasmodium* spp. Evolving a rapid means of host cell entry has been a necessity for malaria parasites as their surface antigens are particularly vulnerable to immune attack. The rapidity of this event combined with the small size of merozoites (~1.5 μm), which is more typical of bacteria than eukaryotes, has made resolution of the individual stages of invasion extremely challenging. The pioneering observations of invasion made in *Plasmodium knowlesi* in 1975 are still relevant today (Dvorak et al., 1975). However, recent advances in the ability to film invasion, including widespread availability of microscopes

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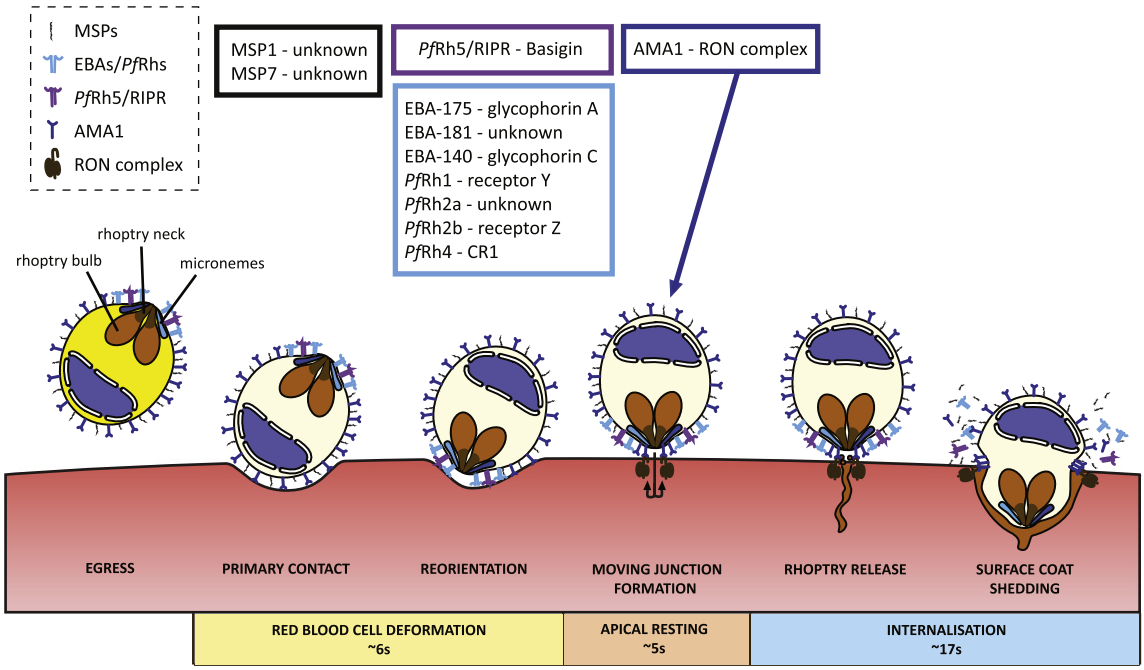

Fig. 1. The host–parasite interactions with a role in erythrocyte invasion by *Plasmodium falciparum*. Receptor–ligand pairs with established roles in invasion are listed in boxes. Parasite encoded proteins are featured in the key. Egress: low environmental potassium triggers a cytosolic calcium increase (yellow) and release of microneme effector proteins onto the surface. Primary contact: merozoites attach reversibly to an erythrocyte possibly via glycosylphosphatidylinositol-anchored merozoite surface proteins (black) and their associated partners. Reorientation: primary contact elicits waves of deformation in the red blood cell, resulting in reorientation of the parasite. This may employ a concentration of apical adhesins such as the erythrocyte binding-like and reticulocyte binding-like (*Pf*Rh) ligands (light blue) or *Pf*Rh5/Rh5 interacting protein (purple) binding to the host cell to hold the parasite in place during moving junction formation. Moving junction formation: parasites remain apically attached to allow translocation of the rhoptry neck protein complex (brown) into the erythrocyte and apical membrane antigen (dark blue) to collect at the apical tip. AMA1–RON complex binding triggers formation of the moving junction apposition between the merozoite and host cell through which the parasite invades. Invasion: during host penetration surface ligands are shed and the parasite invades into the nascent parasitophorus vacuole created by secretion of the rhoptries into the host cell.

with inbuilt cell incubators and high-speed digital cameras with matching computer power, have provided tremendous insight into parasite behaviour and have ultimately simplified dissection of these events (Glushakova et al., 2005; Gilson and Crabb, 2009b; Boyle et al., 2010; Abkarian et al., 2011).

Before merozoites can invade erythrocytes they must exit the host cell in which they are resident. Sequential disruption of the enveloping parasitophorous vacuole and host cell allows non-motile parasites to be ejected at high pressure and disperse among new red blood cells (Glushakova et al., 2005; Chandramohanadas et al., 2009; Abkarian et al., 2011). Merozoites then randomly encounter an uninfected erythrocyte upon which they form a reversible association that is low affinity but specific with parasites only attaching to cells from susceptible hosts (Miller et al., 1979). As was first observed in *P. knowlesi* and later in *P. falciparum*, this initial contact elicits waves of dynamic deformation in the red blood cell, tumbling the merozoite over the surface and allowing it to reorientate to its apical tip (Dvorak et al., 1975; Gilson and Crabb, 2009b). Lew and Tiffert (2007) suggest that host cell signalling may have an active role in this process, namely calcium-activated deformations of the red blood cell cytoskeleton, but effective data is lacking. With each wave the merozoite could establish a stronger point of contact via an apical adhesin gradient preventing backward rolling (Lew and Tiffert, 2007). Alternatively the apical tip may remain at the erythrocyte edge, as it is energetically favourable for membranes to contact at their most curved regions (Farrow et al., 2011). This period of reorientation continues on average for 6s followed by a 5 s apical 'resting period' when an electron-dense circumferential apposition reminiscent of a tight junction forms at the host–parasite interface (Aikawa et al., 1981; Gilson and Crabb, 2009b). This marks a point of irreversible contact and commits the merozoite to invasion, during which the 'moving junction' appears to encircle and migrate over the parasite as it is pulled through via its internal actin-myosin motor (Keeley and Soldati, 2004; Baum et al., 2006). Internalisation of the parasite occurs over a period of approximately 17 s (Gilson and Crabb, 2009b). Also following moving junction formation, rhoptry organelles secrete numerous proteins and lipids into the host cell that are incorporated into the nascent parasitophorous vacuole to accommodate the developing parasite (Lauer et al., 2000; Fig. 1). This pre-invasion stage is a critical mediator of invasion efficiency and appears to be completely conserved over the large evolutionary distance between *P. knowlesi* and *P. falciparum* (Dvorak et al., 1975; Gilson and Crabb, 2009b). Resolution of the mechanisms underlying host cell recognition and attachment will be critical to exploiting the key molecular aspects of red blood cell invasion.

3. Molecular aspects of red blood cell invasion

Characteristic of the phylum Apicomplexa, the invasive zoite not only displays invasion ligands on the cell surface but also stores them within apical organelles that are secreted in response to specific signals. Notably, *P. falciparum* micronemes carry many important surface proteins that are discharged upon red blood cell rupture when low potassium levels in the plasma trigger calcium release from the endoplasmic reticulum (Singh et al., 2010). Super resolution microscopy is now emerging as a powerful means of studying these proteins and their involvement in invasion (Boyle et al., 2010; Riglar et al., 2011). With the dynamics of erythrocyte invasion now well established, together with the identification of many key players, the next challenge is to map out the temporal succession of these molecular events.