The Molecular Basis of Erythrocyte Invasion by Malaria Parasites

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The Molecular Basis of Erythrocyte Invasion by Malaria Parasites

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Plasmodium species cause malaria by proliferating in human erythrocytes. Invasion of immunologically privileged erythrocytes provides a relatively protective niche as well as access to a rich source of nutrients. Plasmodium spp. target erythrocytes of different ages, but share a common mechanism of invasion. Specific engagement of erythrocyte receptors defines target cell tropism, activating downstream events and resulting in the physical penetration of the erythrocyte, powered by the parasite’s actinomyosin-based motor. Here we review the latest in our understanding of the molecular composition of this highly complex and fascinating biological process.

Introduction
Despite increased control measures, malaria remains a major threat, killing an estimated 500,000 people each year (reviewed in Cowman et al., 2010).
Introduction

• There are six species(?) that cause malaria in humans:

✓ Plasmodium falciparum
✓ P. vivax
✓ P. knowlesi
✓ P. malariae
✓ P. ovale curtisi
✓ P. ovale wallikeri

• Colin J. Sutherland et al (2010):
  we propose to name these species *Plasmodium ovale curtisi* (classic type) and *Plasmodium ovale wallikeri* (variant type), in honor of Christopher F. Curtis (1939–2008) and David Walliker (1940–2007), respectively.

• Both P. ovale subspecies have been identified in Ghana, *Myanmar*, Nigeria, São Tomé, Sierra Leone and Uganda

• P. Falciparum is considered the most important because it causes the vast majority of deaths.
Merozoite

• The blood-stage merozoite of Plasmodium spp. is relatively small with a length and breadth of approximately 1–2 μm. Merozoites have a single goal—to invade the erythrocyte—and as such are designed solely for this purpose.

• The merozoite apical end contains organelles and structures, including:

  - **Micronemes** contain adhesins involved in erythrocyte binding.
  
  - **Rhoptries** are released after initial host cell engagement to facilitate the invasion process and form the parasitophorous vacuole.
  
  - **Dense granule** organelles may also have distinct subpopulations, and there is a subset of organelles named Exonemes that release the protease subtilisin 1 (SUB1) into the parasitophorous vacuole, where it proteolytically processes several parasite proteins to facilitate merozoite egress.
Major proteins of the membrane of the human red blood cell
Major proteins of the membrane of the human red blood cell
Mechanical Steps of Erythrocyte Invasion-1

• The surface of the merozoites that first contacts the erythrocyte

• First visualized using video-microscopy of P. Knowlesi and subsequently defined for P. Falciparum.

• This has divided invasion into three phases:
  1. First, initial interaction and deformation of the erythrocyte membrane (Attachment).
  2. Second, apical interaction (Invasion).
  3. Third, Echinocytosis and recovery of the invaded host cell (Sealing).
Mechanical Steps of Erythrocyte Invasion-2

• Previously, it has been unknown whether the erythrocyte actively participates in the invasion process.

• Modeling of the merozoite interaction has been shown that merozoite contact results in increased phosphorylation of the erythrocyte cytoskeleton.

• **Real-time deformability cytometry and flicker spectroscopy** has shown that: binding of the parasite ligand EBA175 to its host receptor, glycophorin A (GPA), increases erythrocyte cytoskeletal tension and reduces the bending modulus of the cell’s membrane which correlate with efficiency of merozoite invasion.
Mechanical Steps of Erythrocyte Invasion-3
Molecular Basis of Erythrocyte Invasion-1

- Merozoite surface proteins (MSPs) could play a role in the first phase of invasion involving the initial interaction of the merozoite with the erythrocyte.

- MSP1 forms a large complex on the merozoite surface with a number of peripheral proteins and there is evidence that it is required for invasion.

- However, recent data have shown that merozoites lacking MSP1 expression can still invade, suggesting it may not be essential for this process.

- Indeed, MSP1 appears to be required for egress from the erythrocyte in which the SUB1 processed form of MSP1 interacts with the spectrin cytoskeleton of the erythrocyte.

- Surface and peripheral proteins may not be involved directly in the invasion process, some do play a role in protecting the merozoite from immune attack.
Molecular Basis of Erythrocyte Invasion-2

• A recent study in P. falciparum has shown Pf92, a member of the 6-cys family, actively recruits Factor H (FH).

• Hijacking FH allows the merozoite to down regulate complement activation on its surface, consequently protecting it from complement lysis.

• MSPs are also targets of acquired immunity.

• Human antibodies to MSP1 and MSP2 promote complement deposition on the merozoite and mediate inhibition of parasite invasion through C1q fixation.
Molecular Basis of Erythrocyte Invasion-3

- Initial tight interaction with the erythrocyte is mediated by two major families of adhesins (have been identified P. Vivax and P. Falciparum):
  - Duffy binding-like (DBL or erythrocyte-binding like [DBL]) protein.
  - Reticulocyte-binding-like protein homolog (Rh or RBL).

- P. falciparum, this family consists of:
  - EBA-175 (binds to GPA)
  - EBA-181 also known as JSEBL (unknown receptor)
  - EBA-140 also known as BAEBL (binds to Glycophorin C [GPC])
  - The RBL or Rh family:
    - PfRh1 (binds to unknown receptor)
    - PfRh2a (binds to unknown receptor)
    - PfRh2b (binds to unknown receptor)
    - PfRh4 (binds to complement receptor 1 [CR1, CD35])
    - PfRh5 (binds to Basigin [BSG, CD147]).
**Molecular Basis of Erythrocyte Invasion-4**

- When the merozoite initiates contact with the erythrocyte it weakly buckles, leading to stronger deformation of the erythrocyte through engagement of the EBAs/PfRhs with their receptors, such as CR1, GPA, GPB, and GPC, and the involvement of the actinomyosin motor.

- Merozoites that **strongly deform the erythrocyte** surface are more likely to successfully invade.

- This essential step in invasion is linked to a Ca2+ flux that has been suggested to emanate from the merozoite into the erythrocytes.

- During invasion, PfRh5 forms a complex with PfRipr (P. falciparum Rh5 interacting protein) and CyRPA (cysteine-rich protective antigen) that both are also required for the generation of the Ca2+ flux.

- This Ca2+ flux is strongly correlated with the observation of echinocytosis, the further release of the merozoite rhoptry contents, and successful invasion.
Molecular Basis of Erythrocyte Invasion-5

- co-localization of all three proteins (PfRh5, PfRipr, and CyRPA) at the merozoite apical tip when the parasite membrane is in close juxtaposition with the erythrocyte membrane such as tight junction formation.

- During this stage in invasion, the merozoite moves through the tight junction propelled by its actinomyosin motor to enter the erythrocyte. At its core the tight junction is composed of AMA1 and RON2.

- AMA1 is found on the surface of the merozoite and binds to RON2, which is part of a larger RON complex, and is one of the first proteins to be injected into the erythrocyte, emanating from the Rhoptry neck.

- Work from the related parasite T. gondii suggests that RON2 embeds itself into the host membrane, becoming the receptor for AMA1.

- An ingenious mechanism used by all apicomplexan parasites to deploy their own ligand receptor pair to facilitate successful invasion.
B

Tight Junction formation
- Rhotry Discharge
- AMA1-RON complex injection

Tight Attachment
- EBAs, RBPs, then Rh5 complex
- Membrane Deformation
- Membrane wrapping
- Pore formation

Penetration
- glideosome activity

Completion
- Sealing of vacuole
- echinocytosis and recovery

Initial Attachment
- MSPs?
Conclusion

• While there has been some controversy over the essentiality of AMA1 to parasite invasion, the vast body of evidence suggests that its interaction with the RON complex is highly important.

• In particular, antibodies that block function of PfRh5 and its partners show the most efficacious inhibition in both in vitro and in vivo models of parasite invasion.

• Collectively, the invasion-inhibitory studies show that targeting multiple steps in invasion is more likely to promote higher levels of parasite invasion inhibition.

• As a result, a multivalent vaccine will be more effective than a single subunit vaccine against P. falciparum.
Thanks for your attention