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Current Opinion

Insights and controversies into the role of the key apicomplexan invasion ligand, Apical Membrane Antigen 1

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ABSTRACT

Apicomplexan parasites are obligate intracellular pathogens that cause a host of human and animal diseases. These parasites have developed a universal mechanism of invasion involving formation of a 'moving junction' that provides a stable anchoring point through which the parasite invades host cells. The composition of the moving junction, particularly the presence of the protein Apical Membrane Antigen 1 (AMA1), has recently been the subject of some controversy. In this commentary we review findings that led to the current model of the moving junction complex and dissect the major conflicts to determine whether a substantial reassessment of the role of AMA1 is justified.

1. Introduction

Apicomplexan parasites represent an important and diverse group of human and animal pathogens, which includes the causative agents of malaria (*Plasmodium* spp.) and toxoplasmosis (*Toxoplasma gondii*). These obligate intracellular parasites have complex life cycles that encompass a succession of developmental stages, often across multiple host species, and they have evolved highly specialised machinery to actively invade host cells with remarkable co-ordination and speed.

Invasive 'zoites' utilise numerous specific ligands to recognise and invade susceptible host cells. *Toxoplasma gondii* tachyzoites are capable of invading a wide variety of cell types that express vastly different surface receptors. In contrast, *Plasmodium* sporozoites and merozoites are highly selective for hepatocytes and red blood cells, respectively, and merozoites from different *Plasmodium* spp. have varying capacities to invade mature erythrocytes (normocytes). Yet despite apicomplexan parasites having specificity for different host cells, the kinetics and molecular aspects of invasion appear to be conserved over a large evolutionary distance within the phylum (Dvorak et al., 1975; Gilson and Crabb, 2009; Sharma and Chitnis, 2013). This points to a pivotal core mechanism that allows these parasites to maintain comparable invasion efficiency.

2. A universal host cell binding mechanism

Five years ago, Besteiro et al. (2009) proposed a remarkable mechanism whereby parasites supply both ligand and receptor to form an intimate membrane junction between the host and parasite during invasion. This junction, first observed over 30 years ago, is described as an electron-dense interface at the point of contact between the parasite and host cell that encircles and migrates down the length of the parasite during internalisation (Aikawa et al., 1978; Riglar et al., 2011). The discovery by Besteiro et al. that a complex of proteins is secreted into the host side of this so-called 'moving junction' (MJ) was a key insight into this unique mechanism of host cell penetration. Formation of an entirely parasite-derived host-anchoring complex would allow parasites to rely less upon the host and thus maintain invasion efficiency across different host cell types. Species-specific adhesins could act upstream of this mechanism to identify a susceptible host cell (reviewed in Harvey et al., 2012), before a conserved multiprotein adhesin complex is deployed to maintain the high level of coordination that is observed across all parasites within the phylum.

Apical Membrane Antigen 1 (AMA1), a micronemal integral membrane protein, is the putative ligand in the MJ, and a complex of rhoptry neck-derived (RON) proteins, RON2, RON4 and RON5 (and RON8 in *T. gondii*), appears to translocate into the host cell to act as a receptor for AMA1.

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2.1. Evidence to support the role of AMA1 and RON proteins in the MJ complex

Binding of AMA1 to the RON protein complex was first observed by Alexander et al. (2005) and several studies have since validated this interaction (Alexander et al., 2006; Besteiro et al., 2009; Cao et al., 2009; Collins et al., 2009; Lebrun et al., 2005; Narum et al., 2008; Richard et al., 2010). AMA1 has a large N-terminal ectodomain that is structurally conserved across genera, folding into three interacting domains with several protruding loops (Bai et al., 2005; Crawford et al., 2010; Hodder et al., 1996; Pizarro et al., 2005). RON2 adopts a membrane-spanning conformation such that a C-terminal loop region is exposed on the surface of the host cell (Lamarque et al., 2011; Srinivasan et al., 2011). RON4 and RON5 (and RON8) have no transmembrane regions and appear to localise entirely within the host cell cytosol to interact with the host cytoskeleton (Riglar et al., 2011; Srinivasan et al., 2011; Takemae et al., 2013). This complex could provide a physical link between the cortical cytoskeletons of both cells to serve as a stable anchoring structure upon which the zoite can apply traction. Tonkin et al. (2011) and Hossain et al. (2012) mapped in detail the binding interface between AMA1 and RON2 in *T. gondii* and *Plasmodium falciparum*, respectively. A hydrophobic trough within domain I of AMA1 forms a binding pocket that accepts the critical loop region in RON2 with significant shape and charge complementarity. In silico modelling illustrates that RON2 displaces a loop in domain II of AMA1 to expose the binding surface, then the exposed RON2 loop can penetrate deep within the hydrophobic groove in AMA1 (Tonkin et al., 2011). This high affinity association would likely withstand mechanical forces and, as such, further supports the role for AMA1-RON complex binding to maintain close contact with the host cell during active invasion.

While the molecular composition of the MJ has been challenging to resolve, largely due to its transient existence over a fleeting internalisation period, there is now considerable evidence to support a role for AMA1 and the RON protein complex in the MJ. Anti-AMA1 antibodies and competitive binding peptides that block the interaction between AMA1 and RON2 inhibit invasion at the stage of MJ formation, when parasites can no longer form intimate contact with the host cell (Treeck et al., 2009; Richard et al., 2010; Srinivasan et al., 2011). Most notably, immunostaining of invading *T. gondii* tachyzoites and *P. falciparum* merozoites demonstrates that the RON complex is located on the host side of the MJ and that the majority of surface-bound AMA1 co-localises with the RON complex in the MJ plane (Alexander et al., 2006; Besteiro et al., 2009; Riglar et al., 2011; Srinivasan et al., 2011). Together, these data support a model whereby zoites secrete essential ligands and their corresponding receptors from distinct organelles to assemble their own machinery for host cell entry.

2.2. Recent challenges to the current model of the MJ

Recently, two major studies have emerged that command a reassessment of the notion that AMA1 plays a universal and essential role in the MJ. Giovannini et al. (2011) targeted the gene encoding AMA1 in *T. gondii* and *Plasmodium berghei* via stage-specific deletion of the 3' untranslated region using a recombinase system. This showed that tachyzoites and sporozoites, but not merozoites, were still able to invade host cells and appeared to form normal MJs. Conversely, disruption of RON4 by the same method completely inhibited invasion in all instances. Together this data suggested that AMA1 is not a functional component of the MJ in tachyzoites or sporozoites, whereas RON4 is essential to the MJ. It is important to note, however, that this gene targeting approach is unlikely to ablate expression as the open reading frame remains and is able to utilise downstream transcriptional termination signals, causing a knockdown effect. It is possible that AMA1 is present in vast excess, as is apparent by the minor peripherally located

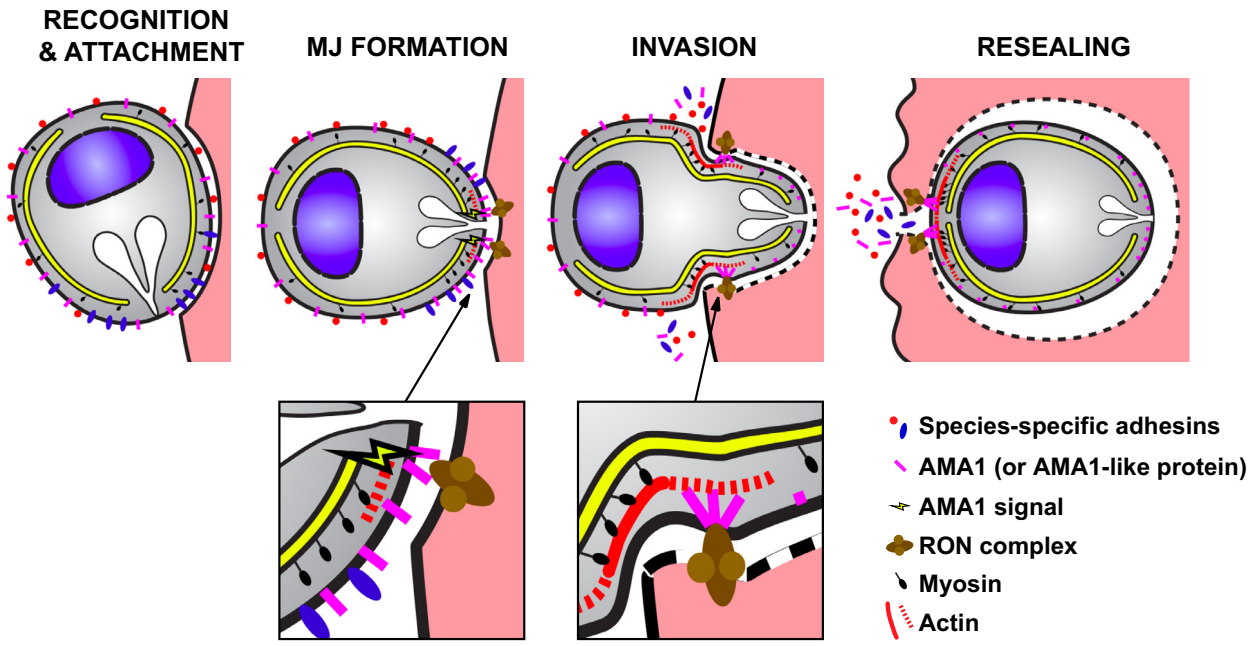

Fig. 1. Putative mechanism of invasion by apicomplexan parasites showing erythrocyte invasion by a merozoite. Host cell recognition, specific attachment and reorientation are likely mediated by several low- and high-affinity binding adhesins that dictate host cell specificity. When apically juxtaposed, the rhoptry neck-derived (RON) protein complex can be translocated into the host cell to act as a receptor for Apical Membrane Antigen 1 (AMA1; or an AMA1-like protein) at the apical tip. This interaction allows intimate contact between the host and parasite (moving junction (MJ) formation), and provides a strong anchor point on the host cell. During invasion, the actomyosin motor utilises the MJ as a traction point to drive penetration. The AMA1 cytoplasmic domain could play a direct role in connecting to the invasion motor or act as a signaling component to coordinate this process. After the zoite has gained entry into the host cell, the MJ may also help to reseal the surrounding host and vacuolar membranes. We speculate that parasites completely lacking AMA1 fail to form a MJ and remain attached to the host cell, but reduced levels of AMA1 allow invasion and instead prevent resealing and subsequent intracellular development.