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Pathogens in focus

Toxoplasma gondii: Microneme protein MIC2

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Abstract

The phylum Apicomplexa contains parasites responsible for a variety of diseases including malaria, cryptosporidiosis, and toxoplasmosis. One of the common features of these parasites is that they contain a set of apical organelles whose sequential secretion is required for the invasion of host cells. Microneme proteins are the main adhesins involved in the attachment to the host cell surface by apicomplexans. The microneme protein MIC2, produced by *Toxoplasma gondii*, is conserved in apicomplexans and serves as a model to understand the first steps of invasion by the phylum. New data about the structure–function relationship of MIC2 reinforce the critical role of this protein in the successful invasion of cells by *Toxoplasma* and reveal potential therapeutic targets that may be used to control toxoplasmosis.

Keywords: *Toxoplasma gondii*; MIC2; ICAM-1; Rhomboid; Aldolase

1. Introduction

Toxoplasma gondii is a member of the phylum Apicomplexa, which contains obligate intracellular parasites including *Plasmodium*, the agent of malaria. *T. gondii* causes toxoplasmosis, a disease that can be fatal for immunocompromised individuals. During natural infections, *T. gondii* initially crosses the intestinal epithelium, disseminates into the deep tissues and traverses biological barriers in the placenta and the blood–brain barrier. Within these immunologically privileged sites it causes severe pathology in the developing fetus and ocular pathology in immunocompetent individuals (Joynson & Wreghitt 2001).

2. Pathogenesis

T. gondii is an intracellular pathogen able to invade most eukaryotic cells. Apicomplexan parasites use a unique mode of locomotion termed gliding motility to rapidly enter host cells by active penetration (Dobrowolski & Sibley 1996; Sibley, 2004). Efficient invasion also requires the polarized secretion of proteins stored in apical organelles (Carruthers & Sibley 1997). The secretion of the microneme proteins from

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apically localized organelles is responsible for the directional attachment of the parasite to the host cell.

Microneme proteins (MICs) contain a variety of adhesive domains including integrin-like (MIC2), thrombospondin type I repeat-like (MIC2 and MIC1), epidermal growth factor-like (MIC3, MIC6, MIC7, MIC8 and MIC9) and lectin-like domains (MIC3 and MIC8), which mediate interactions with host cell surface components (Soldati, Dubremetz, & Lebrun, 2001). Some of the MICs have a transmembrane domain (TMD MICs) that can provide a link between the host cell and internal workings of the parasite (Soldati et al., 2001). TMD MICs associate with themselves or with soluble MICs to form homologous/heterologous complexes, essential for the function of their adhesive properties (Kappe, Kaiser, & Matushchewski, 2003). The diversity of adhesive domains, combined with the capacity of the MICs to form multimeric complexes optimizes the interaction of the parasite with the surface of many cell types.

Upon contact with the host cells, the MICs undergo rapid exocytosis onto the apical surface of the parasite where they initiate tight binding to the surface of the host cell (Fig. 1). As the parasite enters into the cell, the adhesive complexes are translocated towards the posterior pole of the parasite via an actin–myosin dependent process, and hence they are largely excluded from the inside of the host cell. Finally, the TMD MICs are cleaved and the MICs released from the surface of the parasite (Carruthers, Håkansson, Giddings, & Sibley, 2000a). This processing event is mediated by an unknown protease, synthesized by the parasite, whose activity has been named MPP1 (microneme protein protease 1) (Carruthers et al., 2000a).

The microneme protein MIC2 has been extensively studied due to its presumed role in attachment and invasion. MIC2 belongs to a family of transmembrane adhesins originally described in *Plasmodium* as TRAP (thrombospondin-related anonymous protein) (Robson et al., 1988). TRAP plays a crucial role in gliding motility and host cell invasion by malaria sporozoites (Kappe et al., 2003). Similarly, recent genetic and biochemical studies have shown the critical role of MIC2 in the invasion process, as discussed below. In this review, we describe the crucial role of the microneme protein MIC2 in the invasion of the host cells by *T. gondii*.

3. Structure of MIC2

MIC2, a 769 amino acid protein, is composed of two extracellular adhesive domains, an N-terminal A/I (Integrin A) domain (residues 1–270) and six imperfect thrombospondin type I repeats (TSR-1) (residues 271–702) (Lawler & Hynes 1986), a TMD (residues 703–723), and a short cytoplasmic tail (residues 724–769) (Fig. 2) (Wan, Carruthers, Sibley, & Ajioka, 1997). MIC2 interacts with MIC2-associated protein (M2AP). The complex remains stable throughout the trafficking of MIC2 to the micronemes, secretion onto the surface of the parasite, and after cleavage by MPP1 (Rabenau et al., 2001). Recent data have shown that MIC2–M2AP is a hetero-hexameric complex of 450 kDa containing three MIC2–M2AP entities that are associated via interactions between the MIC2 molecules (Fig. 2) (Jewett & Sibley, 2004). The disruption of the M2AP gene leads to the retention of MIC2 in the ER/Golgi, resulting in a drastic reduction in the capacity of the mutated parasite to invade host cells (Huynh et al., 2003). Collectively, these data indicate that the association of M2AP with MIC2 is necessary for proper delivery to the micronemes and suggest that the integrity of the complex is necessary for efficient invasion to occur.

Interestingly, no homologues of M2AP in *Plasmodium* have been reported suggesting that TRAP acts on its own. This observation may reflects differences in (i) the stabilities of MIC2 and TRAP, (ii) the secretion pathways needed to reach the micronemes or (iii) an undetermined function of the MIC2–M2AP complex.

4. Biological functions of MIC2

Deletion of the gene encoding MIC2 has not been possible, suggesting that MIC2 is essential for parasite viability. However, mutation studies of the different domains have shed light on the functions of these adhesions during cell invasion.

4.1. The A/I domain

The A/I domain is found typically in the α chain of integrins like the leukocyte function-associated antigen 1 (LFA-1), Mac-1, some β integrins, von Willebrand Factor, cartilage matrix protein, factor B, and collagen