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Invited Review

Sialic acids: Key determinants for invasion by the Apicomplexa

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ABSTRACT

Sialic acids are ubiquitously found on the surface of all vertebrate cells at the extremities of glycan chains and widely exploited by viruses and bacteria to enter host cells. Carbohydrate-bearing receptors are equally important for host cell invasion by the obligate intracellular protozoan parasites of the phylum Apicomplexa. Host cell entry is an active process relying crucially on proteins that engage with receptors on the host cell surface and promote adhesion and internalisation. Assembly into complexes, proteolytic processing and oligomerization are important requirements for the functionality of these adhesins. The combination of adhesive proteins with varying stringency in specificity confers some flexibility to the parasite in face of receptor heterogeneity and immune pressure. Sialic acids are now recognised to critically contribute to selective host cell recognition by various species of the phylum.

1. Introduction to the role of glycans in host cell invasion by the Apicomplexa

In vertebrates, all cells are decorated with a dense and complex array of glycan structures, collectively termed the glycocalyx, comprising glycoproteins and glycolipids. The glycans consist of chains of sugar molecules, which may be branched and often terminate in a sugar unit belonging to the class of sialic acids (Sias). There is a remarkable array of modifications on Sias, exceeding that of any other common monosaccharide. Additional diversity arises from a variety of glycosidic linkages from C2 to the underlying glycans. The glycans fulfil diverse functions in fertilisation, development, neural plasticity and immune-related processes (Varki, 2008). The ubiquitous distribution of glycans and in particular Sias in the bodies of vertebrates makes them attractive targets for pathogens. These pathogens express adhesins and toxins that bind to glycans with various degrees of specificity and are communally named lectins. For a subset of lectins, sialic acid constitutes a critical component for recognition, which can be specific for one particular sialylated glycoconjugate. Recognition may depend on a specific conformation or modification of the sialic acid (such as methylation, acetylation, sulfation and phosphorylation) or on the linkage to the underlying sugar chain and its composition (Varki, 1997). In addition, the protein or lipid to which the glycan is attached can be important for recognition.

Apicomplexan parasites express an arsenal of adhesins including lectins essential for productive host cell invasion (Fig. 1). In the context of specific receptors these interactions are believed to determine the host cell preference for each parasite and each stage in their life cycle (Cerami et al., 1992; Galinski et al., 1992; Orlandi et al., 1992). Whilst some of the adhesins are conserved amongst several species, others appear to be restricted to a few or a single species, which probably reflects the differences in host and tissue tropism as part of their life cycles (Anantharaman et al., 2007; Templeton, 2007). The purpose of this review is to recapitulate, compare and contrast the role of Sias in host cell invasion by the Apicomplexa. The currently characterised apicomplexan ligands that recognise Sias and the nature of their host receptors are discussed here in the context of their potential contribution to host range specificity.

2. Mechanism of invasion and role of adhesins

Invasion of host cells by apicomplexans is an active and complex process that is fundamentally different from the entry mechanisms of bacteria and viruses that exploit host endocytic uptake pathways. Apicomplexan parasites carry their own machinery for active penetration into the host cell (Carruthers and Boothroyd, 2007). Invasion is initiated by attachment that may occur in any

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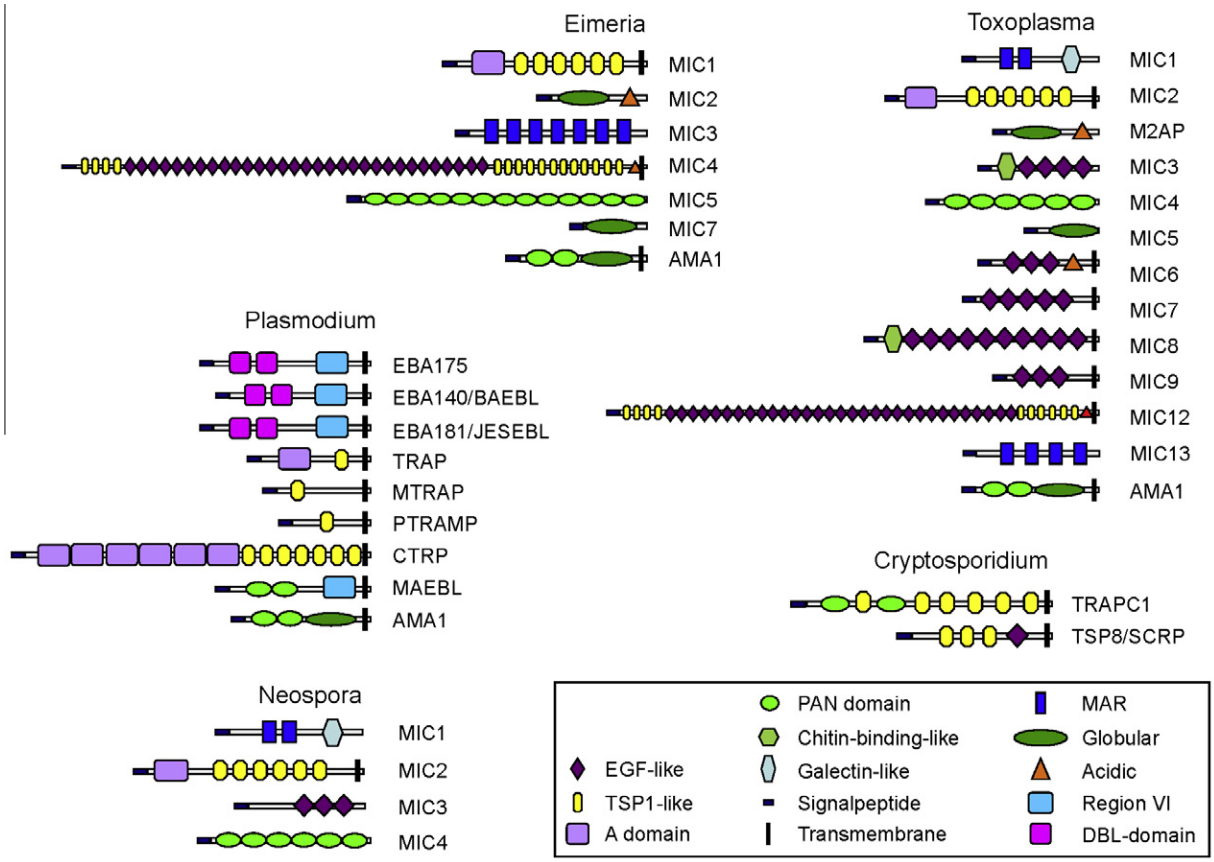

Fig. 1. Representation of a non-exhaustive repertoire of microneme proteins from apicomplexan parasites that exhibit domains involved in protein or carbohydrate interactions. The schematic representation indicates the domain composition of the individual proteins. EGF, epidermal growth factor; TSP, thrombospondin; PAN, plasminogen apple nematode domain; MAR, microneme adhesive repeat. Modified from Tomley and Soldati (2001) and Carruthers and Tomley, 2008.

position of the parasite relative to the host cell and is followed by reorientation such that the parasite apical tip contacts the surface of the host cell. An electron-dense circular structure termed the moving junction is built at the tip, which follows the periphery of the parasite whilst it actively propels itself into the host cell. During this process the host cell plasma membrane progressively invaginates and induces the formation of the parasitophorous vacuole (PV), which is finally sealed behind the parasite. Penetration of the host cell is driven by a parasite actin-myosin motor system located in the space between the parasite plasma membrane and the underlying inner membrane complex (Soldati-Favre, 2008). This motor complex also provides the force for the unique form of substrate-dependent gliding locomotion. Successful host cell invasion relies on the regulated sequential secretion of proteins from two types of membrane-bound organelles, micronemes and rhoptries (Carruthers and Sibley, 1997). Microneme secretion occurs during parasite egress from host cells as well as during gliding locomotion, but mainly upon contact between the parasite and a host cell and is followed by a discharge from the rhoptries that contribute to invasion. Microneme proteins (MICs) are key mediators of cytoadherence, but also play other essential and non-overlapping roles in the invasion process (Carruthers and Tomley, 2008; Soldati-Favre, 2008). Notably, recent studies have highlighted the roles of MICs (TgMIC8 in *Toxoplasma gondii*; PfEBA175 and PfAMA1 in *Plasmodium falciparum*) in the process leading to secretion by the rhoptries (Kessler et al., 2008; Richard et al., 2010; Singh et al., 2010).

The MICs are extensively proteolytically processed during their trafficking along the secretory pathway as well as post-exocytosis. These post-translational events have been studied in detail in *T. gondii* (Dowse and Soldati, 2004). Processing of pro-peptides along the secretory pathway is important for the functionality of some adhesive protein complexes. Only after processing is TgMIC3 able to function as an adhesin through its chitin-binding-like (CBL) domain, but this processing event is dispensable for trafficking (Cerede et al., 2002; El Hajj et al., 2008). Removal of the TgM2AP propeptide is critical for stable assembly and efficient secretion of the TgMIC2–M2AP complex from the micronemes onto the parasite surface (Harper et al., 2006). The cysteine protease cathepsin L (TgCPL) that resides in compartments of the late secretory pathway is a candidate for taking part in proteolytic maturation of some MICs (Larson et al., 2009).

During invasion, the MIC complexes anchored onto the parasite surface are excluded from the forming PV at the level of the moving junction (with the exception of TgAMA1) (Alexander et al., 2005; Howell et al., 2005). For this reason MIC complexes that are in excess or are not part of the moving junction are redistributed towards the posterior end of the parasite during invasion, a phenomenon known as capping, which has been experimentally demonstrated for TgMIC2 and TgMIC3 (Carruthers et al., 1999; Garcia-Reguet et al., 2000). At some point during the entry process, tight interactions formed by the different complexes and host cell receptors have to be disengaged and the MICs removed from the parasite surface. This is effectively achieved by proteolytic shedding, which was first described for TgMIC2. The protease responsible for this activity was termed microneme protein protease 1 (MPP1) and shown to be critical for successful invasion (Carruthers et al., 2000; Brossier et al., 2003). Across the phylum several other MICs as well as resident surface proteins related to adhesive function are shed from the surface either within the transmembrane spanning domain