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Invited Review

Intracellular survival of apicomplexan parasites and host cell modification

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ABSTRACT

The intracellular stages of apicomplexan parasites are known to extensively modify their host cells to ensure their own survival. Recently, considerable progress has been made in understanding the molecular details of these parasite-dependent effects for *Plasmodium*-, *Toxoplasma*- and *Theileria*-infected cells. We have begun to understand how *Plasmodium* liver stage parasites protect their host hepatocytes from apoptosis during parasite development and how they induce an ordered cell death at the end of the liver stage. *Toxoplasma* parasites are also known to regulate host cell survival pathways and it has been convincingly demonstrated that they block host cell major histocompatibility complex (MHC)-dependent antigen presentation of parasite epitopes to avoid cell-mediated immune responses. *Theileria* parasites are the masters of host cell modulation because their presence immortalises the infected cell. It is now accepted that multiple pathways are activated to induce *Theileria*-dependent host cell transformation. Although it is now known that similar host cell pathways are affected by the different parasites, the outcome for the infected cell varies considerably. Improved imaging techniques and new methods to control expression of parasite and host cell proteins will help us to analyse the molecular details of parasite-dependent host cell modifications.

1. Introduction

This review concentrates on the post-genomic era of apicomplexan research. In this period, considerable progress has been made in the field of parasite-dependent host cell modification for *Toxoplasma*, *Theileria* and *Plasmodium* parasites and we therefore focus on these organisms and their effects on their respective host cells. However, it should be mentioned that other Apicomplexa, such as *Eimeria*, *Neospora* and *Cryptosporidium* also have profound effects on their respective host cells and the reader is referred to related literature (del Cacho et al., 2004; Herman et al., 2007; Liu et al., 2008).

The COST Action 857 "Apicomplexan Biology in the Post-Genomic Era" represented an ideal platform for European scientists to exchange ideas and join forces to take large steps forward in the field of parasite–host interactions. A number of very fruitful collaborations started during this COST action, resulting in some major discoveries.

The very dynamic European malaria research community has provided us with important insights into the migration of *Plasmodium* sporozoites in the skin and liver (Mota and Rodriguez, 2004; Amino et al., 2007). Although it is still debated why sporozoites transmigrate through cells, interesting concepts have been suggested by participants of this COST action and will be discussed in this review (Leiriao et al., 2005a; Amino et al., 2008). A collaboration, which was initiated at the COST meeting in Lisbon in 2004 and is still ongoing, resulted in the first description of merosomes as vehicles for the transport of hepatocyte-derived merozoites to blood vessels of the liver (Sturm et al., 2006). This finding now completes our knowledge about the life cycle of *Plasmodium* parasites. Merosome development and other host cell modifications by *Plasmodium* parasites will be summarised by Rebecca Stanway.

Toxoplasma gondii-dependent signalling in host cells is a long-standing interest of Carsten Lüder. He describes here how intracellular *T. gondii* parasites interfere with several host signalling pathways to avoid production of nitric oxide (NO), major histocompatibility complex (MHC)-dependent antigen presentation and induction of host cell apoptosis and he discusses future directions in this field. We will hopefully learn more about *T. gondii*-induced effects on host cell signalling using the large-scale small interference RNA (siRNA) knockdown approach targeting signalling proteins of the host cell, started by the laboratory of Markus Meissner in Heidelberg.

Theileria-induced reversible host cell transformation is probably the most extreme example of how intracellular parasites influence the phenotype of their host cells. This phenomenon has fascinated researchers for decades and scientists involved in the COST Action 857 are global leaders in this field. It is now accepted that the influence of *Theileria* parasites on their host cells is manifold and

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not restricted to one central pathway (Heussler et al., 2002; Dessauge et al., 2005b). Many of the new discoveries came from Gordon Langsley's laboratory in Paris and he, together with Marie Chaussepied, summarises here the recent publications in the *Theileria* field and raises some challenging hypotheses. A very exciting talk at the 2007 COST meeting in Porticcio was given by Dirk Dobbelaere, who reported on kinases involved in both host cell cytokinesis and in the equal distribution of the *Theileria* schizonts to both daughter cells and we hope to see these data published in the near future. The organisers of the COST Action 857 were always successful in encouraging young parasitologists to participate in the annual COST meetings and the selection of authors for this review followed this philosophy. Apart from the principal investigators, two young and enthusiastic post-doctoral scientists, Rebecca Stanway and Marie Chaussepied, joined the team and the review certainly benefited from their input.

2. *Plasmodium* – the parasite dictates life and death of the host cell

The *Plasmodium* parasite must invade and traverse a vast range of different cell types during its life cycle, but has only two true host cells, within which it grows and divides, those being the hepatocyte and the erythrocyte. The parasite must modify both of these host cells to allow its own development and at the same time must prevent host cell death. This section of the review will focus primarily on modification, by the *Plasmodium* parasite, of the host hepatocyte. During the past 4 years of COST Action 857, our understanding of *Plasmodium* development within the liver has advanced greatly but much remains to be understood, particularly on a molecular level.

2.1. Early stage development of the Plasmodium parasite within the hepatocyte

In 2001, data was published that confirmed that sporozoites, on entering the liver, transmigrate through a number of hepatocytes before invading a final one by the formation of a parasitophorous vacuole (PV) (Mota et al, 2001). This process of hepatocyte transmigration has been shown to involve the perforin-like sporozoite protein essential for cell traversal (SPECT), (Ishino et al., 2004) and SPECT2 (also known as *Plasmodium* perforin-like protein 1, (PPLP1) (Ishino et al., 2005)). Additionally, the thrombospondin-related adhesive protein (TRAP)-like protein (TLP) has recently been identified, which appears to play a role in hepatocyte traversal (Moreira et al., 2008), potentially in anchoring the sporozoite to the hepatocyte prior to, and during, movement into the cell. It has been hypothesised that traversal of hepatocytes is necessary to activate sporozoites for invasion of a final hepatocyte by formation of a PV. Sporozoite activation occurs by contact with the host cell cytoplasm and results in regulated exocytosis by the sporozoite of proteins contained within apical secretory organelles (Mota et al., 2002). In support of this hypothesis, it has been shown that stimulation of regulated exocytosis leads to a significantly increased level of *Plasmodium yoelii* sporozoite infectivity to hepatocytes (Ono et al., 2008). However, in in vitro culture, SPECT2-deficient sporozoites, which are unable to traverse hepatocytes, infect hepatoma cells at the same level as wild-type sporozoites (Ishino et al., 2005), showing that activation of parasites by cell traversal is not essential for establishment of infection. Another role and the one most relevant to this review is that transmigration of hepatocytes and the cell wounding this involves, leads to protection of infected cells. Hepatocyte wounding by sporozoites releases hepatocyte growth factor (HGF) (Carrolo et al., 2003) and this binds to c-mesenchymal–epithelial transition factor (c-MET) on the surface of hepatocytes. C-Met ligation activates phosphatidylinositol 3-kinase (PI3-K) to inhibit apoptosis of infected and presumably non-infected hepatocytes (Leiriao et al., 2005a). Supporting this, inhibition of PI3-K at early stages of infection leads to apoptosis of infected cells (Leiriao et al., 2005a,b). Such HGF-c-MET signalling was therefore thought to be responsible for preventing apoptosis in the initial phase of infection. However, when SPECT2 is disrupted, sporozoites are incapable of cell wounding and thus HGF release, but in vitro do not show a reduced survival following infection, despite a presumed absence or great reduction in HGF-c-MET signalling (Ishino et al., 2005). It is therefore not currently possible to definitively determine the role of HGF-c-MET signalling in prevention of host cell apoptosis. We know, however, that prevention of host cell apoptosis initially appears to involve signalling via PI3-K (Leiriao et al., 2005a), but what signals occur upstream of PI3-K remain to be identified.

2.2. Later stage inhibition of apoptosis by Plasmodium in the hepatocyte – lessons from the erythrocyte stage

Following early stages of infection, inhibition of apoptosis becomes independent of PI3-K activity (van de Sand et al., 2005) and it is thought that once the parasite is established in the hepatocyte, it actively inhibits apoptosis of the host cell, most probably via secretion of parasite molecules into the host cell cytoplasm. At 18 h p.i., infected cells are more resistant to external stimuli of apoptosis than non-infected cells (Leiriao et al., 2005a), showing that the presence of the parasite confers resistance to apoptosis. Host cell survival certainly requires the parasite to stay alive, as mutant parasites that invade hepatocytes but do not develop, such as *Plasmodium berghei* parasites lacking the P36p protein, fail to protect the host cell from undergoing apoptosis after the initial phase of infection (van Dijk et al., 2005). Additionally, it has been shown that host cells invaded by irradiated sporozoites die by apoptosis shortly after invasion (Leiriao et al., 2005b).

Secretion of *Plasmodium* proteins into the host cell cytoplasm and onto the host cell surface has best been characterised in the *Plasmodium falciparum* blood stage, where the parasite similarly resides within a PV and must ensure survival of the host erythrocyte until maturity of daughter parasites. Here, secretion into the host cell occurs primarily by use of a *Plasmodium* export element (Pexel) (Hiller et al., 2004; Marti et al., 2004), which appears to be processed during export (Chang et al., 2008). Bioinformatic prediction of such motifs has allowed the generation of a predicted secretome for the *P. falciparum* blood stage, with up to 150 potentially exported proteins (van Ooij et al., 2008). Liver stage parasites must also secrete molecules into the hepatocyte cytoplasm, including those presumably involved in inhibition of host cell death, but which proteins are secreted and which signalling pathways and events they interfere with is not clear. One might predict that secreted proteins include remodellers of the host cell cytoskeleton, required to allow the extensive growth of the parasite. Several proteins secreted into the erythrocyte by the *P. falciparum* parasite bind to elements of the red blood cell cytoskeleton (reviewed in van Ooij and Haldar, 2007). Ring-infected erythrocyte surface antigen (RESA), for example, binds to spectrin beneath the plasma membrane and is thought to stabilise the membrane of the host cell, preventing subsequent merozoite invasion, ensuring proper parasite development and protecting the erythrocyte from heat damage during malarial fever (Mills et al., 2007). *Plasmodium* parasites express a homologue of the secreted *Theileria* protein TaSE (Schneider et al., 2007), which binds to host cell microtubules and it will be interesting to investigate the localisation and function of the *Plasmodium* homologue in infected hepatocytes and erythrocytes. In *P. berghei*, the predicted secretome contains much fewer proteins than that of *P. falciparum*, but the machinery for