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REVIEW

Live and let die: manipulation of host hepatocytes by exoerythrocytic *Plasmodium* parasites

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Abstract The generation of rodent *Plasmodium* strains expressing fluorescent proteins in all life cycle stages has had a big impact on malaria research. With this tool in hand, for the first time it was possible to follow in real time by in vivo microscopy the infection route of *Plasmodium* sporozoites transmitted to the mammalian host by *Anopheles* mosquitoes. Recently, this work has been extended to the analysis of both hepatocyte infection by *Plasmodium* sporozoites, as well as liver merozoite transport into blood vessels. The stunning results of these studies have considerably changed our understanding of hepatocyte invasion and parasite liberation. Here, we describe the most important findings of the last years and in addition, we elaborate on the molecular events during the intracellular development of *Plasmodium* exoerythrocytic forms that give rise to erythrocyte infecting merozoites.

Keywords Exoerythrocytic *Plasmodium* parasites · Merosome formation · Intravital imaging · Programmed cell death

Malaria: Introduction

After more than a 100 years of research [1] malaria remains one of the deadliest infectious diseases in the world. Malaria is caused by apicomplexan parasites of the genus *Plasmodium*. *P. falciparum* is responsible for the most damaging form of the disease, infecting between 300 and 500 million people each year and killing about 1–2 million children under the age of five in sub-Saharan Africa. Forty percent of the world population live in endemic areas and malaria-infested countries suffer from enormous economic losses [2].

The *Plasmodium* life cycle starts with a blood meal of an infected *Anopheles* mosquito and the coincident injection of sporozoites into human skin. After breaching blood vessels of the skin [3] sporozoites are transported by the bloodstream to the liver. There, the parasite infects liver parenchyma cells (hepatocytes) and differentiates into thousands of merozoites. To access the bloodstream, liver-derived merozoites must leave their host cell and cross the endothelium of liver blood vessels. Within red blood cells (RBCs) each parasite replicates, depending on the *Plasmodium* species, into 16 or 32 merozoites, which then get released to directly infect other RBCs. These cycles of infection and release induce strong inflammatory immune responses resulting in the typical periodic attacks of fever. Some parasites eventually develop into gametocytes, which can be transmitted to uninfected *Anopheles* mosquitoes during a blood meal. Exflagellated motile microgamets and macrogamets fertilize inside the mosquito gut and migrate as kinetes through the gut wall to develop into oocysts, which finally release several thousands of sporozoites into the haemolymph of the insect. Within the haemolymph sporozoites circulate throughout the entire body of the mosquito and eventually reach and invade the salivary glands to develop into mature infectious sporozoites [4].

The *Plasmodium* liver stage

Plasmodium-infected hepatocytes are ideal targets for the development of anti-malarial measures, as liver infection is

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still clinically silent. In addition, *Plasmodium* parasites remain for several days in hepatocytes that should allow enough time for the immune system of a vaccinated individual to eliminate the infection at this early stage. Research performed in humans and mice has shown that irradiated or genetically attenuated sporozoites are able to confer protection against challenge with wild type parasites [5–8]. In spite of this knowledge and the recent effort to mass-produce irradiated sporozoites for vaccination trials [9, 10], a commercial vaccine against malaria is still not available. It is therefore of decisive importance to gain more detailed knowledge about the observed protective immune responses and the parasite survival strategies within hepatocytes.

The *P. falciparum* liver stage still remains a black box [11], because in vitro infection of human hepatocytes [12, 13] and in vivo experiments using primates or humanized mice [14] rely on *P. falciparum*-infected *Anopheles* mosquitoes and all aspects are difficult to perform, since they need to be carried out under high (S3) safety laboratory conditions. Therefore, mouse models using *P. berghei* and *P. yoelii* infections have successfully been introduced and the generation of fluorescent rodent parasite strains has opened new avenues to investigate migration and development in the mammalian host [15, 16]. In vitro live imaging of infected cell cultures (Fig. 1) and intravital imaging of surgically exposed livers of infected mice (Fig. 2) allowed us to investigate intracellular development of the parasite and to follow its journey from the skin into the liver and back to the blood stream [3, 17–19].

During the blood meal of an infected mosquito *Plasmodium* sporozoites are injected into the skin. Recent studies using GFP expressing *P. berghei* parasites have shown that only 50% of the sporozoites in the skin actually leave the bite area [3]. Seventy percent of these parasites actively enter blood vessels, whereas the residual 30% penetrate lymphatic vessels and end up in the draining lymph node. Although these parasites do not develop to mature EEF, their presence in the lymph node might substantially influence the immune response against subsequent hepatocyte infections. Whether this dead-end path of sporozoites in the draining lymph node indeed activates relevant cell-mediated immune responses remains to be shown, but it is reasonable to speculate that primed effector cells leave the draining lymph node and eventually reach the liver.

Sporozoites that drift with the blood stream to the liver abruptly slow down once they enter the liver sinusoids [18]. This is probably due to binding of the major surface proteins circumsporozoite protein (CSP) and thrombospondin-related adhesive protein (TRAP) to the liver sinusoid-specific heparansulfate proteoglycans. Originally, these molecules are exposed on hepatocytes and stellate cells and reach the sinusoids through the so-called fenestrae, 100 nm holes in endothelial cells [20, 21]. Parasites glide along the sinusoidal wall [22] and finally cross this barrier by traversing Kupffer cells [23, 24], which are the resident macrophages of the liver. It is still not known why Kupffer cells do not recognize and remove traversing sporozoites by phagocytosis. After the Kupffer cell passage, sporozoites

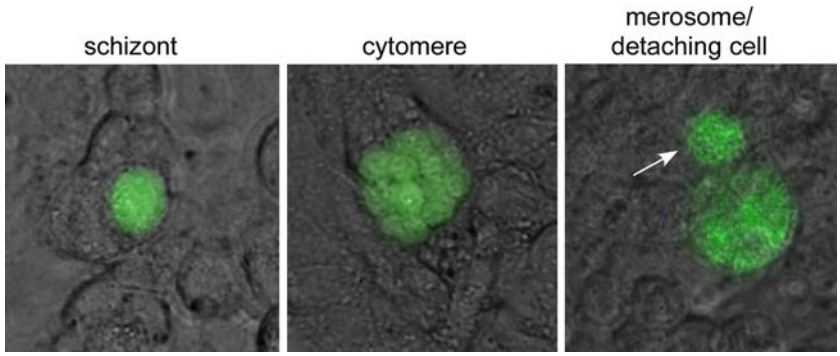

Fig. 1 In vitro live imaging of GFP-expressing *P. berghei*-infected HepG2 cells (merger of IFA and phase contrast image). Different developmental stages of liver stage parasites: *schizont* stage, *cytomere* stage and detached infected cell with a budding merosome (*arrow*)

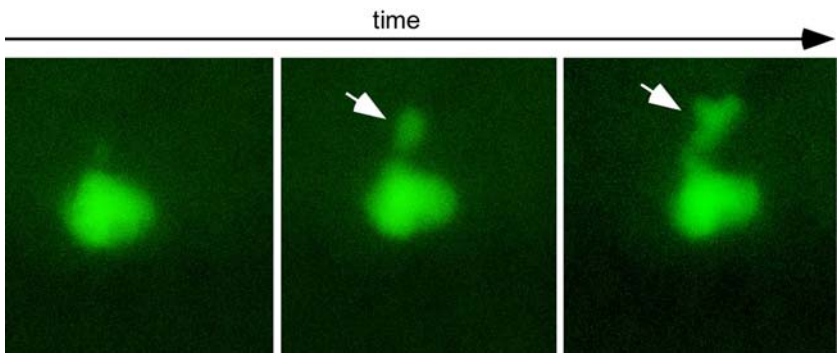

Fig. 2 Intravital microscopy of a *P. berghei*-infected mouse liver. A mouse was infected with GFP-expressing parasites and 45 h later the liver was surgically exposed for microscopical examination. The series of images show the same infected cell in three different phases of merosome formation (*arrows*) over a period of ca. 1 h

migrate through the space of Disse from where they have direct access to hepatocytes. Mota and colleagues [24] have shown that sporozoites transmigrate through several hepatocytes before they infect a final one and start to develop to liver schizonts within a parasitophorous vacuole membrane (PVM). The migration of sporozoites through hepatocytes results in wounding of the cell membrane, which in turn leads to the release of hepatocyte growth factor (HGF). Locally increased levels of HGF are thought to support survival of neighbouring infected hepatocytes via HGF/MET signalling and activation of the corresponding anti-apoptotic PI3 kinase pathway of the host cell [25]. During the following schizogony the parasite grows considerably in size and infected hepatocytes can be several times bigger than non-infected ones. Although this enormous growth is certainly an important stress factor for the host cell and stress normally induces apoptosis, infected cells surprisingly do not exhibit signs of cell death. On the contrary, *Plasmodium* liver schizonts even protect their host cells from programmed cell death [26]. However, this parasite-mediated anti-apoptotic effect differs markedly from the above-mentioned HGF/MET-dependent signalling during the infection process in that it does not depend on PI3 kinase signalling.

So far it is not known to what extent the anti-apoptotic phenotype of infected cells represents an advantage concerning elimination by primed cytotoxic T cells. These killer cells can induce apoptosis in target cells by a death receptor mediated pathway and the granzyme-perforin pathway. A solid protection from cytotoxic effects would support survival of the intracellular parasite and it has indeed been suggested that CD8+ cell-mediated immune responses are restricted to IFN-γ secreting cells, which are thought to inhibit intracellular parasite growth by NO formation, rather than inducing apoptosis of infected cells [27].

By the end of the liver stage several 1000 of merozoites have been formed that must first leave the PVM and then the host cell membrane, before they pass the space of Disse and penetrate endothelial cells to enter blood vessels and finally infect red blood cells. For many years it has been assumed that merozoites would be liberated by rupture of the host cell membrane, but this does not explain how the merozoites cross the extracellular matrix-filled space and the endothelium of the blood vessels. First indications that the process is more complex came from Meis and Verhave [28] who analysed in detail *P. berghei*-infected rat liver sections. They briefly mentioned the occurrence of merozoite-filled structures within sinusoids, but this observation was ignored for many years partly because at the time it was not clear why these structures are formed and whether they are the result of a physiological process. Our own studies confirmed the regular occurrence of merozoite-filled vesicles (merosomes), which are extruded into liver sinusoids (Fig. 2). We could show that merosome formation is indeed part of a physiological process that occurs during merozoite formation of the *Plasmodium* parasite. The cell membrane of merosomes is of host cell origin, which masks them from attack by the numerous phagocytic Kupffer cells in sinusoids. This guarantees a safe journey for merozoites from infected hepatocytes into the blood vessel. In vitro analysis of *P. berghei*-infected hepatoma cells showed that merosome formation is preceded by parasite-dependent host cell death that appears to be a prerequisite for merozoite liberation.

Die another way: parasite-mediated host cell death

As long as *P. berghei* schizonts develop within the PVM, the host cell does not exhibit any signs of cell death. As mentioned above, during schizogony the parasite even blocks the apoptotic machinery of its host cell [26]. Only in the very late phase of the hepatic stage, when schizonts differentiate into thousands of merozoites, can the first signs of host cell death be detected [19]. Upon PVM destruction the host cell detaches and looses contact with neighbouring cells. Hollingdale et al. [29] already described the in vitro formation of merozoite-filled vesicles, but the molecular details of the process were only identified much later [19]. Once merozoites mix freely with the host cell cytoplasm, a protease-dependent destruction of host cell organelles can be observed. All this could be induced theoretically simply by removing the parasite-dependent block of host cell apoptosis, but the mechanism appears far more complicated. Caspases, as central executers of apoptosis, were found to be only weakly activated in floating infected cells and specific caspase inhibitors had no effect on PVM destruction, detachment of infected cells, and formation of merosomes. However, the more general cysteine protease inhibitor E64 blocked PVM destruction (Fig. 3) and all subsequent events. So far it is not known whether the corresponding proteases are of parasite or host cell origin, but there are arguments for both possibilities, as described below.

Cathepsins and autophagy

Apart from caspases, there are other cysteine proteases known to induce programmed cell death in mammalian cells (for a review see [30]). Cathepsins are normally located within lysosomes, but eventually they can be found in the cell cytoplasm, where they mediate cytochrome *c* release from mitochondria [31]. Damage of mitochondria and cytochrome *c* release is also a feature of detached *P. berghei*-infected cells. Furthermore, cathepsins are inhibited by E64 and not by caspase inhibitors

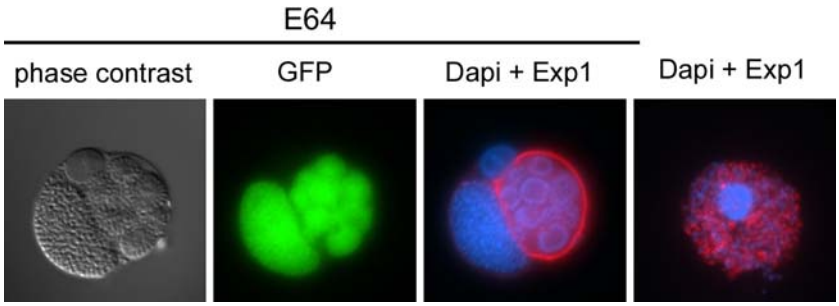

Fig. 3 Protease inhibition preserves the structure of the PVM in *P. berghei*-infected detached HepG2 cells. Infected cells in the process of detachment were either treated with cysteine protease inhibitor E64 (*left* images), or left untreated (*right* image). The E64 treated infected cell carries two GFP-expressing parasites at different stages: merozoite stage (*left* parasite) and cytomere stage (*right* parasite). Exp1 (*red*) labels the PVM (E64 treated cell), or the remnant of it in an untreated control cell (*right* image)

and they are known to play a central role in autophagy, or type II programmed cell death [32]. Autophagy occurs normally within starving cells and has initially been characterized as a rescue process [33]. Limited self-digestion provides nutrients and energy to the starving cell, but if the conditions do not improve, autophagy finally leads to cell death. It is reasonable to assume that the enormous growth of intracellular *Plasmodium* schizonts eventually results in a lack of nutrients and induction of autophagy. Indeed, *Plasmodium*-infected detached HepG2 cells exhibit autophagic features like the lack of caspase activation during cell death, the integrity of the cytoskeleton (our unpublished data) and the condensed, but not fragmented nucleus (Table 1). Although detached infected cells share some features with cells undergoing apoptosis, parasite-induced cell death more clearly resembles autophagy. A hallmark of accidental cell death, or necrosis is the early loss of membrane integrity. Since the membrane integrity of detached, infected cells is maintained as long as the intracellular merozoites are viable, this form of cell death is highly unlikely.

Plasmodium SERA proteases

E64-sensitive cysteine proteases inducing host cell death could on the other hand originate from the parasite. *Plasmodium* is known to express many cysteine proteases that are involved in various processes such as haemoglobin hydrolysis and erythrocyte invasion (for a review see [34]). Even more importantly, cysteine proteases have also been shown to play an essential role in merozoite egress from infected RBCs [35, 36] and it can be assumed that liver-derived merozoites use a similar strategy in order to be released from hepatocytes. It has been speculated that the SERA (**SE**rine **R**epeat **A**ntigen) proteases, a family of papain like cysteine proteases, are involved in merozoite egress from erythrocytes [37–39]. The SERA protease gene family consists of at least five (*P. yoelii*) and up to nine (*P. falciparum*) genes. Several SERA proteases are highly expressed at the end of the blood stage development until merozoites are released [40, 41] and for most of them secretion into the parasitophorous vacuole (PV) has been described [40, 42, 43]. Additionally, a *P. berghei* SERA protease has been shown to

Table 1 Features of *P. berghei*-induced host cell death compared with two well-characterised forms of programmed cell death, autophagy and apoptosis and additionally, necrosis as an unordered, accidental form of cell death

Features of cell death	*P. berghei*-induced host cell death	Autophagy	Apoptosis	Necrosis
Caspase dependent	No	No	Yes	No
Preserved membrane asymmetry	Yes (initially)	Yes (initially)	No	No
Membrane integrity	Yes	Yes	Yes	No
DNA fragmentation	No	No	Yes	No
Degradation of cytoskeleton	No	No	Yes	Yes
Nuclear condensation	Yes	Yes	Yes	Yes
Cytochrome c release from mitochondria	Yes	Yes	Yes	Yes
Cell detachment	Yes	Yes	Yes	Yes

The fact that the integrity of the host cell membrane is maintained in infected cells during the process of cell detachment renders necrosis-like cell death unlikely

mediate sporozoite egress from oocysts in the mosquito midgut [44]. Together, SERA proteases appear to be key molecules for parasite liberation from their corresponding host cells. It will now be a major challenge to determine the role of SERA proteases during merosome formation and host cell death in infected hepatocytes.

Conclusions and future options

The recent findings shed some light on important and fascinating details of the *Plasmodium* liver stage and our understanding of the life cycle of the parasite has changed accordingly (Fig. 4). Yet still many questions remain to be answered. How do merosomes cross the endothelial cell layer of liver sinusoids and how are merozoites finally released from merosomes? Which parasite and host cell molecules are involved in the inhibition of apoptosis that occurs throughout the parasite growth and host cell death upon merozoite formation? Genetic manipulation of parasites and siRNA techniques to down regulate host cell signalling pathways are valuable tools to investigate parasite–host interactions at a molecular level. Intravital imaging using transgenic mice expressing GFP in various cell types of the immune system [45], infected with red fluorescent parasites will undoubtedly help us to analyse the immunological responses during merosome formation.

A possible scenario is that hepatocyte-derived extrusions, which reach the sinusoids through the fenestrae (100 nm holes in endothelia), become enlarged and filled with parasite material. Although the size of the fenestrae at 100 nm is just 1/10 of the size of a merozoite, it is conceivable that they are flexible enough to allow parasite transport into the forming merosomes. Transmigration of lymphocytes through endothelial cells (diapedesis) has been suggested as a form of cellular passage [46] and at this stage we cannot exclude that infected cells use diapedesis to enter liver sinusoids.

Alternatively, similar to homing cells of the immune system, infected cells might induce a limited disruption in the cell–cell adhesions of neighbouring endothelial cells, which then allows merosome budding into the sinusoid lumen.

Whatever way merosomes pass the endothelial barrier, the striking fact that parasite material can be transported from the infected cell to the growing merosome and sometimes back to the cell strongly suggests the involvement of the host cell cytoskeleton. This in turn argues for an ordered action of cysteine proteases inducing cell death, while preserving the cytoskeleton. In vitro analysis of infected hepatocytes treated with cytoskeleton-modifying drugs will provide us with important information on which components of the cytoskeleton might be involved in the process.

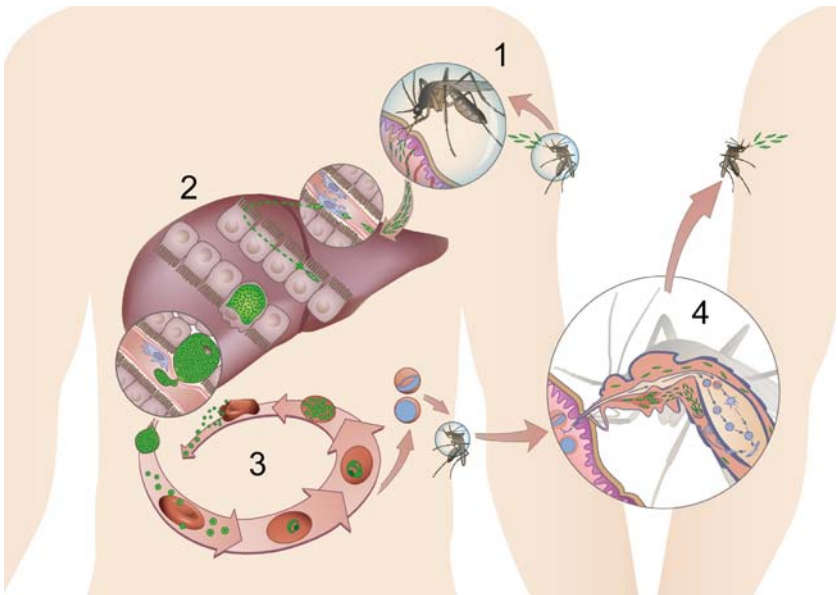

Fig. 4 Revised life cycle of *Plasmodium* parasites in mammals. *1* Inoculation of *Plasmodium* sporozoites during blood meal of an infected *Anopheles* mosquito. *2* Sporozoites reach the liver with the blood stream and glide along sinusoidal endothelia before breaching through Kupffer cells and several hepatocytes before finally developing into liver schizonts within an hepatocyte. Upon merozoite formation, merosomes are extruded into liver sinusoids. *3* Liver-derived merozoites are liberated and infect red blood cells. Repeated infection cycles occur with some parasites developing into gametocytes. *4* Fertilisation in the mosquito gut and development to infective sporozoites in the salivary glands. Picture courtesy of Maria Mota

Proteases appear to be key players in the process of parasite liberation from erythrocytes and hepatocytes and the recently discussed strategy to develop specific protease inhibitors as a new generation of anti-malarias [34] might be extended to prevent merozoite release from both cell types. In comparison to vaccination strategies using attenuated sporozoites that do not develop into schizonts, inhibition of parasite liberation from infected hepatocytes would allow complete development of the parasite and presentation of additional *Plasmodium* antigens to the immune system. Whether this results in a more potent immune response against subsequent *Plasmodium* infections and better protection rates awaits further investigations.

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References

1. Laveran A (1881) De la nature parasitaire des accidents de l'impaludisme. CR Acad Sci Paris, pp 627–632
2. Sachs J, Malaney P (2002) The economic and social burden of malaria. Nature 415:680–685
3. Amino R, Thiberge S, Martin B, Celli S, Shorte S, Frischknecht F, Menard R (2006) Quantitative imaging of *Plasmodium* transmission from mosquito to mammal. Nat Med 12:220–224
4. Frischknecht F, Baldacci P, Martin B, Zimmer C, Thiberge S, Olivo-Marin JC, Shorte SL, Menard R (2004) Imaging movement of malaria parasites during transmission by *Anopheles* mosquitoes. Cell Microbiol 6:687–694
5. Mueller AK, Camargo N, Kaiser K, Andorfer C, Frevert U, Matuschewski K, Kappe SH (2005) *Plasmodium* liver stage developmental arrest by depletion of a protein at the parasite–host interface. Proc Natl Acad Sci U S A 102:3022–3027
6. Mueller AK, Labaied M, Kappe SH, Matuschewski K (2005) Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine. Nature 433:164–167
7. Nussenzweig RS, Vanderberg J, Most H, Orton C (1967) Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. Nature 216:160–162
8. Renia L, Gruner AC, Mauduit M, Snounou G (2006) Vaccination against malaria with live parasites. Expert Rev Vaccines 5:473–481
9. Hill AV (2006) Pre-erythrocytic malaria vaccines: towards greater efficacy. Nat Rev Immunol 6:21–32
10. Luke TC, Hoffman SL (2003) Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. J Exp Biol 206:3803–3808
11. Frevert U (2004) Sneaking in through the back entrance: the biology of malaria liver stages. Trends Parasitol 20:417–424
12. Karnasuta C, Pavanand K, Chantakulkij S, Luttiwongsakorn N, Rassamesoraj M, Laohathai K, Webster HK, Watt G (1995) Complete development of the liver stage of *Plasmodium falciparum* in a human hepatoma cell line. Am J Trop Med Hyg 53:607–611
13. Mazier D, Beaudoin RL, Mellouk S, Druilhe P, Texier B, Trosper J, Miltgen F, Landau I, Paul C, Brandicourt O et al (1985) Complete development of hepatic stages of *Plasmodium falciparum* in vitro. Science 227:440–442
14. Morosan S, Hez-Deroubaix S, Lunel F, Renia L, Giannini C, Van Rooijen N, Battaglia S, Blanc C, Eling W, Sauerwein R et al (2006) Liver-stage development of *Plasmodium falciparum*, in a humanized mouse model. J Infect Dis 193:996–1004
15. Franke-Fayard B, Trueman H, Ramesar J, Mendoza J, van der Keur M, van der Linden R, Sinden RE, Waters AP, Janse CJ (2004) A Plasmodium berghei reference line that constitutively expresses GFP at a high level throughout the complete life cycle. Mol Biochem Parasitol 137:23–33
16. Natarajan R, Thathy V, Mota MM, Hafalla JC, Menard R, Vernick KD (2001) Fluorescent *Plasmodium berghei* sporozoites and preerythrocytic stages: a new tool to study mosquito and mammalian host interactions with malaria parasites. Cell Microbiol 3:371–379
17. Amino R, Thiberge S, Shorte S, Frischknecht F, Menard R (2006) Quantitative imaging of *Plasmodium* sporozoites in the mammalian host. C R Biol 329:858–862
18. Frevert U, Engelmann S, Zougbede S, Stange J, Ng B, Matuschewski K, Liebes L, Yee H (2005) Intravital observation of *Plasmodium berghei* sporozoite infection of the liver. PLoS Biol 3:e192
19. Sturm A, Amino R, van de Sand C, Regen T, Retzlaff S, Rennenberg A, Krueger A, Pollok JM, Menard R, Heussler VT (2006) Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science 313:1287–1290
20. Frevert U, Sinnis P, Cerami C, Shreffler W, Takacs B, Nussenzweig V (1993) Malaria circumsporozoite protein binds to heparan sulfate proteoglycans associated with the surface membrane of hepatocytes. J Exp Med 177:1287–1298
21. Pradel G, Garapaty S, Frevert U (2002) Proteoglycans mediate malaria sporozoite targeting to the liver. Mol Microbiol 45:637–651
22. Bergman LW, Kaiser K, Fujioka H, Coppens I, Daly TM, Fox S, Matuschewski K, Nussenzweig V, Kappe SH (2003) Myosin A tail domain interacting protein (MTIP) localizes to the inner membrane complex of *Plasmodium* sporozoites. J Cell Sci 116:39–49
23. Baer K, Roosevelt M, Clarkson AB Jr, van Rooijen N, Schnieder T, Frevert U (2007) Kupffer cells are obligatory for *Plasmodium yoelii* sporozoite infection of the liver. Cell Microbiol 9:397–412
24. Mota MM, Pradel G, Vanderberg JP, Hafalla JC, Frevert U, Nussenzweig RS, Nussenzweig V, Rodriguez A (2001) Migration of *Plasmodium* sporozoites through cells before infection. Science 291:141–144
25. Carrolo M, Giordano S, Cabrita-Santos L, Corso S, Vigario AM, Silva S, Leiriao P, Carapau D, Armas-Portela R, Comoglio PM et al (2003) Hepatocyte growth factor and its receptor are required for malaria infection. Nat Med 9:1363–1369
26. van de Sand C, Horstmann S, Schmidt A, Sturm A, Bolte S, Krueger A, Lutgehetmann M, Pollok JM, Libert C, Heussler VT (2005) The liver stage of *Plasmodium berghei* inhibits host cell apoptosis. Mol Microbiol 58:731–742
27. Doolan DL, Hoffman SL (2000) The complexity of protective immunity against liver-stage malaria. J Immunol 165:1453–1462
28. Meis JF, Verhave JP (1988) Exoerythrocytic development of malarial parasites. Adv Parasitol 27:1–61
29. Hollingdale MR, Leland P, Schwartz AL (1983) In vitro cultivation of the exoerythrocytic stage of *Plasmodium berghei* in a hepatoma cell line. Am J Trop Med Hyg 32:682–684
30. Johnson DE (2000) Noncaspase proteases in apoptosis. Leukemia 14:1695–1703
31. Stoka V, Turk B, Schendel SL, Kim TH, Cirman T, Snipas SJ, Ellerby LM, Bredesen D, Freeze H, Abrahamson M et al (2001) Lysosomal protease pathways to apoptosis. Cleavage of bid, not pro-caspases, is the most likely route. J Biol Chem 276:3149–3157
32. Lockshin RA, Osborne B, Zakeri Z (2000) Cell death in the third millennium. Cell Death Differ 7:2–7

33. Levine B, Yuan J (2005) Autophagy in cell death: an innocent convict? J Clin Invest 115:2679–2688
34. Rosenthal PJ (2004) Cysteine proteases of malaria parasites. Int J Parasitol 34:1489–1499
35. Salmon BL, Oksman A, Goldberg DE (2001) Malaria parasite exit from the host erythrocyte: a two-step process requiring extraerythrocytic proteolysis. Proc Natl Acad Sci USA 98:271–276
36. Wickham ME, Culvenor JG, Cowman AF (2003) Selective inhibition of a two-step egress of malaria parasites from the host erythrocyte. J Biol Chem 278:37658–37663
37. Delplace P, Bhatia A, Cagnard M, Camus D, Colombet G, Debrabant A, Dubremetz JF, Dubreuil N, Prensier G, Fortier B et al (1988) Protein p126: a parasitophorous vacuole antigen associated with the release of *Plasmodium falciparum* merozoites. Biol Cell 64:215–221
38. Hodder AN, Drew DR, Epa VC, Delorenzi M, Bourgon R, Miller SK, Moritz RL, Frecklington DF, Simpson RJ, Speed TP et al (2003) Enzymic, phylogenetic, and structural characterization of the unusual papain-like protease domain of *Plasmodium falciparum* SERA5. J Biol Chem 278:48169–48177
39. Li J, Matsuoka H, Mitamura T, Horii T (2002) Characterization of proteases involved in the processing of *Plasmodium falciparum* serine repeat antigen (SERA). Mol Biochem Parasitol 120:177–186
40. Aoki S, Li J, Itagaki S, Okech BA, Egwang TG, Matsuoka H, Palacpac NM, Mitamura T, Horii T (2002) Serine repeat antigen (SERA5) is predominantly expressed among the SERA multigene family of *Plasmodium falciparum*, and the acquired antibody titers correlate with serum inhibition of the parasite growth. J Biol Chem 277:47533–47540
41. Miller SK, Good RT, Drew DR, Delorenzi M, Sanders PR, Hodder AN, Speed TP, Cowman AF, de Koning-Ward TF, Crabb BS (2002) A subset of *Plasmodium falciparum* SERA genes are expressed and appear to play an important role in the erythrocytic cycle. J Biol Chem 277:47524–47532
42. Delplace P, Fortier B, Tronchin G, Dubremetz JF, Vernes A (1987) Localization, biosynthesis, processing and isolation of a major 126 kDa antigen of the parasitophorous vacuole of *Plasmodium falciparum*. Mol Biochem Parasitol 23:193–201
43. Li J, Mitamura T, Fox BA, Bzik DJ, Horii T (2002) Differential localization of processed fragments of *Plasmodium falciparum* serine repeat antigen and further processing of its N-terminal 47 kDa fragment. Parasitol Int 51:343–352
44. Aly AS, Matuschewski K (2005) A malarial cysteine protease is necessary for *Plasmodium* sporozoite egress from oocysts. J Exp Med 202:225–230
45. Mempel TR, Scimone ML, Mora JR, von Andrian UH (2004) In vivo imaging of leukocyte trafficking in blood vessels and tissues. Curr Opin Immunol 16:406–417
46. Millan J, Hewlett L, Glyn M, Toomre D, Clark P, Ridley AJ (2006) Lymphocyte transcellular migration occurs through recruitment of endothelial ICAM-1 to caveola- and F-actin-rich domains. Nat Cell Biol 8:113–123