Host ER stress during malaria parasite infection

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After transmission by *Anopheles* mosquitoes, malaria parasite sporozoites target the liver, where they infect hepatocytes and multiply thousands of times. The release of new parasites into the blood stream then initiates symptomatic red blood cell infection. Although successful replication within hepatocytes is critical for host infection, little is known about parasite–hepatocyte interactions that ensure parasite survival and development. In this issue of *EMBO Reports*, the Mota group describes a beneficial role of the host ER stress pathway for *Plasmodium* survival in infected hepatocytes [1]. They demonstrate that proteins and transcripts that act in the unfolded protein response (UPR) are elevated in hepatocytes in response to infection. Reversing these perturbations by eliminating the splicing of XBP1 or knockdown of CREBH is detrimental to parasite development. These findings are of significant interest in light of recent findings that elucidate other aspects of liver-stage parasite–hepatocyte interactions and raise new, intriguing questions for the field (Fig 1).

See also: P Inácio *et al* (August 2015)

The success of obligate intracellular pathogens is dependent on their capacity to remain innocuous, evade or counteract host defenses. Although the liver stage is clinically silent, recent reports show that parasite infection of hepatocytes is detected by the host [2] and elicits innate responses that can negatively impact parasite survival during a subsequent infection prior to adaptive responses. The most successful of pathogens can utilize host defenses to thrive. Examples of this are

Leishmania parasites and *Chlamydia* bacteria, which thrive in the host cell phagosomal environment, despite its canonical role in pathogen elimination. For other pathogens, co-opting host defenses cannot be observed on the cellular level but is apparent at the molecular level. Do malaria parasite liver stages co-opt hepatocyte responses to their benefit? Inácio and colleagues now explore how hepatocytes react to *Plasmodium* infection, and identify an induction of the UPR [1]. They then demonstrate that activation of this pathway is beneficial for parasite survival in the liver.

The UPR pathway induces endoplasmic reticulum (ER) stress in response to a wide variety of cellular perturbations. These include disruptions of the secretory pathway, nutrient depletion, and accumulation of free fatty acids or reactive oxygen species. Many of these changes are induced by intracellular pathogens, so it is not surprising that ER stress is induced by cellular pathogens and viruses alike. ER stress is a protective mechanism that restores homeostasis by increasing the capacity for protein folding within the ER and temporarily decreasing transcriptional and translational responses. However, if induction is prolonged, ER stress can also lead to hepcidin production—with its ensuing changes in iron metabolism—and an inflammatory response (induced by JNK and NFκB) [3]. Sustained ER stress can also induce apoptosis [3].

Plasmodium has evolved mechanisms to counteract the negative downstream effects of ER stress during infection of hepatocytes. For example, liver stage parasites protect infected host cells from apoptosis by increasing the levels of Bcl-2 family proteins [4,5]. Furthermore, the induction

of NFκB activity can be directly inhibited by the circumsporozoite protein, which is the major parasite surface protein of the sporozoite [6]. How the liver stage parasite might protect against hepcidin production in response to ER stress remains an open question. It has been shown that hepcidin is also produced during blood stage infection, and this production negatively impacts liver stage parasites by redistributing non-heme iron out of hepatocytes and into liver non-parenchymal cells [7].

Somewhat surprisingly, Inácio and colleagues report that the induction of ER stress is good for liver stage parasite development [1]. Although the blocking mechanisms described above may partially explain why infected hepatocytes are able to survive the insults of ER stress, they do not explain why ER stress might be advantageous for the parasite. The authors show that the benefit from ER stress is not present after 14 h of infection, before substantial parasite replication is observed, but after 24 h, when parasite DNA replication has begun. This suggests it is the maintenance of infection and the transition to the growth and replication phase that benefits from host cell ER stress. Several cellular changes occur during this time, including the sequestration of late endosomes and the host ER around the parasitophorous vacuole membrane (PVM) [8], which ensconces the parasite during liver stage development. Endoplasmic reticulum localization to the PVM suggests that the trigger to upregulate the UPR response might be direct protein–protein interactions between PVM and host proteins. In this case, the benefit of activation of the ER stress pathway might be to provide resolution to any protein instability that arises from heterologous protein–protein interactions

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Figure 1. ER stress lies at the intersection of several *Plasmodium*-induced changes.

Plasmodium liver stage parasites induce hepatocyte ER stress during infection, which can elicit NFκB, hepcidin and apoptosis. Liver stages have mechanisms to counteract at least some of these effects. ER stress can also suppress antigen presentation and increase fatty acid metabolism in the hepatocyte. CSP, circumsporozoite protein; UPR, unfolded protein response; PPM, parasite plasma membrane; PVM, parasitophorous vacuole membrane.

between parasite and host. Although the specifics of the downstream pathways that are important for parasite infection remain unknown, the hypothesis that ER stress supports parasite growth by regulating lipid metabolism, particularly that of phosphatidylcholine—which the parasite requires in abundance [9]—is worth exploring. Finally, there is evidence that during hepatitis C virus infection of hepatocytes, ER stress can diminish antigen presentation [10]. This raises the possibility that liver stage parasites might have evolved a mechanism to ensure that they remain undetected by

preexisting T-cell responses, which might be present in individuals in malaria-endemic areas. This is consistent with data suggesting that presentation of parasite antigens by infected hepatocytes through the major histocompatibility complex class I (MHC-I) is dependent on the transporter associated with antigen processing (TAP). Parasite-induced ER stress could reduce antigen presentation by the infected hepatocytes, thereby rendering it less visible to the immune system.

Inácio and colleagues have provided the first evidence that the host hepatocyte UPR

pathway is important for malaria parasite liver stage survival and development [1]. While the molecular aspects of how and why the parasite initiates this pathway remain unexplored, the induction of ER stress might provide a conduit to integrate the signals and macromolecular changes that have been described to occur in infected hepatocytes during *Plasmodium* liver stage infection. It remains crucial to elucidate the most critical points of interaction that drive parasite development in the hepatocyte host. Host pathways perturbed by the parasite represent possible points of susceptibility and may be suitable targets for the development of host-based malaria intervention strategies. Many host pathways are already the targets of drug development efforts, and perturbing them is unlikely to give rise to parasite drug resistance. Identifying essential host factors will represent a major step forward in our understanding of parasite biology and also provide opportunity to prevent the onset of clinical malaria.

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