Abstract

The malaria parasites are the one among the most biologically sophisticated and wilier organisms afflicting humans from many centuries. The mankind still remains unsuccessful in conquering the full control over the widespread prevalence of malaria causing significant morbidity and mortality. Although malaria can be diagnosed and treated, the combat against emerging drug resistances towards first-line antimalarial drugs has increased the urgency to identify new drug targets and vaccine candidates against malaria to develop novel therapeutics. Heme metabolism is central to malaria parasite biology. The parasite acquires heme from host hemoglobin in the intraerythrocytic stages and stores it as hemozoin to prevent free heme toxicity. The parasite can also synthesize heme de novo, and all the enzymes in the pathway are characterized. To study the role of the dual heme sources in malaria parasite growth and development, we knocked out the first enzyme, δ-aminolevulinate synthase (ALAS), and the last enzyme, ferrochelatase (FC), in the heme-biosynthetic pathway of Plasmodium berghei (Pb). The wild-type and knockout (KO) parasites had similar intraerythrocytic growth patterns in mice. We carried out in vitro radiolabeling of heme in Pb-infected mouse reticulocytes and Plasmodium falciparum-infected human RBCs using [4-14C] ALA aminolevulinic acid (ALA). We found that the parasites incorporated both host hemoglobin-heme and parasite-synthesized heme into hemozoin and mitochondrial cytochromes. The similar fates of the two heme sources suggest that they may serve as backup mechanisms to provide heme in the intraerythrocytic stages. Nevertheless, the de novo pathway is absolutely essential for parasite development in the mosquito and liver stages. PbKO parasites formed drastically reduced oocysts and did not form sporozoites in the salivary glands. Oocyst production in PbALASKO parasites recovered when mosquitoes received an ALA supplement. PbALASKO sporozoites could infect mice only when the mice received an ALA supplement. Our results indicate the potential for new therapeutic interventions targeting the heme-biosynthetic pathway in the parasite during the mosquito and liver stages. Further, we immunized mice with PbALASKO sporozoites and examining liver stage protection. The numbers of memory CD8+ T cells were found to be increased in PbALASKO immunized mice in comparison with naïve mice post immunization showing that the liver stage protection in PbALASKO immunized mice is mediated by CD8+ based memory response. The results indicate the potential of PbALASKO parasite as a genetically attenuated sporozoite vaccine candidate for malaria.