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INFECTIOUS DISEASE

Blocking malaria parasite invasion and egress

Growing resistance of the malaria-transmitting parasite, *Plasmodium falciparum*, to existing therapies has increased the need for new therapeutic approaches. Two new papers in *Science* now report vital roles for plasmepsins IX and X (PMIX and PMX) in *P. falciparum* invasion and egress and show that small-molecule inhibition of these aspartic proteases exhibits antimalarial efficacy in mouse infection models.

The ten plasmepsins encoded in the *P. falciparum* genome are key contributors to malaria pathogenicity, being involved in diverse cellular processes. PMIX and PMX, which are expressed at various parasite stages, have emerged as promising antimalarial targets as they fulfil indispensable, albeit unknown, functions.

Goldberg and colleagues therefore set out to characterize the roles of PMIX and PMX in blood-stage *P. falciparum* parasites. Reduction of PMIX or PMX expression in early ring-stage parasites using a new conditional knockdown technology decreased replication, confirming a critical role for these enzymes in parasite survival.

Analysis of cell cycle progression of these knockdown cell lines implicated PMIX in erythrocyte invasion and PMX in both egress and invasion. Further subcellular localization studies revealed that PMIX localizes to and acts on the biogenesis of rhoptry secretory organelles that are involved in invasion; whereas PMX localizes to exonemes and controls maturation of subtilisin-like serine protease (SUB1), which plays a crucial role in egress.

> “small-molecule inhibition of these aspartic proteases exhibits antimalarial efficacy in mouse infection models”

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Next, the authors investigated whether three previously identified aminohydantoin aspartic protease inhibitors target PMIX and/or PMX. The compounds phenocopied the PMX knockdown parasites, acting in a PMX-dependent manner and blocking SUB1 processing. Notably, oral administration of one of the aminohydantoins, CWHM-117, has previously been shown to suppress parasitaemia in mice infected with *P. chabaudi*.

Meanwhile, Soldati-Favre and colleagues studied the role of aspartic proteases during egress and invasion using the previously identified compound 49c, a hydroxy-ethyl amine peptidomimetic competitive aspartic protease inhibitor, which has previously demonstrated efficacy in *in vitro* and *in vivo* malaria infection models.

In *P. falciparum* cultures, 49c acted during late schizogony, preventing invasion and egress through inhibition of SUB1 maturation, blockade of parasitophorous vacuole membrane rupture and abrogation of processing of apical membrane antigen 1 (a component of a moving junction that is critical for invasion).

Using conditional expression systems and recombinant active PMIX and PMX, the authors revealed essential roles for these plasmepsins in erythrocyte invasion and egress, through effects on processing of rhoptry proteins and microneme/exoneme proteins, respectively; these actions were inhibited by 49c.

In vivo, daily injection of mice infected with *P. berghei* with 49c for 4 days cleared parasites from peripheral blood. After initial treatment, circulating schizonts first accumulated in the blood, confirming blockade of parasite egress from erythrocytes.

Compound 49c also inhibited hepatic stage egress *in vitro* as well as *in vivo*. When mice infected with *P. berghei* sporozoites were treated with 49c, the liver load was prolonged and blood-stage development was strongly delayed compared to untreated mice.

Furthermore, 49c targeted the sexual stage of the malaria parasite, which is required for transmission. Analysis of gametocytes isolated from *P. berghei*-infected mice revealed that 49c prevented the conversion of mature gametocytes into fertile gametes by preventing gamete egress and decreasing male exflagellation. In addition, treatment with 49c after fertilization prevented maturation of CelTOS, a micronemal protein involved in cell traversal. As a consequence, a single treatment of infected mice before a mosquito blood meal blocked oocyst formation in the mosquito midgut.

Taken together, these studies reveal the vital roles of PMIX and PMX in malaria invasion and egress, demonstrating the potential of these enzymes to be therapeutically targeted.

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ORIGINAL ARTICLES Nasamu, A. *et al.* Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasion. *Science* **358**, 518–522 (2017) | Pino, P. *et al.* A multistage antimalarial targets the plasmepsins IX and X essential for invasion and egress. *Science* **358**, 522–528 (2017)