Development of a Cell-Free Culturing System for Liver-Stage Malaria Parasites

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Malaria is caused by an intracellular eukaryotic pathogen from the genus *Plasmodium*. When infected *Anopheles* mosquitoes take a blood meal, infectious sporozoites enter into the host skin and travel to the liver. Liver-stage development is a crucial bottleneck in the malaria life-cycle, so effective medical interventions to prevent this massive amplification in parasite number are vital to prevent the onset of symptomatic disease. Currently, the field lacks the ability to effectively study the transition from early-to-late liver-stage development utilizing existing *in vitro* culturing methods. Mouse malaria parasites (*P. yoelii*) are a great model organism to study human malaria (*P. falciparum*), but *P. yoelii* liver-stage development cannot be completed in full using *in vitro* techniques. We cultured uninfected mouse hepatoma cells (Hepa 1-6 cells) and treated them with a cholesterol-dependent cytolysin, known as perfringolysin O (PFO), to selectively permeabilize the hepatocyte's plasma membrane. PFO is an exotoxin from the anaerobic bacterium *Clostridium perfringens,* that binds to cholesterol within the cell membrane at colder temperatures and forms a pore (30 nm in diameter) at physiological temperatures. Our goal is to eventually treat *P. yoelii*-infected Hepa 1-6 cells with PFO to isolate the parasitophorous vacuole so it can be cultured using a cell-free *in vitro* system. This procedure would allow us to examine the regulatory mechanisms underlying liver-stage development, which would open new areas of investigation into liver-stage specific drug therapies.

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