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Abstract of Dissertation submitted by MEDARD ERNEST

Title: The Toll-Like Receptor 2 agonist PEG-Pam2Cys as an immunochemoprophylactic and immunochemotherapeutic against the liver and transmission stages of malaria parasites

Japanese Title: マラリア原虫の肝臓期と伝播期に対する Toll 様受容体 2 のアゴニスト PEG-Pam2Cys を用いた免疫化学予防と免疫化学療法

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Introduction

After a period of maturation and development in mosquitoes, malaria parasites are injected into the blood stream of the human host via the bite of an infected mosquito. These sporozoites migrate to the liver were they infect hepatocytes and develop into thousands of merozoites. Merozoites are then released into the blood stream following rapture of infected hepatocytes. Such release and infection of the erythrocytes results into clinical malaria and deaths which are currently estimated to be around 400,000 annually.

It has been proposed that a treatment regimen that will be able to effectively eliminate the malaria parasite at the liver stage will be a blue print to malaria eradication efforts. This is because the liver stage is a clinically silent stage and therefore an ideal target to kill the parasite before any causality. Such an idea has inspired numerous studies to investigate the malaria parasite infection at the liver stage. A recent publication has shown that Plasmodium RNA is detected by the host cytosolic pathogen recognition receptor mda5, this triggers the type 1 interferon response (Liehl et al. 2014). It has also been shown that sporozoites can stimulate Toll Like Receptor 2 (TLR2) possibly via Kupfer cells (Zheng et al. 2016). Together these findings, along with others, show that pre-erythrocytic stage parasites can be killed by the innate immune response of the vertebrate host, but do not stimulate this response sufficiently for this to be protective.

To date, Primaquine and Tafenoquine are the only drugs that have been licensed to treat the liver stages of malaria parasites, although they have some serious side effects in glucose-6-phosphate dehydrogenase (G6PD) deficient patients in which severe hemolysis may occur. My work has postulated on the possibility of using a TLR2
agonist, S-(2,3-bis(palmitoyloxy)propyl cysteine (Pam2Cys) to directly stimulate the host innate immune response. I thus hypothesized that the TLR2 agonist Pam2Cys could work as both an immunochemotherapeutic to clear liver stage parasites and immunochemoprophylactic to prevent the establishment of parasites in the liver following sporozoites challenge.

**Material and Methods**

In order to investigate the role of Pam2Cys as an immunochemoprophylactic or immunochemotherapeutic, groups of mice were intravenously inoculated with an optimum dose of Pam2Cys or PBS followed by injection of sporozoites six hours after Pam2Cys inoculation, similarly, other groups of mice were inoculated with sporozoites followed by an optimum dosage of Pam2Cys 24 hours after sporozoite inoculation. Liver parasite burden was assessed by reverse transcription PCR targeting the 18s ribosomal gene to quantify gene copy number and thus liver parasite burden.

To investigate the effect of Pam2Cys with regards to innate immune cytokines and cells, mice were inoculated with 10nm of Pam2Cys followed by immune cell analysis by flow cytometry. Immune cytokines were analyzed in blood of mice injected with Pam2Cys by Elisa. The ability of Pam2Cys to block transmission was assessed by feeding mosquitoes to Pam2Cys injected mice followed by assessing oocyst burden in mosquitoes.

**Results**

Results from these experiments have revealed that Pam2Cys is able to significantly reduce liver parasite burden either when sporozoites were injected first before treating with Pam2Cys or when Pam2Cys is injected followed by sporozoites. Furthermore, inoculation of Pam2Cys resulted in an increased number of natural killer T-cells (NKT) in mice livers. Of note, it was found that Pam2Cys injection reduced the oocyst burden in mosquitoes fed to Pam2Cys treated mice.

**Discussion**

The results presented in my thesis suggest a novel way of using a TLR 2 agonist to prevent and treat malaria parasites at the liver stage and also to block transmission to mosquitoes. It is unlikely that parasite would develop resistance against Pam2Cys as it does not directly target the parasite itself. Further research on how to increase the immunogenicity of Pam2Cys possibly by conjugating it with a potent malaria antigen is critical. Moreover, studies on characterizing the compound’s toxicity to the vertebrate host should be carried out.