Genetically Attenuated Parasite Malaria Vaccine

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Introduction
Malaria is a disease with significant morbidity and mortality in humans caused by five known species of Plasmodium parasites that use female Anopheles mosquitoes as a vector (Longley, Hill, & Spencer, 2015). It is estimated that about half of the world’s population lives in areas endemic for malaria, mainly in the poor regions in sub-Saharan Africa (Karunamoor-thi, 2014). In recent years, significant gains have been made in reduction of both malarial incidence and death rates via vector control methods such as mosquito nets and insecticides (Longley, Hill, & Spencer, 2015). However, developing parasitic resistance to insecticides and antimalarial drugs undermines current disease prevention and treatment efforts. Vaccination against Plasmodium could provide another means of malaria prevention. However, the plasmodium parasite has a complex life cycle with two hosts and several life stages. Malaria is transmitted by inoculation of the sporozoite (SPZ) stage of Plasmodium into a human host via an infectious Anopheles mosquito bite. These SPZs migrate to the liver of their human hosts where they invade hepatocytes, transforming into liver stages (LS) and subsequently to pathogenic merozoites released into the blood. The pre-erythrocytic (PE) stage of the parasite is a crucial target for potential malaria vaccines as it is silent clinically and elimination of PE parasites would prevent blood stage infection and consequently both disease and transmission (VanBuskirk, et al., 2009).

Many studies on malaria utilize genetic research on P. falciparum, mostly via mouse ortholog studies. However, I could not find a single paper that compared the entire genomes of all sequenced malarial parasites – that infect humans – for conserved sequences that would most likely be from genes crucial in development of the parasite and could be used as potential targets for genetically attenuated parasite (GAP) vaccines. These vaccines have been shown to elicit complete protection against PE infection of malaria in mice and are a promising lead to future vaccines (Sack, Keitany, Vaughan, Miller, Wang, & Kappe, 2015).

Hypothesis
H0: There are no conserved regions of the Plasmodium genome that contribute to development in the PE stages and could be exploited for potential vaccines.
Hα: There are conserved regions of the Plasmodium genome that contribute to development in the PE stages. Identifying these conserved regions would provide potential therapeutic research towards the production of GAPs for immunization experiments.

Specific Aims
The first aim of this study will be to identify those genes conserved in Plasmodium species affecting humans. This will be accomplished via bioinformatic study to elucidate conserved sequences in corresponding genes of the genomes of all, or some, human-infectious plasmodium species. The second aim will be to use the identified gene(s) to create a novel GAP malaria vaccine. The sequence(s) found to be highly conserved would be mapped to their corresponding gene(s), spliced into transfection plasmids containing luciferase-coding sequences, and amplified and purified by PCR. Subsequent injection into mice at varying time and dosage increments will be conducted, with LS development attenuation measured by qRT-PCR at multiple time increments. The luciferase-tagged transfection plasmids will enable in vivo imaging of the luciferase-fused tagged protein expression in the mice. Through these techniques, I will determine if there are conserved genomic regions in Plasmodium parasites that can be subsequently exploited to create novel malaria vaccines.

Experimental Procedure
Identification of Conserved Plasmodium Genes
Full genome sequences of P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi will be compared. Software programs such as Clustal Omega and VISTA can be used to line up multiple genetic sequences to analyze sequence conservation (Useful, 2015). Once the conserved regions are known, the gene(s) that the sequences correspond to can be elucidated. Programs such as the Conserved Domain Architecture Retrievalal Tool (CDART) can be used to identify similarities of the coded proteins via sensitive domain profiles (Useful 2015). Given that the entire genomes of the parasites are being analyzed, there are no preliminary elimination criteria other than those genes that have already been adequately described and studied.

Creation of Modified Parasites
Once the conserved genomic regions have been elucidated, the corresponding genes can be individually deleted via a double crossover replacement strategy as outlined by Mueller et al. (2005) into dual Renilla and Firefly luciferase plasmids. This luciferase reporter system can be later utilized to spatio-temporally analyze promoter activity in transfected organisms (De Niz, et al., 2015). After confirmation of successful mutant plasma creation and amplification via PCR, the mutant plasmids can then be transfected into each Plasmodium species to create mutant lines for all identified conserved-sequence genes. These mutant parasites can individually be grown in humanized mice models carrying human hepatocytes by injecting them with BS parasites and subsequently using the mice to feed mosquitoes after observation of gametocyte exflagellation in the blood (De Niz, et al., 2015). The salivary glands of the mosquitoes can then be dissected to obtain salivary gland SPZs for subsequent in vitro and in vivo infections.

Plasmodium GAP Vaccine Development
Humanized mice carrying human hepatocytes will be injected with the mutant SPZs via Hydrodynamic Tail Vein Injection (HTVI) or used to feed mutant infectious mosquitoes not dissected previously. These chimeric mice, described by VanBuskirk et al. (2009), have been shown to be adequate animal models for those parasites that develop only in human hepatocytes, such as P. falciparum. At varying time intervals post-infection, the mice will be anesthetized and administered luciferase substrate intraperitoneally, subsequently positioned in an imaging chamber, and software used to measure and analyze photons emitted by the regions of interest (Chen, et al., 2014). This technique allows visualization of stage-specific activity and high levels of bioluminescence in the liver are expected for those mutants affecting LS and PE development. To show if the luciferase activity reflects the mutant gene activities at the mRNA level, transcriptional profiles of the mutant genes will be studied with qRT-PCR analysis using WT Plasmodium profiles as controls (De Niz, et al., 2015). qRT-PCR will also be conducted at various time increments to analyze LS attenuation of the parasites.

The stage-specificity, if any, of the identified mutant parasites is crucial in developing GAP vaccines. Thus far, late liver stage attenuated GAP vaccines have seen the most success in providing patency to subjects (Chen, Keitany, Vaughan, Miller, Wang, & Kappe, 2015). The elucidated mutants will then be subjected to vaccination testing via varying prime-boost regimens and subsequent challenge by intravenous injection of infectious WT-SPZs (Mueller, Labeled, Kappe, & Matuschewski, 2005). Evaluation of protection will be evaluated by blood smear to detect development of BS parasitaemia at various time points following malarial infection. Those that show development of successful patency against Plasmodium will be evaluated for protracted protection by re-challenge with varying doses of infectious SPZs at progressively later time periods.

Conclusion
The above experiments will either support or refute my hypothesis that Plasmodium parasites affecting humans have conserved genomic Sequences. The presence of conserved genomic regions would be suggestive of crucial components in parasite development, which could possibly be manipulated to yield GAP malaria vaccines. While malaria is easily preventable and there are treatment options available, drug resistance continues to rise and thus it is crucial to discover vaccines against this complex and deadly disease.

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References
Target Hepatocytes for Killing In Vivo and Contribute to Protection Elicited by Whole-Parasite Vaccination. Public Library of Science One, 9 (7).


