Gene sequencing to support malaria relapse is induced by primary infection, not a new source

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Introduction:

Malaria affects over 450 million people (Wykes, 2011). The *Plasmodium* responsible affects humans during the blood stage of its life cycle; in this stage, the *Plasmodium* is able to replicate within the hosts erythrocytes and further the infection (Wykes, 2011). While the initial presence of *Plasmodium* may be eliminated from the host's system by either an immune response or drug treatment, relapse of malaria has been known to occur (Manandhar, 2013, Wykes, 2011). Certain strains of malaria have demonstrated avoidance of the host's immunity and lay dormant in host cells, even after the infection has cleared (Wykes, 2011). The sporizoites of strain *P. berghei* invade mice peritoneal macrophage cells *in vitro* (Vanderberg, 1990) as well as survive and replicate in CD317+ dendritic cells, which may later transfer the infection to previously uninfected erythrocytes (Wykes, 2011).

Recent studies have been able to provide more easily accessible information about malaria; the DNA sequence of five different strains is available for access (Frech, 2011). This has provided understanding for more than 200 genes, involving cell-mediated interaction to the host, surface proteins that help to evade the host immune response (Bradbury. 1988), and similarities between sequences correlated with erythrocyte entry (Frech, 2011). Information like this provided on UniProt databases allows the comparison of gene sequences between varieties of *Plasmodium* with ease. Gene sequencing has also been used to examine the effect of human single nucleotide polymorphisms on susceptibility in high-risk malaria regions (da Silva Santos, 2012). Using gene sequencing research, models of past studies, and the finding that *Plasmodium* has the ability to invade and replicate in cells other than erythrocytes while being sheltered from the host immunity, *Plasmodium* rebound can be studied and compared to initial infections.

The goal of this experiment is to fill the gap in knowledge as to whether repeat infection is caused by newly introduced *Plasmodium*, or those of prior infection being sheltered by host cells. Building off of previous research, it is hypothesized that if the gene sequence of a *Plasmodium* from an initial infection is aligned, and the gene families are compared of the same individual experiencing relapse, then the result will show the second bout of infection was induced by the original *Plasmodium*, and not a new source of infection. This study will help further the understanding of the disease by allowing a deeper insight into the cause of infectious rebound. This information can be considered for research of a vaccine as well as potentially revive more extreme treatment options such as erythrocyte exchange transfusions in severe cases, which may not respond to standard treatment (Gilks, 1998).

Specific Aims:

The specific aim is to understand whether relapse of *Plasmodium* presence in the blood is caused by previous infection, or the host being infected for a second time by a new source. This important study stems from a lack of knowledge about relapsed infection, *Plasmodium* "hiding," and how replicating in other host cells may affect treatment. First, we will test our methods, verified by identical testing in mice representing a positive control, followed by surveying a sample of humans experiencing their first infection with the disease who have volunteered to return for further testing if repeat symptoms arise. Blood samples will be taken from those experiencing initial infection as well as for those with returning symptoms. Both samples will involve a blood smear to verify infection followed by isolating a small sample of the *Plasmodium*, sequencing the genes families, and comparing the initial and second infection. It is expected that we will find the *Plasmodium* that caused the first infection to also be responsible for the relapse.

Experimental Proposal:

Initial experimentation will be used to verify the methods on naive mice. This is to demonstrate that a positive result may be obtained. Fifteen naive mice will be used: five as a negative control, ten as a positive control. The negative control will demonstrate both manufacturer and lab conditions are both sufficient not to expose untested mice to the possibility of contracting malaria. Mice positive for Plasmodium (pMice+) will be infected through the tail with green florescent protein marked *P. chabaudi*. Green florescent protein will be used to aid in visualization of live *Plasmodium* in the blood stage. It has shown to be reliable in previous studies of biological processes (Wykes, 2011; Franke-Fayard, 2004), and that it performs similarly to the wild type in terms of lifecycle characteristics (Franke-Fayard, 2004). Using a transgene introduced into the *Plasmodium*, it will integrate GFP into the genome of the parasite (Franke-Fayard, 2004).

P. chabaudi was chosen for being a non-lethal strain, specific only to mice, which has been seen to cause rebound within approximately 20 days (Wykes, 2011). The pMice+ displaying symptoms of rebound (as indicated by a daily blood smear)(Wykes, 2011) will be further examined by taking a blood sample, extracting *Plasmodium* DNA, and using DNA nanoball sequencing to obtain the gene sequence. The initial gene sequence and the gene sequence from the rebound will be formatted into a FASTA and/or UniProt format and aligned using BLAST (basic local alignment search tool) and/or ClustalW. This should indicate the initial injected *Plasmodium* is identical to that of the *Plasmodium* collected after rebound.

After conformation of successful method, the study will be applied to patients with existing malaria. This is to better understand the reason behind rebound. Sample size will include 50 individuals, regardless of sex, who have previously been undiagnosed with malaria. Treatment must not be underway before the first sample is taken. A small blood sample will be taken and identical methods will be used to identify the strain. The *Plasmodium* DNA will be extracted with a commercial brand DNA extraction kit (Manandhar, 2013) and will be subjected to DNA nanoball sequencing (Anderson, 2010) paired with the patient's case report. Any patients showing repeat symptoms within an eight-month time span will return to first receive a follow-up blood test where the *Plasmodium* from the rebound sample will be sequenced and compared using ClustalW to align multiple gene families to that of initial infection. The time span is based off of previous work showing 17% relapse from individuals with *P. vivax* within six months (Manandhar, 2013). Results should indicate some portion of the original patient pool

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that returned had the presence of *Plasmodium* in the blood due to the initial strain remaining in the host's cells.

There is potential for slight variation to be seen within the aligned sequences caused by SNP (da Silva Santos, 2012). If a high conservation of genes is observed, it may be concluded that the strain is the same. If there is a large discrepancy between aligned sequences, it may indicate the second wave of infection was caused by a second source of infection. Further studies will still need to be performed to verify that even highly aligned sequences were not the result of a second infection of an identical strain. In terms of ethics, the most humane way to avoid such a situation would be to provide the patients each with a mosquito net, instruct them on basic precautions to avoid contracting the parasite, and finally determining an acceptable percentage of similarity between *Plasmodium* of the same species and that of *Plasmodium* that has replicated from a previous generation within the same host.

Conclusion:

Research on malaria is heavily geared towards finding a cure and a vaccine. The work of others in the field has provided a building block to work off of. The goal of this research is to further close the gap and give a deeper understanding of why patients experience repeat battles with malaria. Information from this study should help further advance treatment plans and help understand why certain treatments may not be working, simply by indicating that the initial treatment may not be completely eliminating the *Plasmodium* from the blood and voiding replicating parasites from host cells. Studies which may find a way to overcome this mechanism bring hope into allowing treatments such as blood transfusions or drawing out Plasmodium from host cells to be performed with safety and confidence.

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