Development of a Pre-Hepatocytic Stage Live Attenuated Malaria Vaccine

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Introduction

Malaria is a serious health concern in the world today with about 3.3 billion people living in high-risk areas around the globe (cdc). Malaria is a disease caused by a *Plasmodium* parasite. This parasite has a complicated life cycle, including developmental stages within its vector, the Anopheline mosquitoes (Ishino et al. 2004). Creating a vaccine against this disease is one of the most important areas of research today.

The first step for *Plasmodium* to cause infection in a human after the mosquito bites is for the parasite to move through the bloodstream and invade liver cells. To invade liver cells the *Plasmodium* must first utilize cell-traversal abilities (Ishino et al. 2005) to pass through the sinusoidal layer that separates the circulating blood from the liver cells (Ishino et al. 2004). Having no cilia or flagella, the *Plasmodium* utilize organelles called micronemes and the proteins they secrete to pass through this layer (Kaiser et al. 2004).

One such protein, found by is S13 (Kaiser et al. 2004). This protein has a domain similar to perforin membrane attack complex proteins and is highly conserved between *Plasmodium* species (Kaiser et al. 2004). Conservation between species is indicative of a vital gene for the life cycle of the parasite. Researchers believe that S13, also called sporozoite microneme protein essential for cell traversal (SPECT2), is involved in rupturing the plasma membrane of Kupffer cells, phagocytes in the sinusoidal layer. This is in order for the parasite to pass through these cells to hepatocytes (Ishino et al. 2004). Malaria vaccines are ideal in this pre-liver stage because it would prevent any invasion of the *Plasmodium* to any of the host hepatocytes or erythrocytes that the parasite infects in the life cycle. Therefore this research will focus on using the vital protein for hepatocyte invasion, S13/SPECT2, in the development of an attenuated vaccine.

Hypothesis

Since an attenuated pre-hepatocyte invasion vaccine would make the host immune to any sort of infection by the malaria *Plasmodium*, finding a gene vital to the transition from sporozoite blood stage to liver stage would be the ideal option. This study hypothesizes that using the perforin-like protein S13/SPECT2 to develop an attenuated vaccine will protect against all forms of *Plasmodium* invasion since this is at the beginning of the infectious cycle.

Specific Aims

The first aim of this study is to create a successful knockout or gene disruption, of the S13/SPECT2 locus. The second aim of this study is to use the attenuated sporozoite to immunize a rodent model, which will subsequently be challenged with wild-type (WT) sporozoites.

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Research Methods & Design

Creation of S13/SPECT2 disruptants
A rodent *Plasmodium* species, *Plasmodium bergehii*, would be used in this experiment. A targeted gene disruption of the S13/SPECT2 locus will be created using homologous recombination. The first step is to amplify small fragments of the gene using polymerase chain reaction (PCR). Specific primer pairs can be found in the previously conducted study by Ishino et al. (2004). These small fragments will then be cloned onto a selectable marker. In this case the marker will give the S13/SPECT2(-) resistance to the antimalarial drug pyrithamine. The S13/SPECT2 locus will then be cleaved with the restriction enzyme NcoI and double-crossover recombination will insert the new fragment with the pyrithamine marker into the genome (Figure 1). The above procedure is followed per the previous SPECT2 locus disruption by Ishino et al. (2004).

Confirmation of the presence of S13/SPECT2(-) can be done in several ways. One way is by Southern blot analysis as carried out by Ishino et al. (2004). WT sporozoites would have a band appearing at about 3.7 kbp. If the gene disruption was successful the S13/SPECT2 (-) should show a band near 7.8 kbp (Ishino et al. 2004). This is because the addition of the marker would add about 4.1 kbp to the locus.

Attenuation and Immunization with S13/SPECT2 (-)
Once the disruptants have been confirmed, they will be injected into rodents via the tail vein and their infectivity observed. To assess whether the sporozoites were able to cross the sinusoidal barrier and invade liver cells, parasitaemia will be monitored via blood smears. Previous studies have found that SPECT2 disruptants had significantly lower rate of infection compared to WT sporozoites (Ishino et al. 2004). Furthermore, Ishino et al. found that the majority of SPECT2(-) sporozoites were unable to leave blood circulation, therefore staying in the non-pathogenic stage until death (2004). I would therefore predict these same results to occur. This would preliminarily indicate attenuation of the sporozoite *Plasmodium* and open the possibility to test this as an attenuated vaccine.

Once attenuation has been confirmed, immunization of rodents with the S13/SPECT2 (-) sporozoites can take place. Different doses of the disrupted sporozoites will be used in order to determine the minimum dosage needed for immunization. The need for boosters of varying dates after initial immunization and varying booster dosages will also be examined. Rodents injected with WT sporozoites will act as a control and be expected to develop the malaria disease within a standard timeframe. Finally, the immunized rodents will be challenged with WT sporozoites and the parasitaemia again monitored with blood smears.

The experimental procedure described above follows the design of Mueller et al. in their study of UIS4 sporozoite protein (2005). In this previous study they found that mice immunized with their UIS4(-) were conferred complete protection when challenged with WT sporozoite stage *Plasmodium* (Mueller et al. 2005). This would also be the predicted outcome of the proposed immunization experiment using S13/SPECT2 (-).

Possible Complications
One possible complication or pitfall of the previously described experiment would be if making the S13/SPECT2 (-) causes some sort of unpredicted malfunction in the rest of

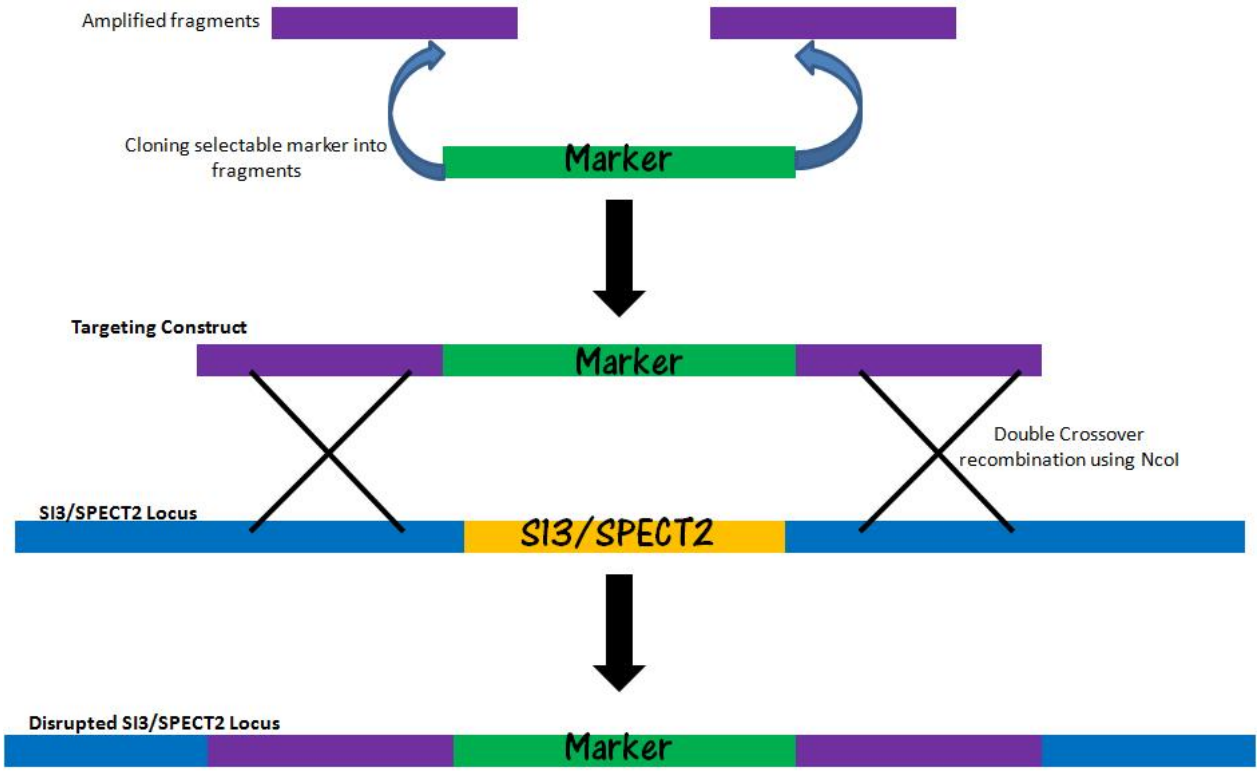

Figure 1: Targeted Disruption of S13/SPECT2 gene. This diagram shows the selectable pyrithamine marker being integrated into the S13/SPECT2 locus by a double crossover event. The recombination both disrupts the gene locus and makes the sporozoites selectable by conferring pyrithamine resistance.

the *Plasmodium* life cycle other than entrance into the liver. In a study previously conducted by Ishino et al. they examined this very possibility and found that there was no difference between their SPECT2(-) and the WT sporozoites in growth rate or development in the mosquito vector (2005). We would thus predict the same for our disruptants.

Also, although the previous study found that the parasitaemia of the SPECT2 (-) were significantly lower than WT, there was not a zero infection rate. This means that the sporozoites are somehow able to get through the sinusoidal cell layer even without the membrane attack complex present in the S13/SPECT2 protein. This is unexplained and may pose a problem if the vaccine is able to cause full malaria infection.

Conclusion

An effective malaria vaccine would be extremely valuable to those living in high-risk areas around the globe. By utilizing this S13/SPECT2 gene it not only promises potential immunity, but since this will prevent the *Plasmodium* from ever entering the liver, it will stop the parasite before it truly invades its host. In keeping the *Plasmodium* out of the liver and in the circulatory system it allows more exposure to immune system cells. This could aid the immune system in generating a stronger, more targeted response. Also, since the *Plasmodium* will be in the sporozoite stage in the blood, other antimalaria medications will be more effective since the *Plasmodium* will not be hidden within our own body cells.

References

1. Centers for Disease Control and Prevention. CDC, n.d. Web. 11 Dec. 2012. <http://www.cdc.gov/MALARIA/>.

2. Ishino, Tomoko, Yasuo Chinzei, and Masao Yuda. "A *Plasmodium* sporozoite protein with a membrane attack complex domain is required for breaching the liver sinusoidal cell layer prior to hepatocyte infection." *Cellular Microbiology* 7.2 (2005): 199-208.

3. Ishino, Tomoko, Kazuhiko Yano, and Masao Yuda. "Cell-passage Activity is Required for the Malarial Parasite to Cross the Liver Sinusoidal Cell Layer." *PLOS Biology* 2.1 (2004): 77-84.

4. Kaiser, Karine, et al. "Differential Transcriptome Profiling Identifies Plasmodium Genes Encoding Pre-Erythrocytic Stage-Specific Proteins." *Molecular Microbiology* 51.5 (2004): 1221-32.

5. Mueller, Ann-Kristin, et al. "*Plasmodium* Liver Stage Developmental Arrest by Depletion of a Protein at the Parasite-Host Interface." *PNAS* 102.8 (2005): 3022-27. Print.