The use of algal chloroplasts to express *Plasmodium falciparum* Circumsporozoite Protein and Pfs25 surface antigens in a vaccine aimed to induce protective antibody responses in mice and mosquitoes

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Introduction

Malaria is a major issue in tropical and subtropical regions of the world. There are nearly 200 million new cases of malaria and 1 million deaths per year (Wiki, 2012). *P. falciparum* causes the most deadly form of the disease and is transmitted by the female *Anopheles* mosquito (Wiki, 2012). The circumsporozoite protein (CSP) is a prominent surface antigen of the *P. falciparum* parasite during its erythrocytic stage. The CSP has been a prime target for vaccine development to fight blood stage infection; among the most promising is the RTS,S viral vector vaccine by GlaxoSmithKline, but has not been effective enough to be licensed (Ballou, 2009). The uses of bacterial and viral vectors have been common among attempts to develop an effective vaccine but remain expensive to produce and not always effective at expressing the target antigen, which may explain why none have been licensed (Danuvillee et al., 2010). Recent research suggests that algal chloroplasts may be utilized as effective recombinant system for *Plasmodium* surface antigens (Danuvillee et al., 2010). The algae are cheap and easy to produce in large quantities, genetically tractable, and have been used to produce protein subunit vaccines (Danuvillee et al., 2010).

A study published last spring by Gregory et al. (2012) and colleagues used the algae *Chalamydomonas reinhardtii* to express the *P. falciparum* surface protein 25 (Pfs25) in a vaccine and found that the vaccine elicited antibody responses in mice and blocked sexual development of the parasite in mosquitoes (Gergory et al., 2012). The Pfs25 is a surface protein expressed during the sexual stages of *P. falciparum* (Gergory et al., 2012). Previous research has shown that *C. reinhardtii* can effectively express *P. falciparum* surface antigens and induce antibody response in mice and mosquitos (Gergory et al., 2012). Furthermore, over the years, the CSP antigen's immunogenicity has been shown to elicit strong antibody responses in numerous studies; however using *C. reinhardtii* to express CSP and Pfs25 antigens together has not been studied and serves as a potential vaccine candidate (Okitsu et al., 2007) (Wang et al. 1995).

Given this, I would like to develop a vaccine against *P. falciparum* using the *C. reinhardtii* chloroplasts to express recombinant CSP and Pfs25 surface antigens.

Hypothesis

I hypothesize that using *C. reinhardtii* chloroplasts as a recombinant system to express the CSP and Pfs25 surface antigens will elicit increased antibody responses in mice and mosquitos, preventing *P. falciparum* infection and transmission. The Pfs25 antigen has been shown to elicit heightened antibody responses in mice and mosquitos but CSP has not been used in a chloroplast system (Gergory et al., 2012). Given that the chloroplasts can be readily manipulated genetically, we predict that the CSP antigen will be effectively expressed and thus increase antibody responses in hope of preventing infection of blood cells in mice.

Specific Aims

1. Use *C. reinhardtii* chloroplasts as a recombinant system to effectively produce specific P. falciparum surface protein antigens and elicit increased antibody production in mice and mosquitos. We will assess the immunogenicity of the recombinant Pfs25 and CSP surface proteins by measuring the amount of antibodies produced in vaccinated mice. Our hypothesis predicts that there will be significant level of antibodies produced against the specific antigen.
2. To determine whether the heightened antibody production will inhibit the various stages of the parasite, we will test to see if vaccinated mice show resistance to sporozoite inoculation and if mosquitos fed antisera from vaccinated mice will experience hindered transmission. This will be done by testing the blood of the mice for sporozoite development and by dissecting mosquitos to see if any ookinetes develop in the mosquitos.

Experimental Proposal

In the first experiment we will measure the immunogenicity of the chloroplast recombinant vaccine. The experiment will require a total of eighty mice, all of which will be assigned to one of four vaccine variants. The mice will either be given the CSP + Pfs25, CSP without Pfs25, Pfs25 without CSP, or the negative control that does not express either. Vaccinating two different groups with each antigen alone will allow us to determine if immunogenicity is greater when the two antigens are not combined in the recombinant chloroplast system. Twenty mice will be assigned to each group and we will wait two weeks before analyzing mice antisera. Antibody titers will be measured by ELISA against affinity purified CSP and Pfs25 (Gergory et al., 2012). This will allow us to quantify the relative amount of antibodies produced in response to the various vaccine assignments (Gergory et al., 2012).

The second part of the experiment will depend on the results measured by ELISA. If our hypothesis is correct that the CSP + Pfs25 vaccine will induce heightened antibody response, then the antisera from the CSP + Pfs25 vaccinated mice will then be fed to female *Anopheles* mosquitoes along with *P. falciparum* gametocytes to determine if the antibodies to the CSP+Pfs25 antigens will block transmission. The female *Anopheles* mosquitoes will then be dissected after two weeks to analyze oocyte production (Gergory et al., 2012). The control mosquitoes will be fed only the *P. falciparum* gametocytes.

If the CSP+Pfs25 vaccine does in fact elicit increased antibody responses, we will also test if the extent of immunogenicity elicited by CSP+Pfs25 vaccine is enough to provide resistance to sporozoites. The immunized and control mice will be administered 5000 live sporozoites to ensure that enough sporozoites are present for infection to occur. A hemocytometer will be used to analyze the blood cells and determine whether infection has been hindered.

Potential Outcomes

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Given that previous studies, such as those done by Okitsu et al. (2007) and Wang et al. (1995), have shown increased antibody production with CSP and with Pfs25 vaccination, we predict that if the chloroplast can effectively express both recombinant antigens, we should expect to see increased antibody responses in mice vaccinated with CSP+Pfs25; furthermore, we expect to see little to no antibody production in the control mice. However, the genetic manipulation of recombinant CSP into chloroplasts has not been previously attempted; therefore, we cannot guarantee that CSP can be expressed by the algal chloroplasts. In the previously mentioned study done by Gregory et al. (2012), the Pfs25 chloroplast vaccine was effective in inducing antibody responses in mice and blocked transmission to mosquitoes. We expect our results to follow this same trend, unless the presence of CSP in the vaccine hinders expression of the Pfs25. If the CSP+Pfs25 vaccine does elicit increased antibody responses compared to the control, we would hope that this would provide resistance or at least some delay in blood cell infection.

Conclusion

This experiment would be the first to incorporate the CSP antigen in the *C. reinhardtii* Pfs25 recombinant chloroplast vaccine. It is important to experiment with the CSP antigen given its strong immunogenicity for antibodies that provide protection against infection of the blood cells. Incorporating CSP and Pfs25 into a single *C. reinhardtii* recombinant chloroplast vaccine that can effectively express both antigens and elicit antibody responses would provide protection to the individual being vaccinated, as well as block transmission in the mosquito. It is possible that this type of vaccine could be efficiently produced in mass quantities and at low cost, making it a very attractive candidate for vaccine design and development (Gergory et al., 2012).

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