Evolutionary Consequences of the Plasmodium falciparum Sporozite Vaccine in Humans

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Introduction:

Malaria is a blood-borne disease that infects millions across the world each year. It causes anemia and is deadly in humans once it enters the bloodstream, as it accumulates in areas of the body. There are various species of parasite that cause malaria, all belonging to the genus *Plasmodium*. The specific *Plasmodium* that causes the most malaria-related deaths in humans is *Plasmodium falciparum* (Kyes et al., 2001). All four species of the *Plasmodium* genus are dependent on mosquitoes to serve as vectors to reach their human hosts and complete their life cycle (Kyes et al., 2001). When the parasite enters the bloodstream, it enters and changes the structure of erythrocytes, which allows infected erythrocytes to evade the host's immune response (Fairhurst et al., 2005).

The most successful method for obtaining high-level, sustained, and protective immunity against the pre-erythrocytic stages of the parasite is through the immunization of *Plasmodium* falciparum (Pf) sporotzites (SPZ) (Luke et al., 2003). The safest way to establish this immunization is through the administration of attenuated, aseptic, purified, and cryopreserved PfSPZ's (Luke et al., 2003). The live attenuated parasites protect through the liver's CD8+ T Cell immunity (Epstein et al., 2011). It has been demonstrated that through using intravenous administration of PfSPZ's, a strong immune protection is established in humans (Seder et al., 2013). Previous studies have addressed the concern that a malaria vaccine will lead to antigenic escape and will lead to erosion in vaccine efficacy (Barclay et al., 2012). The risk of virulence evolution is an issue when developing malaria vaccines both for those who are vaccinated and those who are unvaccinated (Barclay et al., 2012). The factor of virulence evolution has been linked to both serial passage and a blood-stage vaccine in rodents (Barclay et al., 2012) but not to a pre-erythrocytic stage vaccine and not in humans.

Hypothesis:

The research will test the thesis that *P.falciparum* will undergo virulence evolution in humans due to serial passage. It will also test the thesis that humans vaccinated with the PfSPZ vaccine will have a greater evolution of virulence in serial passage.

Specific Aims:

The primary goal of this study is to use a serial passage experiment to observe the virulence evolution of the *P.falciparum* parasite in unvaccinated humans, then to observe the change in humans vaccinated with the PfSPZ vaccine. We will test the prediction that *P.falciparum* will increase in virulence in unvaccinated humans by using a serial passage experiment. The serial passage experiment will be carried out by transferring the parasites from one human to the next by needle. Any increase of virulence of *P.falciparum* will be determined by measuring the anemic response, determined using flow cytometry, and by parasite density, found through a blood smear. The prediction that vaccinated humans will lead to more virulent parasites will be determined by the same method. There will be two groups, one inoculated intravenously with the PfSPZ vaccine and the other inoculated intravenously with adjuvant of the vaccine to serve as a control. We expect to find that virulence will increase for all serial passage experiments, though there will be a greater increase in those vaccinated with the PfSPZ vaccine.

Experimental Proposal:

This experiment will test the thesis that virulence evolution can occur in humans with the human malaria parasite *P.falciparum*. If it is found that *P.falciparum* can undergo virulence evolution in humans, the next step is to test the hypothesis that said evolution is stronger in those vaccinated with the PfSPZ vaccine. The experiment will start by compiling human subjects. It is understood that there is an inherent risk with exposing human subjects to *P.falciparum*, but understanding the changes that occurs in this human parasite is integral to understanding the behavior of the parasite. As soon as testing is complete, infected subjects will be treated with appropriate drugs such as chloroquine or quinidine. The procedure for these experiments follow the protocol established in previous studies (Barclay et al., 2012).

Serial Passage A

Serial Passage Experiment A will involve the transfer of 0.1 ml of diluted blood containing $5x10^5$ parasites between subjects every seven days. This first serial passage experiment is merely to derive a more virulent parasite lineage from the ancestral parasite. This allows us to test whether the derived parasite or the ancestral parasite is more virulent. Serial passage A will be done for 30 series in order to allow enough series for any changes in virulence to occur. In order to test for virulence, two different methods will be used. Seven days after infection, the subject infected initially and the subject from the final series will have blood samples collected and a blood smear and flow cytometry performed. A blood smear is a test that provides information about the shape and number of blood cells. A drop of blood is placed on a slide while another slide is placed at a 45° angle and dragged across the slide containing the drop of blood. The blood is then spread thin which allows for the counting of parasites. It is from the blood smears that parasite densities are measured and an increase in parasite density is congruent with an increased virulence. The anemic affect is measured using flow cytometry in which the blood is placed in a tube and a laser is used to count the different number of cells and which types the cells are. The flow cytometry measures the number of erythrocytes and this is related to anemia. The lower the number of erythrocytes, the greater the virulence of *P.falciparum.*

Serial Passage B

Serial Passage Experiment B will involve the comparison of two serial passages, one involving those inoculated intravenously with the PfSPZ vaccine contacting $1.35x10^5$ PfSPZs and those inoculated intravenously with the

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adjuvant of the PfSPZ vaccine to serve as a control. For each lineage, 0.1 ml of diluted blood containing $5x10^5$ parasites is transferred by needle between subjects every seven days. This experiment is to observe the change in virulence that occurs due to the PfSPZ vaccine. Serial Passage Experiment B will take place for 21 series. Blood samples will be drawn seven days after exposure to the 0.1 ml of diluted blood containing $5x10^5$ parasites, and virulence will be measured using blood smears and flow cytometry. Samples will be drawn for the initial subjects in both the control and the vaccinated trials, as well as after 10 series and 21 series. The reasoning for this intermediate sample is to observe the difference in virulence along different points in the series. If there is a great change in virulence between series 10 and 21 in the subjects, it is safe to hypothesize that a greater virulence is possible after additional series and that an apex has not been at the final series.

Conclusion:

This research can further the understanding of the how the malaria parasite P.falciparum can evolve in human subjects. Furthermore, understanding how parasites' virulence can evolve in vaccinated individuals will increase the understanding of the effect of vaccination on unvaccinated individuals. Just as antibiotics have lead to the creations of "super bugs" such as MRSA and antibiotic resistant TB, it is possible that vaccines can lead to a more virulent pathogen. Previous studies have demonstrated the malaria parasites do evolve in mice. If it is proven that the PfSPZ vaccine can lead to the formation of more virulent P.falciparum, then new research has to be done in order to prevent it. Perhaps since the PfSPZ vaccine involves attenuated, aseptic, purified, and cryopreserved PfSPZs, it is possible that the immune response will be strong enough to prevent the survival of any transferred parasites in the serial passage experiments. If this is the case then more support is lent to the effectiveness of the PfSPZ vaccine.

References

Barclay, V. C., Sim, D., Chan, B. H. K., Nell, L. A., Rabaa, M. A., et al. (2012). The evolutionary consequences of blood-stage vaccination of the rodent malaria plasmodium chabaudi. *PLOS Biology, 10,* 1-11.

Epstein, J. E., Tewari, K., Lyke, K. E., Sim, B. K. L., & Billingsley, P. F. (2011). Attenuate malaria vaccine designed to protect through hepatic CD8+ T cell immunity. *Science, 334*, 475-480.

Fairhurst, R. M., Baruch, D. I., Brittain, N. J., Ostera, G. R., & Wallach, J. S. (2005). Abnormaldisplay of PfEMP-1 on erythrocytes carrying haemoglobin c may protect against malaria. *Nature, 435,* 1117-1121.

Kyes, S., Horrocks, P., & Newbold, C. (2001). Antigenic variation at the infected red cell surface in malaria. *Annual Review of Microbiology, 55*, 673-707.

Luke, T. C., & Hoffman, S. L. (2003). Rational and plans for developing a non-replicating, metabolically alive, radiation-attenuated plasmodium falciparum sporozite vaccine. *Journal of Experimental Biology, 206*, 3803-3808.

Seder, R. A., Chang, L., Enama, M. E., Zephir, K. L, Sarwar, U. N., et al. (2013). Protection against malaria by intravenous immunization with a nonreplicating sporozite vaccine. *Science, 314,* 1359-1365.