The winding road to developing a malaria vaccine. Study hypothesis.

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Abstract

In Africa, a child dies every 30 seconds from malaria, a vector-borne parasitic disease caused by *Plasmodium* spp, with higher mortality and severe forms of disease more frequently associated with *Plasmodium falciparum* infection. By looking at the natural resistance to malaria conferred by sickle cell trait, we hypothesize that a malaria therapeutical vaccine targeting the erythrocyte stage of the parasite through erythrocyte sickling could reduce parasite density and control the progression and severity of disease, thus decreasing the morbidity and mortality associated with severe forms of malaria.

Keywords **Malaria, falciparum, infection, sickle cell, vaccine.**

Introduction

In Africa, a child dies every 30 seconds from malaria, a vector-borne parasitic disease caused by *Plasmodium* protozoa.[1,2] Typically, the parasites invade the red blood cells and are responsible for fever with chills and headaches which can associate coma or lead to death, especially when the infection is unrecognized and untreated or when the parasite poses resistance issues. Out of the five *Plasmodium* species known to cause malaria in humans, *Plasmodium falciparum* is associated with the highest mortality.[3]

In the context of increasing resistance of *Plasmodium* spp to chloroquine and other antimalarial agents such as sulfadoxine-pyrimethamine,[4] the quest for a malaria vaccine has been long and sombre but recent development in medical research has led us to the point where we can consider the development of a vaccine to be feasible.[2]

When working on such a vaccine, it is fundamental to look at both the human body's response to the infection and the life cycle of the parasite in the vector (female *Anopheles* mosquitoes) and in the human host, particularly since different stages of the parasite life cycle have been shown to express different antigens.[2,5]

The immune system is key to fighting off attacks by *Plasmodium* spp, through various mechanisms. In the first months of life, protection from malaria is conferred by the acquired maternal antibodies,[6] and later on, in children older than one year of age, it has been shown that the odds of developing clinical malaria are lower in the high-transmission *Plasmodium falciparum* areas. This phenomenon appears to be due to premunition, defined as "resistance to new infections because of an existing infection with other strains."[7] It is therefore apparent that naturally-acquired immunity can be effective, however partial and short-lived it may be, dependent on continuous antigenic stimulation.[2]

Most prophylactic vaccines under research target the pre-erythrocytic stage of the parasite – the sporozoite and liver stage – and try to elicit immunity to certain surface antigens expressed on infected hepatocytes or to parasite DNA, while vaccines targeting the sexual stage of *Plasmodium* aim to prevent parasite transmission to new hosts.[2]

A different approach is that of erythrocytic or blood stage vaccines which can be considered therapeutic vaccines since they aim to decrease parasitic load in order to control the progression and severity of disease.

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Study hypothesis

The typical approach to vaccination is that of providing the immune system with either a non-virulent strain of the pathogen or with antigenic particles able to elicit a strong, long-lived antibody response that will prevent sporozoites from invading hepatocytes and/or a strong cellular immunity (which would inhibit intra-hepatic parasite development).[2]

However, the hypothesis for this study stemmed right from the textbook definition of the term "immunity: a condition of being able to resist a particular disease especially through preventing development of a pathogenic microorganism."[8]

Certain genetic conditions have been associated with resistance to *Plasmodium* spp infection, as is the case with sickle cell trait (HbAS) or the potentially fatal form of sickle cell disease (hemoglobin SS, HbSS).[9] In an attempt to determine the mechanism whereby HbAS is protective against *Plasmodium falciparum* malaria, Gong et al. have performed a study on a cohort of 601 children with ages between 1-10 years old,[10] suggesting an innate mechanism of protection against high parasite density at younger ages and an acquired mechanism of protection from establishment of parasitemia in children with HbAS, at older ages. Despite the already well-described apparent risk of severe outcomes of disease in patients with HbSS,[9] this study showed that in parasitemic children with HbAS the progression to symptomatic malaria was lower, particularly at higher ages (roughly 9 years old).[10]

Plasmodium spp sporozoites pass to humans through infecting mosquito bites and they are taken up by liver Kupffer cells, which play an important role in conditioning the immune response and can act as antigen presenting cells.[11] Those which escape destruction invade the hepatocytes and start heavily replicating, passing through the schizont and the merozoite stages. Injured hepatocytes[12,13] release the merozoites into the blood stream, where they invade the circulating erythrocytes.[6] The parasites then metabolize hemoglobin, freeing the heme and using globin as a source of amino acids.

Given that the sickle cell gene causes a substitution of the amino acid valine for glutamic acid at one point in the beta-polypeptide chain of the hemoglobin molecule, the new hemoglobin (hemoglobin S) becomes insoluble when reduced and precipitates inside the erythrocyte envelope, distorting the cell into the shape of a sickle. As the malaria parasite enters the sickled cell, it forms an impervious or insoluble complex.

The hypothesis for developing a malaria vaccine is that sickling of red blood cells protects against malaria. In vitro studies have shown that under low oxygen tension, sickle trait red cells infected with *Plasmodium falciparum* tend to sickle more than uninfected cells,[6,14] which alters the intraerythrocytic conditions and disrupts parasite metabolism,[15] killing the parasites primarily at the large ring stage.[6,16] Given that sickle cells have been shown to undergo eryptosis (suicidal erythrocyte death),[17] selective sickling of infected sickle trait red cells could be yet another factor involved in reducing the parasite burden.[6]

Sodium dithionite induces sickling in vitro and adding sodium dithionate to sickled cells increases their sickling.[18] We hypothesize that by administering immunizing doses of sodium dithionite it is possible to induce sickling in humans and protect them against the lethal form of *Plasmodium falciparum* malaria. Thiomersal needs to be used as a preservative. The aim of the study is to determine whether sickling also takes place in subjects without the genetic mutation for sickle cell trait and to check if artificially sickled erythrocytes display the same resistance to *Plasmodium* spp parasites as do the genetically sickled ones. We also aim to determine how long the transition to sickle cells would last, if this is a reversible process or one that is maintained during the life cycle of the erythrocytes exposed to the agent. Given the limited field literature, additional data on this topic is stringently needed but we consider this approach to be a feasible hypothesis for further research.

Discussion

This approach to a malaria therapeutical vaccine is based on the hypothesis that targeting the erythrocyte stage of the parasite could reduce the parasite density in order to control the progression and severity of disease, thus decreasing the morbidity and mortality associated with severe forms of malaria. Given the well-described risks associated with homozygous sickle cell disease, it is extremely delicate to set cut-off estimates for what could be a reasonable amount of sickled cells for malaria protection and what could, on the other hand, pose a certain risk either through anemia, hypoxia, or through the advent of sequestration crises.[9] The impact of such a vaccine on the survival of erythrocytes is also a matter which requires further studies and it is important to evaluate and describe the safety profile of such an approach.[19]

One other risk described in patients with HbSS is related to auto-infarction, which plays an important role in the deterioration of the splenic function, reducing its ability to clear infected erythrocytes.[9] Recent data suggest that malaria is one of the conditions that associate eryptosis.[17] In order to ensure its optimal growth, *Plasmodium* spp uses oxidative stress[20] to open sodium and calcium channels in the host red blood cells. However useful for parasite development, calcium is also responsible for triggering suicidal death of infected erythrocytes, through cell shrinkage, cell membrane blebbing and phospholipid scrambling,[21] leading to recognition and degradation by macrophages,[17] which may to a certain point compensate for the decreased splenic function.

As with all study hypotheses, the risk/benefit ratio related to this vaccine approach needs to be thoroughly studied and all results must be interpreted with circumspection, in the context of the existing literature data. More work needs to be undertaken in vitro since the road to developing a vaccine for malaria is indeed a long and winding one. However, we do consider that our hypothesis justifies further study, particularly at a time when we have to deal with increasing resistance to antimalarial drugs and potentially life-threatening outlines of *Plasmodium falciparum* malaria.

Conclusions

In the context of limited literature data on in vivo sickling of erythrocytes with sodium dithionite and thiomersal, we consider it necessary to perform further testing in order to gather the preliminary data for developing a therapeutical malaria vaccine which would target the erythrocyte stage of the parasite. This could help limit the progression and severity of the disease and could contribute to lowering malaria associated morbidity and mortality, particularly in cases with decreased antimalarial susceptibility and consequent issues of resistance to current treatment options.

Conflicts of interest All authors – none to declare.
Author contributions All authors had equal contributions.

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