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A Research and Development in Malarial Vaccine: Systems of Genetically-Based Resistance to Malaria

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Abstract – More than 30years after the first report of successful vaccination against malaria using radiation-attenuated sporozoites, an effective malaria vaccine is not yet available. However, field and experimental data indicate that it can be developed. An astonishing amount of data has accumulated concerning parasite biology, host-parasite interactions, immunity and escape mechanisms, targets and modulators of immune responses. Nevertheless, so far this knowledge has not been enough to make us understand how to properly manipulate the whole system to build an effective vaccine. In this article, we describe candidate antigens, mechanisms, targets and trials performed with potential malaria vaccines and discuss the approaches, in vivo and in vitro models, constraints and how technologies such as DNA vaccination, genomics/proteomics and reverse immunogenetics are providing exciting results and opening new doors to make malaria vaccine a reality.

Malaria remains one of the most prevalent parasitizes worldwide. About 350 to 500 million febrile episodes are observed yearly in African children alone and more than 1 million people die because of malaria each year. Multiple factors have hampered the effective control of this disease, some of which include the complex biology of the Plasmodium parasites, their high polymorphism and their increasingly high resistance to antimalarial drugs, mainly in endemic regions. The ancient interaction between malarial parasites and humans has led to the fixation in the population of several inherited alterations conferring protection against malaria. Some of the mechanisms underlying protection against this disease are described in this review for hemoglobin-inherited disorders (thalassemia, sickle-cell trait, HbC and HbE), erythrocyte polymorphisms (ovalocytosis and Duffy blood group), enzymopathies (G6PD deficiency and PK deficiency) and immunogenetics variants (HLA alleles, complement receptor 1, NOS2, tumor necrosis factor-α promoter and chromosome 5q31–q33 polymorphisms).

Malaria, a vector-borne infectious disease, is currently a grave and universal concern with a significant social, economic, and human cost, mainly in developing countries. In addition, the emergence and spread of resistance to antimalarial therapies have further aggravated the global situation. Currently most of the research is focused on development of antimalarial drugs, drug resistance, and novel formulations to maximize the therapeutic effect. A number of novel molecules potentially active against malarial parasites are being developed. A vaccine is still viewed as a critical part of a long-term malaria control strategy. In the last several years various studies have shown significant progress in the development of vaccines against malaria. Advancement in vaccine technology and immunology is being used to develop malaria subunit vaccines that would open up new vistas for effective treatment and control of malaria.

The development of an effective malaria vaccine represents one of the most important approaches that would provide a cost-effective intervention in addition to currently available malaria control strategies. An overview on progress in antimalarial vaccines is presented.

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INTRODUCTION

Malaria has been known to students of human disease from the dawn of history. Observers had followed the association between water collections and the prevalence of the disease since early times. The discovery by Ronald Ross, in Secunderabad, of the role of the female Anopheles mosquito in sustaining the infection has become a part of medical lore. The subsequent researchers solved the complex life cycle of the malarial parasite.

Malaria is seen in all the continents to a certain extent. While infections caused by P. vivax, P. malariae and P. ovale are rarely life threatening or overwhelming, P. falciparum infections can be fatal due to the peripheral vascular localization of the mature trophozoites. The placental and juvenile infections by P. falciparum are associated with high

mortality, particularly in Sub Saharan Africa. Elsewhere the blockage of cerebral and splanchnic vasculature by parasitized erythrocytes gives rise to the manifestations of Malignant Tertian Malaria. The complex sequence of reactions that result in the clinical manifestations are well described.

At present malaria is endemic between the Tropics of Cancer and Capricorn. The density of infection is the result of the interplay of a number of factors. These include the prevalent parasite species, vector transmission efficiency, and availability of good primary health care and the economic development of the population. Probably the worst combination is seen in Africa where P. falciparum accounts for a majority of the infections. There, Anopheles gambiae is the major vector, primary health care is relatively poor, and the countryside has the poorest communities of the world.

This region accounts for nearly a million deaths a year predominantly in infants and pregnant women. Malaria is responsible for declining Gross Domestic Product and perpetuation of poverty.

The use of insecticides to interrupt the transmission cycle promised a lot. In fact, the World Health Organization launched the Malaria Eradication Programme the world over in the early 1950s. Spraying of diclophan (DDT) in the homes brought about a dramatic decline in the incidence rates. In fact, in some temperate areas the infection was truly eliminated.

However, rising drug and insecticide resistance, among other factors led to a resur gence of malaria. In India, the low level of infection in 1965 resurged to an annual incidence of 6.4 million in 1976.7 The worsening trend continues despite the use of newer drugs, better insecticides and a slow but steady improvement in health infrastructure, particularly in Africa.8 The remedies to reverse the present situation are: 1) better sanitation, engineering solutions to mosquito breeding 2) proper town and house planning 3) drug research along with the development of newer insecticides and 4) vaccine.

The first two options are highly capital intensive and it is unlikely that the states endemic for malaria would be in a position to afford them. It is nearly a decade since any new antimalarial drug was developed. The publication of the complete genome of P. falciparum9 has opened up the research field. The major stumbling block, however, is the lack of funds. The low paying capacity of malaria victims makes drug development unattractive for pharmaceutical firms. Efforts are being made to remedy the situation by setting up organizations like the Medicines for Malaria Venture, Roll Back Malaria consortium and with the help of philanthropic organizations lik e the Bill and Melinda Gates Foundation. However, the issues of delivery at an affordable price and ensuring easy availability still remain.

At best the drug initiatives would be medium term measures since the parasite would develop resistance sooner or later. Even simple methods aimed at decreasing mosquito bites like impregnated bed nets have not been fully effective due to leaching of the insecticide during wash and development of parathyroid resistance by the mosquito. Development of a malaria vaccine would, therefore, be a long term measure that would ultimately be cost effective too. Scientists have been working for decades to develop a preventive malaria vaccine. While they have successfully demonstrated that such a vaccine is possible, many challenges continue to impede progress on the road to an effective malaria vaccine. As a result, the Malaria Vaccine Advisory Committee to the World Health Organization (WHO), coordinated by the WHO Initiative for Vaccine Research (IVR), called for a collective effort to explore and address the challenges. This effort resulted in the Malaria Vaccine Technology Roadmap process.

The Malaria Vaccine Technology Roadmap process was jointly sponsored by the Bill & Melinda Gates Foundation, the PATH Malaria Vaccine Initiative (MVI), and the Wellcome Trust. A Roadmap Working Group, consisting of representatives of the sponsors and IVR, guided the process. Members of the malaria vaccine funders group served as active participants and advisors. Energetics Incorporated assisted with the coordination of the process.

The world urgently needs a malaria vaccine to relieve the human suffering associated with the parasitic disease that kills more than one million people—most of them African children—every year. Hundreds of millions more people suffer from the effects of malaria. While drugs, insecticide-treated bednets, and other interventions are being used to reduce malaria's impact, the disease remains a tenacious adversary. A safe, effective, and affordable malaria vaccine would create a powerful public health benefit by closing the gap left by other interventions.

Despite the disappointing results of malaria vaccine trials so farl2, vaccination still remains the most potentially powerful new weapon to be used against this major parasitic disease of mankind. The first generation of vaccination experiments, in the 1970s, has used either X-irradiated or killed extracellular stages of the parasite (sporozoites, merozoite or gametes): these experiments have confirmed the hypothesis that a malaria vaccine was feasible, but have also pointed out the difficulties ahead. In the second wave of experiments, which is still continuing, research has concentrated on purified parasite antigens and the study of their protective value: although a large number of molecules have been identified, with their genes cloned and sequenced, the protective value of these molecules is generally difficult to establish. Over recent years, new techniques have been described which are likely to provide new approaches to the very difficult problem ofproducing a vaccine against this parasite, and much

progress has been made in our basic understanding of what is needed to elicit an effective immune response. This paper discusses some of the current trends in the search for a rational malaria vaccine, taking into account the results obtained in the development of vaccines against other infectious agents and what can be learned from recent immune logical, parasitological, molecular biological and epidemiological findings.

THE MALARIA PROBLEM

Mankind has stepped into the 21st century and diseases such as malaria still represent a major threat to populations in many parts of the world. The exact extent of the malaria problem is not known, but several estimates provide a gloomy picture of the situation. It is estimated that between 400 and 900 million febrile episodes occur every year only in African children, with a minimum of 750,000
deaths (probably up to 3 million). In addition, nonsevere cases yet cause considerable morbidity in acute or chronic disease, with serious socio-economical consequences. Sub-Saharan Africa is the most affected region in the world, but malaria is also a serious problem in several other places, such as South-East Asia, Oceania, Middle East and Latin America. Historically, in the late 1940s, there was great
optimism in the fight against malaria, mainly owing to the introduction of dichlorodiphenyltrichloroethane (DDT) for vector control and of chloroquine as a very efficient antimalarial drug. These and other available control tools prompted the World Health Organization (WHO) to launch a campaign for complete malaria eradication. The campaign was very successful in places such as Mediterranean countries and even many regions in the tropics. In Brazil, for instance, the number of cases per year dropped from nearly 6 million in the 1940s to around 37,000 in 1962 and became restricted to the Amazon region. But since then, malaria has seen resurgence and/or is spreading in many areas; in the Amazon region of Brazil the number of cases per year increased from nearly 37,000 in 1962 to around 600,000 in the late 1990s. Indeed, already in the 1960s it became clear that eradication was not feasible, and the WHO strategy was switched, aiming to control rather than to eradicate.

Environmental conditions, population habits and living conditions, migratory movements of people to endemic areas, regional development projects, resistance of parasites to drugs and of mosquitoes to DDT, among other factors, greatly favored the maintenance of malaria in the endemic regions. All the difficulties concerning malaria control justify the search and adoption of new tools and measures to minimize the impact of malaria on the affected populations. One strategy is the development of a malaria vaccine.

MALARIA VACCINES

The concept for the development of a malaria vaccine arose from experimental immunization studies with irradiated sporozoites. Human volunteers immunized with irradiated attenuated *P. falciparum* sporozoites developed a protective immune response against subsequent malaria infections (Clyde 1975; Rieckmann et al. 1974) . This observation supported the idea that vaccination against malaria infection should be possible in principle. Disadvantages associated with attenuated vaccines, like difficulties in producing large amounts of irradiated sporozoites, have led to the search for protective antigens of different life cycle stages of the malaria parasite. Despite intensive research during the last twenty years, there is still no effective vaccine available (Facer and Tanner 1997) . The complexity of the parasite life cycle, imperfect tools to assess the efficacy of immune responses and limited knowledge of the factors that determine the outcome of an infection are still the main obstacles in developing such a vaccine.

The hope for developing a malaria vaccine is based on field and experimental observations, showing that immune protection against malaria can be achieved: (a) individuals living in areas of intense transmission naturally acquire clinical immunity first against severe disease and then against clinical manifestations; partial ant parasite immunity is also developed, and
adults may have parasites in the blood but usually at very low densities without causing symptoms; (b) immunity can be passively transferred from immune to no immune individuals through the administration of immunoglobulins, showing that protection against blood-stage infection is largely mediated by antibodies; (c) immunization of humans, primates and mice with radiation-attenuated sporozoites or with recombinant antigens can induce partial or sterile (100% effective) ant parasite immunity.

Nevertheless, the belief that a malaria vaccine can be developed has been often questioned by the fact that natural immunity takes many years to be acquired, and this happens only under continuous and heavy contact with the parasite, as in holo- and hyper endemic areas; in addition, immunity is partial (no sterile) and short lived, if continuous boosting is not present. For most of the pathogens against which vaccines have been developed, such as measles and mumps, no immune individuals surviving an infection develop strong long-lived sterile immunity. In those cases, the vaccine just does what the nature is able to do. In the case of malaria, a vaccine has to be more efficient than nature. Could that be possible? Some observations indicate that it could be.

First, the fact that only adults are immune to malaria in holo and hyper endemic areas seems to be related

to the host age or, in a better context, to its developmental state, rather than a consequence of long-term exposure. Although children are not able to develop efficient protective immunity against the parasite, they can quickly develop immunity against severe disease. In addition, the increased resistance to malaria has been shown to be directly related to pubertal hormone levels. These observations indicate that immunity is achieved in a ore efficient manner than previously thought. It is largely dependent on how the response is modulated, and it may be easier to develop an efficient vaccine for adults than for children. However, as children must be the main focus for vaccination in many communities, it is of key importance that the mechanisms behind their low ability to develop immunity be clarified, so that a vaccine may be effective irrespective of the vaccine's age. Second, strong protective immunity is induced after a single inoculation of radiation-attenuated sporozoites but not after repeated natural challenge with normal sporozoites.

One of the possible reasons for this phenomenon is that irradiated sporozoites invade hepatocytes and express liver-stage antigens, and, because they do not progress beyond the liver stage, they become a source of persistent intrahepatic antigens. It is reasonable to think that a synthetic vaccine having the same property might also induce protective immunity. These observations raise optimism in the expectation that an artificial intervention - a vaccine - may be more efficient than nature in inducing protective immunity against malaria.

PLASMODIUM MEROZOITE PROTEINS: VACCINE CANDIDATES

Malaria vaccines can be classified as pre-erythrocytic, erythrocytic stage, or transmission blocking vaccines. Pre-erythrocytic stage vaccines target the liver stage of *Plasmodium,* erythrocytic stage vaccines target the blood stage, and transmission blocking vaccines target the sexual stages of the parasite and prevent transmission of *Plasmodium* from the infected host to the mosquito.

Several decades ago the promise of a malaria vaccine was spurred on by evidence that protective immunity could be achieved by immunization with live-attenuated sporozoites in both mice and humans. At the time, the idea of an irradiated sporozoites vaccine was thought to be impractical and so the alternative was to identify sporozoites antigens that could be potential vaccine targets. This lead to the identification of the circumsporozoite protein, CSP and thrombrospondin-related adhesion protein, TRAP. Since that time most vaccine development projects against the liver stage of malaria have primarily been focused on CSP and TRAP, essentially ignoring the many thousand other antigens expressed during the liver stage.

Currently, the RTS,S vaccine is considered to be the most promising malaria vaccine. This vaccine is composed of a recombinant protein against the C-terminus of PfCSP fused to a hepatitis B virus surface antigen. In a study conducted in children (age 1-4 years) from Mozambique, a clinical phase IIb trial demonstrated that individuals immunized with the RTS,S vaccine displayed a reduction of clinical malaria by 35% and the incidence of severe malaria was decreased by 50%; the partial protection induced against the clinical symptoms of malaria lasted for approximately eighteen months.

Another clinical phase IIb trial was conducted using the same vaccine in Tanzania. The vaccine was administered to infants at 8, 12, and 16 weeks of age and demonstrated reduction of clinical disease by 65% in these infants. There is currently a debate within the malaria research community concerning RTS,S as a promising malaria vaccine. Some researchers have questioned whether the location of the trials has affected the rates of malaria infections, as the studies have been conducted in areas of low disease burden. Performing the same trials in areas of high disease burden may affect the incidence of clinical malaria observed after vaccination. More research needs to be performed to address this and other questions concerning this malaria vaccine. However, these recent vaccine trials give renewed hope that an effective malaria vaccine can and will be discovered.

The specific *Plasmodium* species is listed along with the target antigen and the current clinical stage of the specific vaccine. Data compiled from WHO vaccine development tables. others are soluble proteins (PfMSP-3, 6, 7, and 9). However, most members in the family contain at their carboxy-terminus at least one epidermal growth factor (EGF)-like domain (the exception being PfMSP-2).

It has been postulated that MSPs function in the initial contact of the merozoite with the host erythrocyte because of the even distribution of MSP-1 over the surface of the merozoite. Although there has been extensive research into this family of proteins, the specific function(s) of the MSPs are still unknown. However, it has been reported that MSP-1 binds the protein band 3 on the surface of erythrocytes. Despite the lack of comprehensive understanding concerning the role of MSPs in invasion, several protein family members (PfMSP-1, MSP-2, MSP-3, and MSP-4) have been developed as potential malaria blood-stage vaccine candidates.

CONCLUSION

Malarial parasites have coevolved together with the human host for thousands of years, which have led them to constitute an important driving evolutionary force behind common erythrocyte variants, such as thalassemia, sickle-cell disease, HbC, HbE, gluose-6-phosphate deficiency and other erythrocyte anomalies. Host–parasite interactions have led to a

host's relative resistance to the parasite and parasite strain-specific susceptibility or virulence.

As has been shown in this review, the molecular bases of hemoglobinopathies vary greatly. However, some common characteristics can be found in support of the malaria hypothesis, such as the coincidence between the global distribution of hemoglobinopathies with that of malaria as well as the increase in various regionally specific mutations during the last 5000 years despite their different molecular origins, which points toward a common cause.

The different hemoglobinopathies-related alleles could be related to the beginnings of human colonization in different areas of the world, since activities such as agriculture, trade and fishing would have led to the parasite becoming dispersed to different geographical areas and thus to the fixation of new alleles related to such hemoglobinopathies in the population.

As different mechanisms conferring protection against malaria are widely found among different populations, it is plausible to suppose that populations have evolved and developed different genetic variants which are related to resistance to the disease. This, in turn, could imply that the maintenance of these alleles in the population has been due to the effects of positive selection arising from relatively recent human history (i.e. when human populations were migrating from Africa).

All vaccines which have failed in different phases or are in the pipeline toward success have been covered in this review. Many clinical trials and unresolved issues related to stability, immunogenicity and targeting to the site of the parasite life cycle give hope for further development of anti malarial vaccines. The development of a vaccine of therapeutic and protective benefit against the malaria parasite requires a novel approach and to date there are no vaccines available that can effectively target a parasitic infection. Traditional approaches to vaccine development against malaria have met with limited success. The search for an efficacious vaccine against malaria is ongoing and it is now widely believed that to confer protection a vaccine must induce very strong cellular and humoral immunity concurrently, but the vaccine, which has been promised to be 'just round the corner' for many years, remains elusive.

Development of an effective and deployable malaria vaccine seems technically feasible in the view of most malaria researchers. New vaccine delivery methods and adjuvants could continue to increase the antibody and cellular immunogenicity of subunit vaccination. The development of a vaccine to protect human subjects against malaria is a feasible goal and the emergence of DNA vaccine technology offers a simple approach to formulating such a multivalent vaccine.

Highly purified subunit vaccines require potent adjuvants in order to elicit optimal immune responses and therefore an efficient adjuvant is also needed. A vaccine that would reduce both mortality and morbidity secondary to *P. falciparum* infection would be a valuable resource in the fight against this disease.

A safe, effective and affordable malaria vaccine is expected to provide a long-lasting approach to prevent infection, reduce disease severity, prevent death and interrupt transmission. A vaccine that completely prevented infection, even for a relatively short time, would be very satisfactory for travelers.

There is no doubt that vaccines against malaria will be made: the need for such tools in the armamentarium for malaria control has been established, the expertise and technology required is available and, most importantly, there is a considerable 'international will' for their production. By all accounts, however, this development is likely to take many years.

While safety criteria alone need to be considered before releasing a 'traveler's vaccine', far more stringent criteria need to be imposed on the release of a vaccine to be used in populations living in endemic areas, especially if the objective is to interrupt malaria transmission, because a failure in a vaccination programme at a population level would have dramatic consequences for that population.

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