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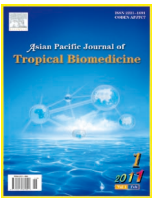

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Malaria vaccines: looking back and lessons learnt

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ABSTRACT

The current status of malaria vaccine approaches has the background of a long and arduous path of malaria disease control and vaccine development. Here, we critically review with regard to unilateral interventional approaches and highlight the impact of socioeconomic elements of malaria endemicity. The necessity of re-energizing basic research of malaria life-cycle and *Plasmodium* developmental biology to provide the basis for promising and cost-effective vaccine approaches and to reach eradication goals is more urgent than previously believed. We closely analyse the flaws of various vaccine approaches, outline future directions and challenges that still face us and conclude that the focus of the field must be shifted to the basic research efforts including findings on the skin stage of infection. We also reflect on economic factors of vaccine development and the impact of public perception when it comes to vaccine uptake.

1. Introduction

According to the WHO a child dies of malaria every 30 seconds and in 2008 there were approximately 250 million cases of malaria, causing nearly one million deaths. The persistence of malaria in tropical countries, especially Sub-Saharan Africa, is bound to the climatic surrounding that provides an optimal environment for the mosquito vector[1]. Still, closely interlinked to the impact of disease burden remains the poor economic standing of endemic countries which impedes adequate interventional control[2]. Evaluating the current status of malaria disease control we must also take into account lessons learned in the past. Numerous historical examples display the importance of social networks in malaria control. In Italy in the late 19th century drug availability was reinforced through imposing quinine taxes on landlords and schools were founded providing general education as well as information about malaria. A remarkable decrease of malaria associated mortality could be obtained by this novel approach.

Further historical investigations have elucidated how the political standing of countries paid contribution to and possibly interfered with disease control as well. At the period of the Cold War dichlorodiphenyltrichloroethane (DDT) was extensively applied on the basis of American funding and constituted a means of winning over local populations in the war against communism. Consistently a lack of dedication to the political lead of the US could induce the opposite. Evidence of this assessment is provided by the cut of foreign aid to India in the 1970s. The Indian government had agreed on a friendship treaty with the Soviet Union in 1972 as these were times of war with Pakistan[3].

Among the lessons learned in the past attempts of disease control is also how a unilateral approach, as performed with DDT for instance, will not be successful in controlling disease on its own. Inconsequent insecticide application resulting in insecticide resistance is one of the major impediments to this form of disease control. The need for a programme that ensures an overall socioeconomic and health improvement of malaria-struck populations has become increasingly clear. Still, however, malaria disease control is characterized by a large focus on a biomedical micro-cosmos with anti-malaria drugs and insecticide interventions, which can undoubtedly be successful in limiting disease impact but will not abolish the circumstances of poverty that provide the necessary basis for disease endemicity.

It appears even more unreasonable to believe that a vaccine is at any price the best solution for malaria disease control. Huge investments in the most prominent vaccine candidate RTS, S were performed, despite the weak performance of the candidate in several trial sites. Therefore the cost-effectiveness of this vaccine candidate has been the subject of massive criticism. The success of vector control programmes, annually supported by approximately 1 billion dollars, is said to have efficiently and markedly improved the situation in numerous endemic areas. The programmes including insecticide-treated bed nets, artemisinin combination therapies and insecticides are

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reported to have decreased the severity of malaria by 90% (measured were cases of death and admissions to hospitals). Critical reports estimate RTS, S would only improve these numbers by a meagre rate of 3%. Therefore conclusions were drawn as to which an only partially effective vaccination is, in comparison with other malaria control measures, less efficient[4].

Critically speaking, one might suggest that it is primarily the long duration of 30 years of research and investment that account for the continuating of research on this vaccine candidate. After all we must regard economic factors as vitally contribution to the decision-making of pharmaceutical companies and quite frankly. Malaria vaccine development appears an altogether unattractive branch of investment. The limited profit that can be expected from a vaccine candidate is well illustrated by the following numbers. The highest-revenue-generating vaccine in the US, a conjugate pneumococcal vaccine for children yields an annual gross sale of approximately 1 billion dollars. In comparison drugs for cardiac diseases or obesity reach revenues of annually 7 billion dollars or more[5].

Furthermore several malaria researchers such as Ross and Grassi have rendered the malaria research platform an attractive one for ambitious scientists. This represents an advantage as competition accelerates science but can also be considered to have negative side effects. The hunt for personal prestige hampers collaboration between scientists and bears the danger of losing track of the broader context of disease control.

Additionally some scientists appear to have been pressured by the investing pharmaceutical companies and therefore tend to offer overly optimistic hope of reaching the aim of successful vaccination soon. An illustrative example might be that of scientist William Trager, who already in the 1970s stated in an unofficial context:"*We must promise that a vaccine is on the horizon or else research funding will quickly dry up*"[6]. Evidence suggests that this form of pressuring might in fact have slowed the research pathway and triggered the generation of various vaccine candidates that lacked basic research background.

2. How far advanced is malaria vaccine development really?

Impediments to successful vaccination are represented by the fact that malaria is a parasitic disease. We must bear in mind that no vaccine against any parasitic disease exists so far. The *Plasmodium*'s genomic complexity with approximately 5 400 protein-coding genes, as well as our lack of natural immunity to malaria, renders this an issue very different from infectious diseases that were successfully combated by vaccination[7].

However, various candidates against the different life stages of the *Plasmodium* have been developed. Attempts to target the erythrocyte stages of the life cycle appear unwise as they are subjects of antigenic variation, meaning they can easily evade the human immune system.

Nevertheless a recent systematic review with meta-analysis of 33 different studies has reported that antibodies against MSP proteins (MSP-119 and MSP-3) exhibit the strongest association with lower incidence of malaria and protection[8].

Clearly this attempt of imitating clinical immunity to malaria, that is also mostly associated with high titers to several merozoite-stage antigens, can milden clinical episodes of malaria and decrease parasite density in the blood, but it is no real disruption of the life cycle[9]. Therefore, the problem of the human parasite reservoir remains unsolved. Equally important a population group that is highly susceptible to severe malaria episodes, pregnant women, are not regarded by these approaches. Pregnant women experience severe episodes of disease even if they had lived in a malaria-endemic area and acquired clinical immunity before[10]. We can expect equal results with a vaccine approach that aims to imitate natural forms of immunity without regarding the specific antibodies that were observed to protect unborn babies in multigravidas[11].

Furthermore, the development of dormant forms of the *Plasmodium* within the liver, observed with *Plasmodium vivax (P. vivax)* and *Plasmodium ovale* (*P. ovale*), is not taken into account by post-liver vaccine approaches.

Concerning the approach of transmission-blocking there were various ideas ranging from the blocking of the gamete's interactions to the blocking of the sporozoites' invasion of the salivary gland[12,13] Generally these different kinds of transmission-blocking vaccine are prophesied to only extend disease control if applied in combination with other interventional methods such as exposure prophylaxis and vector control[14]. Alternatively their application in combination with poorly efficacious pre-erythrocyte vaccines or blood-stage vaccines might be proposed[15].

Additionally we must bear in mind how malaria has displayed to be a highly dynamic disease in the past and we might expect its adaptation to a new vector or an alteration of certain proteins that are required for the interaction with the vector. And what is more, a transmission-blocking vaccine does not impede the infection of the individual, but can only reduce transmission on the long run and after mass immunization. This renders the approach unattractive for western travellers, who majorly drive vaccine development efforts.

A further very appealing approach was concerned with a vaccine targeting especially pregnant women. Due to the high susceptibility of pregnant woman for malaria this appears to be a very sensible approach, which might markedly improve disease rates of mothers and the general health of their newborn children. Syncytiotrophoblasts inside the placenta can also be defined as a sort of endothelium that expresses chondroitin sulphate A (CSA) supporting *Plasmodium* adherence. The process of adherence is accompanied by an eventual sequestration of the placenta [10]. The sequestered placenta appears to be dysfunctional which increases the unborn child's risk of low birth weight and mortality[16].

Studies suggest that multigravidas are less likely to have placental infections and experience lower levels of parasitaemia. First hypotheses stated how they, in comparison to primigravidas, possibly benefit from the development of anti-adhesion antibodies that offer a certain degree of protection[10]. Antibodies against parasite lines expressing *var2csa* could be identified as dominant for the protective effect. In the presence of such antibodies the parasite-encoded variant surface antigens bind to chondroitin sulphate A and the accumulation of parasite-infected erythrocytes is prevented. Based on these findings the development of an anti-adhesion vaccine was suggested to significantly lower disease burden for mothers and children[11]. Again a clear impediment that has so far limited research on this presumably strongly disease- limiting vaccine is its unsuitability for the western market.

Despite these various vaccine approaches there are several elements of the *Plasmodium's* life cycle that still require extensive investigations as they might provide for

unrecognized intervention targets. Clearly the investment in certain research areas strongly influences the acceleration or deceleration of research. The EU support provides an illustrative example of this. In 2009 malaria basic research was supported with 15.9 Euros. Research was coordinated by the French Pasteur Institute and concentrated on parasite's genetics, cell biology and metabolism, pathogenesis, immunology and the mosquito vector. Malaria vaccine development was supported with only a little less, 13.5 Euros[17]. In 2006 resources were invested in 16 candidates that were in clinical development[18]. The value of these inventions is controversial especially with regard to the poor performance of the most advanced vaccine candidate RTS, S. It offers a protection rate of only 30%[19]. Critics disapprove of the cost-demand intensive testing of ineffective vaccine candidates and demands the generation of superior vaccine candidates based on basic research[20].

Proponents of the various vaccine approaches argue how the very successful smallpox vaccine was also developed with little knowledge concerning host immunology, however, the genomic complexity of the *Plasmodium* clearly impedes a comparison. Several ineffective vaccine approaches of the past provide evidence of the fact that basic research on the *Plasmodium's* life cycle is obligatory.

Latest findings with regard to the skin stage of infection appear appealing. A study conducted with *Plasmodium yoelii (P. yoelii)* sporozoites that were injected intradermally into mice gave evidence that suggested most of the sporozoites leave the site of deposit after more than 1 hour. Presumably sporozoites that have remained inside the skin for more than 3 hours are eradicated by a mechanism that waits to be explored[21]. The sporozoites continue their journey through the random invasion of either blood vessels or lymphatic vessels to the skin-draining lymph node where CD8+ T-cells experience activation through dendritic cells. This was reflected in mice experiments with *Plasmodium berghei (P. berghei)* expressing a green fluorescent protein[22]. A trial including the removal of the skin-draining lymph node lead to a decreased immune response to attenuated *P. yoelii* sporozoites and thus shed light on the fact that these CD8+ cells are primed already in the lymph node and not at later stages[23]. The DCs are capable of presenting a fragment of the sporozoites to the CD8+ cells via MHC I in contrast to the common antigen presentation via MHC II. The application of MHC I, described with the term of cross-presentation, is of huge relevance as it enables the DCs to activate CD8+ cells. The exact mechanism and location of the DCs' and sporozoites' interaction still remains to be elucidated.

Due to the parasite's tremendous capacity of immune evasion, including its ability to traverse epidermal cells, to induce antigenic variation in erythrocytes or to impede apoptosis in hepatocytes, the first confrontation of the parasite and our immune system represents a highly intriguing research field. Why is our immune system unable to combat infection at this point?

Rather generally the target of the skin, highly equipped with immune cells, appears a very suitable target for vaccine application. After all the approach to intramuscular vaccination was made in times in which our knowledge concerning the immune system was very incomplete. Nowadays it seems unwise to ignore the density of dendritic cells in the skin that represent the initiators of a successful immune response.

Apart from its suitability with regard to the *Plasmodium's* life cycle this approach might inter alia also solve the problem of immunosenesence. A reduced number of dendritic cells and a reduced natural killer cell cytotoxicity could be documented for the elderly resulting in an increased susceptibility to infection and a reduced effectivity of vaccination[24]. Especially with regard to the western world this is a subject of substantial importance. Our societies will be markedly characterized by an aging of the population. Therefore it is crucial to adapt our vaccination attempts to these demographic developments. And clearly the necessity of research on senescence was already recognized. *E.g.* the Max-Planck institute in Germany only shortly founded a centre concerned with the biology of aging in Cologne medical school and provided it with an annual budget of 15 million Euros.

Apart from a novel approach of vaccine application that will presumably enhance immune responses the vaccine research platform offers several attempts of so-called DNA vaccination, especially for diseases such as malaria against which no vaccine could be evolved so far. Veterinary medicine creates a role model pathway that will expectedly be followed by human medicine. The DNA vaccines and therapies that were both released in 2007 must be especially underscored. The Canine Melanoma Vaccine and growth hormone releasing hormone (GHRH) which is applied in swine. The two vaccines target very different problems. The Melanoma Vaccine is a form of tumour therapy; by contrast GHRH is a preventive vaccine, which has decreased prenatal mortality and morbidity[25].

The DNA vaccination is based on the following construction. A plasmid, which is a circular piece of bacterial DNA, genetically modified to produce proteins of a certain microbe or virus, is inserted into a human cell. The host cell expresses proteins on the basis of this new DNA and the immune system recognizes these as foreign [26]. Most DNA vaccines that are subjects to current research programmes do not integrate into the host's cellular DNA, they only enter the nucleus[25].

There is wide agreement that one of the major immunological advantages of DNA vaccination is the activation of cytotoxic T lymphocyte (CTL)-cells. CTL-cells require antigen-presentating cells through MHC I which is either achieved through direct transfection of the adenomatous polyposis coli (APCs) by the plasmid vaccine or through cross-presentation[25].

Arguments in favour of DNA vaccination include the attractive possibility of easily addressing not only one part of the *Plasmodium's* life cycle but several as genes coding for numerous parts of the life cycle can potentially be integrated into a vector.

A further pivotal argument in favour of DNA vaccination is of economic nature. Its inexpensive and pure manufacturability does not encounter the various production hurdles of other vaccine approaches[25].

Still there is a widely accepted consensus among many scientists that not only CTL cells or antibodies on their own lead to a successful immune protection. Therefore research has been forwarded to generate vaccine candidates that provoke CD8+ responses as well as antibody responses, thus activating both arms of the immune system.

In a US study from 2004 human volunteers were at first immunized with a *Plasmodium falciparum* (*P. falciparum*) circumsporozoite protein (CSP) DNA vaccine which resulted in an activation of CD8+ cells and higher levels of IFNY. The subunit vaccine RTS, S (see chapter on preliver stage vaccines) applied on its own gives rise especially to CSP-antibodies and activates CD4+ cells. In this trial the volunteers that had previously received the DNA vaccination also received RTS, S twice, 8 weeks apart, resulting in an

antibody response and an activation of CTL-cells. Research confirmed that DNA vaccination itself did not give rise to an antibody response but primed for boosting of the antibody response[27]. The molecular basis of this priming process is the activation of specific memory T-cells that persist even when the antigen is eliminated and rapidly expand when exposed to the same antigen in the boosting vaccination[28]. In order to describe these phenomena adequately the term of heterologous prime-boost has evolved[27].

3. What can we do?

Surely, however, the eradication of infectious diseases is a goal calling for more than the development of a vaccine. In western countries we clearly face other pivotal hurdles. Both anti-vaccine movements as well as a lowered consciousness of disease risks have lead to a suboptimum vaccination coverage concerning measles. A 2-year study with 32 European countries shed light on the fight that the incidence of measles has had an upsurge in Europe. Affected were priorily unvaccinated or incompletely vaccinated children. Among the 5 countries with the highest incidence were Romania, UK, Switzerland, Italy and Germany[29].

Taken together it stands out that a major task of the future will be an improvement of the public understanding of vaccination and the massive impact of infectious diseases[30]. Especially in schools and kinder gardens improved information politics have proven to be highly effective and require further extension.

However, the presented lack of awareness of life-threatening infectious diseases is not only significant with regard to already vaccine-preventable diseases. It is also significant with regard to malaria. Western societies have the power to primarily drive malaria vaccine development through financial investments. Still, apart from the huge investments of the Bill and Melinda Gates Foundation, Glaxo Kline Smith is the only major pharmaceutical company investing in a malaria vaccine and the underlying cause is in fact very obvious. Pharmaceutical companies favour the development of vaccines or medications that can be profitably sold on a western market. The western world does tend to invest in vaccine development for diseases such as cervical cancer that in comparison to malaria cannot be regarded as a serious threat to humanity. Undoubtedly vaccinations against several chronic diseases and neoplasm's will evolve and presumably lead to the further heightening of our life expectancy.

However, the evolution of Public Private Partnerships, such as the Malaria Vaccine Initiative of the Bill and Melinda Gates Foundation, which is based on a mobilization and collaboration of public and private investors, does offer hope for enhanced malaria research in the future.

Due to the many concerns that were uttered with regard to the unfavourable market situation for vaccine developers a study was conducted for the private-sector vaccine research and development for the period of 1995-2008 displaying results that clearly contradict the generally pessimistic view. In the observed period a doubling of the global number of vaccine originators as well as a doubling of the number of prophylactic vaccine products in development could be recorded. Even so, the authors' state that the future of vaccine development remains uncertain as a growth in phase III trials of vaccine development could not be reported yet[31]. Nonetheless, the study might predict a flourishing vaccine market in the future that will hopefully not only comprise vaccines for the western market.

Still, the impact of malaria does not appear to greatly affect western societies. As numbers displaying the prevention measures taken by western travellers revealed, many individuals are simply unaware of their risk of infection when travelling to the third world. Equally impressive is the lack of knowledge concerning our professionals. An impressively high percentage of imported malaria cases remain unrecognized, even though correct diagnostic measure would quickly lead to the correct diagnoses. Malaria is simply underestimated. Therefore we have a considerable rate of approximately 3, 9 per cent lethality in Germany.

So apart from a moral obligation that has so far not been too influential to any kind of economic decision-making, why should western societies start to take more responsibility?

Clearly there are numerous reasons, but the most important are probably that we should care because the ongoing climate change increases the risk of malaria-spreading in western countries and even more importantly it is undoubted that the eradication of malaria will improve the economic situation of third-world countries and turn them into strong economic partners in the future.

4. What happens with the parasite while we are researching?

The red cell defences are a reminder of the remarkably long history of malaria. As formulated by microbiologist Rene Dubos: "Given enough time a state of peaceful coexistence eventually becomes established between any host and parasite". So quite independent from the question whether human kind accomplishes the goal of finding a successful malaria vaccine or not, we might expect that at some point natural defences that have already evolved against *P. vivax* for instance (the Duffy antigen) will also evolve with regard to the currently most feared *Plasmodium* species: *P. falciparum*.

However, only recently it became clear that not only our human organism might adapt to the parasite but that also new parasites are adapting and becoming capable of infecting humans.

In 2004 intriguing findings were made on Malaysian Borneo[32] based on microscopy patients were diagnosed with what seemed like rather atypical forms of *Plasmodium malariae* (*P. malariae*)[33]. The description of atypical was accounted for by the strikingly high incidence of *P. malariae* and reports on an unusually high parasitaemia[32].

These observations triggered a further specification of investigation with PCR methods that were to determine whether this was an atypical form of P. malariae or a whole new Plasmodium species[33]. Previously unidentified, 106 cases that had been misdiagnosed as *P. malariae* were actually cases of *Plasmodium* knowlesi (*P. knowlesi*)[32].

Reports on human infections with *P. knowlesi* from Thailand, Myanmar, the Philippines and Singapore have lead scientists to the suggestion *P. knowlesi* might be the 5th human malaria parasite[32,34]. The counterview is that infection with *P. knowlesi* remains a zoonotic infection as its transmission from human to human by mosquitoes could not be proven. Additionally, its survival in monkeys was proposed to be totally independent from human existence. Apart from *P. knowlesi* 2 other forms of simian malaria must be of interest for our future investigations: *Plasmodium cynomolgi* and *Plasmodium inui*. Importantly they are transmitted by *Anopheles* groups whose habitat is

not restricted to forests, such as *Anopheles stephensi* and *Anopheles sundaicus*. Adding to their possible threat to humankind they are transmitted between humans by these mosquitoes[35].

5. Future perspectives

We might say that malaria remains a serious and dynamic threat and new research ideas and tasks should be generated with regard to vaccine technology. Moreover it is an urgent requirement for the future to motivate young scientists to engage themselves more strongly in the field of malaria vaccine development. Means of strengthening interest in this research area could for instance be a stronger representation of tropical medicine and disease in the medical curriculum as well as a more dominant representation and advertisement of the vaccine research platform in medical schools.

Conflict of interest statement

We declare that we have no conflict of interest.

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