Available online at www.sciencedirect.com
ScienceDirect
Vaccine 25 (2007) 1567–1580
Vaccine
www.elsevier.com/locate/vaccine

Review

A review of human vaccine research and development: Malaria☆

Marc P. Girard[a,*], Zarifah H. Reed[b,1], Martin Friede[b,2], Marie Paule Kieny[b,3]

[a] *University Paris 7, 39 rue Seignemartin, FR-69008 Lyon, France*
[b] *Initiative for Vaccine Research, World Health Organization, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland*

Received 23 September 2006; accepted 25 September 2006
Available online 5 October 2006

Abstract

The last several years have seen significant progress in the development of vaccines against malaria. Most recently, proof-of-concept of vaccine-induced protection from malaria infection and disease was demonstrated in African children. Pursued by various groups and on many fronts, several other candidate vaccines are in early clinical trials. Yet, despite the optimism and promise, an effective malaria vaccine is not yet available, in part because of the lack of understanding of the types of immune responses needed for protection, added to the difficulty of identifying, selecting and producing the appropriate protective antigens from a parasite with a genome of well over five thousand genes and to the frequent need to enhance the immunogenicity of purified antigens through the use of novel adjuvants or delivery systems. Insufficient clinical trial capacity and normative research functions such as local ethical committee reviews also contribute to slow down the development process. This article attempts to summarize the state of the art of malaria vaccine development.

Keywords: Plasmodium; Vaccine; Clinical trials; Africa; Sporozoites; Merozoites

Contents

☆ The authors alone are responsible for the views expressed in this publication, which does not necessarily reflect the views of the World Health Organization.

* Corresponding author. Tel.: +33 478 748 531.
E-mail addresses: marc.girard36@wanadoo.fr (M.P. Girard), reedz@who.int (Z.H. Reed), friedem@who.int (M. Friede), kienym@who.int (M.P. Kieny).
[1] Tel.: +41 22 791 47 60.
[2] Tel.: +41 22 791 43 98.
[3] Tel.: +41 22 791 35 91.

0264-410X/$ – see front matter © 2006 World Health Organization. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2006.09.074

1. Introduction

Malaria is by far the world's most important tropical parasitic disease, killing more children aged less than 5 years than any other disease, especially in Africa [1]. Malaria is caused by the protozoan parasite *Plasmodium*, which is transmitted by the bite of the female mosquito of any one of the 50 species of *Anopheles* mosquitoes, of which the best known is *A. gambiae* [2,3]. Four species of *Plasmodium* can cause human malaria: *P. falciparum*, responsible for the greatest number of deaths, *P. vivax*, which has the widest geographical distribution, *P. ovale* and *P. malariae* [4]. The life-cycle and parasite–host interaction of each *Plasmodium* species determines the severity and pathogenesis of clinical disease [5]. As recently as the early 1900s, human malaria was endemic across every continent except Antarctica. By the 1950s, control programs based on the use of insecticides led to its elimination from North America, Europe and Australia, but the disease still remains endemic in Africa, South-East Asia and the Western Pacific region, where more than 40% of the world's population lives.

The battle to control malaria has been fought on several grounds, including efforts to implement improved diagnosis and prophylactic chemotherapy as well as integrated vector control through the use of insecticide-treated bednets and residual house spraying [6]. However, prevalence as well as resurgence of malaria continues to be evident worldwide, much of it due to drug-resistant parasites and insecticide-resistant vectors. New malaria drugs such as chemical derivatives of artemisin are too expensive to be affordable by poor countries and still in limited supply [7]. Therefore, the development of a safe, effective and affordable malaria vaccine is a critical global public health priority [8].

Eliciting protective immunity to malaria appears to be achievable, as judged by the fact that natural immunity to the parasite progressively develops with frequent exposure [9,10] and that this immunity can be transmitted by passive transfer of antibodies [11]. As early as 1967, experimental immunization with irradiated sporozoites was shown to generate protective immunity [12]. Proof-of-concept demonstration that protection can indeed be induced through vaccination with a subunit vaccine was recently obtained in children [13,14]. Progress in developing a malaria vaccine has, however, remained slow [15]. This is in part due to the fact that the *Plasmodium* parasite has more than 5200 genes that could code for a protective antigen [16,17], making identification of candidate vaccine antigens a real quagmire, which is rendered even more complex by the fact that these antigens are differentially expressed during the life-cycle of the parasite and that many of the antigens display a high degree of variability. Moreover, the same antigens can be developed into different types of vaccines in a wide variety of ways. Thus, more than 50% of the approximately 75 candidate vaccines in active development today, are based on just three antigens that were cloned 20 years ago [18]: the circumsporozoite protein (CSP), the merozoite surface protein (MSP) and the apical membrane antigen 1 (AMA1) [19–21].

There are reasons to believe that an efficacious malaria vaccine may have to include multiple antigens and target multiple stages of the parasite life cycle. Combination approaches however meet with many challenges, including possible competition between antigens and increased cost and complexity. In addition, the development of a malaria vaccine is met with technical, logistical and financial obstacles, including the scientific, medical and ethical considerations to be taken into account when planning and conducting clinical trials and the fact that the market for a malaria vaccine is viewed as commercially nonattractive. However, in view of the emergence of drug-resistant strains of malarial parasites, the need for the development of safe and effective vaccines for the control of malaria has never been greater, particularly vaccines against the parasite blood stage that is the cause of the clinical symptoms and mortality [22,23].

This article will attempt to summarize the state of the art of *P. falciparum* malaria vaccine development.

2. Disease burden

It is estimated that the global number of clinical cases of malaria is about 350–500 million per year, with an estimated annual death toll of over 1.1 million deaths. Approximately 800,000 deaths occur among children under 5 years of age in Africa, especially in remote rural areas with poor access to health services. Most affected countries are Uganda, Tanzania, Malawi and Mozambique as well as Namibia, where more than 200 cases are reported per 1000 inhabitants per year [1]. In these countries, malaria is by far the leading cause of absenteism and death, even with the advance of AIDS. By contrast, the small number of cases still found in Europe (about 50,000 cases in 1999 [24]) and even smaller number in the USA (∼1500 cases [25]) are mostly imported cases in travelers or military personnels.

Transmission of malaria is affected by climate and geography, and often coincides with the rainy season. Global warming and other climatic events such as "El Niño" also play their role in increasing the risk of disease, presumably because the associated weather disturbances influence vector

breeding sites. Quantitative leaps in malaria incidence coincident with ENSO (El Niño/Southern Oscillation) events have been recorded in South America, Africa, and in Pakistan and Sri Lanka in Asia [26]. Thus, the disease has now spread to highland areas of Africa. A change in risk of malaria also can be the unintended result of economic activity or agricultural policy that changes the use of land, such as the creation of dams, new irrigation schemes, commercial tree cropping and deforestation [27,28]. Urban malaria is increasing due to unplanned development around large cities, particularly in Africa and South Asia. Injection or transfusion of infected blood or use of contaminated needles and syringes (e.g. injecting drug users) may also transmit malaria.

The incubation period from infection to symptoms ranges from 7 days to 18 days but may be as long as 40 days with *P. malariae*. Symptoms associated with malaria include high fever, malaise, chills, profuse sweating, headache, cough, arthralgia and myalgia, often accompanied by nausea and vomiting and at times diarrhoea. These attacks may last up to 12 h and classically recur every 48 h in tertian malaria (*P. falciparum*, *P. vivax* and *P. ovale*) or every 72 h in quartan malaria (*P. malariae*), in synchrony with the release of merozoites from lysed red blood cells (RBC). Anemia and splenomegaly most often develop after some days. *P. vivax* malaria, also referred to as "benign tertian malaria", is rarely fatal because parasitemia is low grade. The main problems are fever and anemia, occasionally with massive splenomegaly.

Severe disease cases, which occur more frequently with *P. falciparum* because of its ability to infect a greater percentage of erythrocytes and to cytoadhere to capillary walls [5], are characterized by acute renal failure with hypotension and shock, or cerebral malaria with unrousable coma and convulsions, or pulmonary edema. These occur most commonly in populations that have not acquired immunity to malaria, such as young children and travelers [29]. Case fatality rates among untreated children and non-immune adults can reach 10–40% or higher. Fatally afflicted children often die less than 72 h after developing symptoms. In those children who survive, profound sequellae often affect physical and intellectual development [30]. Malarial sickness is one of the principal reasons for poor school attendance. Other high-risk groups are women during pregnancy, non-immune travelers, refugees, displaced persons and laborers entering endemic areas. Pregnancy-associated malaria is precipitated by the accumulation of parasites in the placental intervillous spaces and causes maternal anemia and low birth weight. It also is a major factor contributing to abortions and at times maternal deaths. Pregnant mothers who have malaria and are HIV-positive also are more likely to transmit HIV to their newborn. In sub-Saharan Africa, at least 25 million pregnant women are exposed to malaria every year.

Malaria therefore exacts an enormous toll in lives, in medical costs, and in days of labor lost [31]. For the individual, costs include the price of treatment and prevention, and lost income. In rural areas, the rainy season is often a time of intense agricultural activity, when poor families earn most of their annual income. Malaria can make these families even poorer, hitting young adults especially hard: a single bout of the disease costs an estimated equivalent of 10 working days. The estimated costs of malaria, in terms of strains on the health systems, are enormous: in endemic countries, as many as 3 out of 10 hospital beds are occupied by victims of the disease. In 2002, 40% of outpatients at Mozambique clinics and 60% of children admitted to hospitals suffered from malaria. The direct and indirect cost of malaria in sub-Saharan Africa is estimated to be 1–5% of GPD, a cost of about $12 billion a year [32,33].

3. Parasitology

The agents of human malaria are four species of *Plasmodium* protozoa: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* [4]. All are transmitted by *Anopheles* mosquitoes. Their only reservoir is humans, except for *P. malariae*, which is common to man, the African apes and probably some South-American monkeys. Other species of *Plasmodium* are known (*P. yoelii*, *P. berghei*, *P. chabaudi*, *P. cynomolgi*), that are adapted to their wild mammalian host and do not multiply in humans.

All strains of *Plasmodium* have a complex life cycle that begins when a female mosquito injects sporozoites into the blood of an individual when taking a blood meal. The sporozoites enter the bloodstream and within less than 30 min migrate to the liver and invade hepatocytes. Sporozoites mature in the liver over a period of 6–16 days. This period, known as the liver stage, or *pre-erythrocytic stage*, ends with the release into the blood of tens of thousands of merozoites, which invade RBCs where they multiply and mature over a period of a few days. There, they successively undergo a ring-form stage, a trophozoite stage and a schizont stage. The schizont eventually bursts, lysing the RBC and releasing new generation merozoites which immediately proceed to invade new RBCs to repeat the cycle. This is the blood stage or *intra-erythrocytic stage* of the disease, which is characterized by the classical acute febrile episodes that occur every 48–72 h, in coincidence with the synchronized lysis of infected RBCs and the release of newly matured merozoites.

Some of the merozoites eventually develop into *sexual-stage* gametocytes, which, after being taken up by an anopheline mosquito, mature and sexually combine in the insect host to generate a zygote. The latter develops into an oocyst, from which new sporozoites are produced that migrate to the salivary glands of the mosquito, ready to reinitiate the cycle.

Pregnancy-associated malaria is caused by *P. falciparum*, which expresses unique variant surface antigens (VSA) that allow the parasite to bind chondroitin sulfate proteoglycan receptors on the surface of placenta syncytiotrophoblasts [34,35].

The complete nucleotide sequences of the *Plasmodium* parasite vector has been determined. The *Plasmodium* genome is A–T rich, unlike most of the microbial organisms or animal cells used to express recombinant antigens, and it

also shows quite different codon usage. Optimal expression of recombinant *Plasmodium* antigens therefore requires the use of synthetic genes reconstructed with optimized codons best suited for the expression systems used for production.

4. Vaccines

4.1. General considerations

As mentioned above, several lines of evidence suggest that a prophylactic malaria vaccine for humans is feasible. Firstly, immunization of naive human volunteers with irradiated (and thus attenuated) sporozoites was shown to confer 90% sterile protection against experimental infection following laboratory-bred, sporozoite-infected mosquito bites [36,37]. Secondly, naturally acquired immunity progressively builds up during the first two decades of life in people living in malaria-endemic countries. This immunity primarily impacts the severity of clinical disease, and appears to be linked to continuous antigenic stimulation, waning rapidly when exposure ceases [10,38,39]. Thirdly, protection has been elicited by passive transfer of hyperimmune immunoglobulins from malaria-immune adults into malaria-naïve human volunteers [11].

Key obstacles to the development of a vaccine include the lack of immune correlates of protection, the lack of reliable and predictive animal models, and the developmental and antigenic variability of the parasite. An additional obstacle has been the paucity of expertise in GMP vaccine development in the non-industrial centers where much of the malaria vaccine research has been undertaken so far, and the lack of vaccine development platforms to develop clinically acceptable adjuvants able to induce cell-mediated immunity as well as epitope- or antigen-presenting systems. Much work has nevertheless been done to determine which protective antigens or epitopes should be used in the construction of recombinant, subunit or synthetic malaria vaccines.

The traditional approach to develop malaria vaccines has focused on the targeting of one of the different stages of parasite development, whether the pre-erythrocytic, the asexual (intra-erythrocytic) or the sexual stage. *Pre-erythrocytic vaccine* strategies aim to generate an antibody response able to neutralize sporozoites and prevent them from invading the hepatocyte, as well as to elicit a cell-mediated immune response able to interfere with the intra-hepatic multiplication cycle of the parasites, e.g. by killing the parasite-infected hepatocytes. This type of vaccine would be ideal for travelers because it would prevent the advent of any form of clinical disease. *Asexual blood-stage* (*erythrocytic stage*) vaccine strategies aim to elicit antibodies that will inactivate merozoites and/or target malarial antigens expressed on the RBC surface through antibody-dependent cellular cytotoxicity and/or complement lysis; and also are meant to elicit T-cell responses able to inhibit the development of the parasite in RBCs. By decreasing the exponential multiplication of merozoites, this type of vaccine would mostly serve as a disease-reduction vaccine in endemic countries [40]. As for vaccines that target the *sexual stage* of the parasite, they do not aim to prevent illness or infection in the vaccinated individual, but to prevent or decrease transmission of the parasite to new hosts. This 'transmission-blocking' vaccine can be seen as a true altruistic vaccine.

Besides these classical approaches, novel approaches being currently undertaken include the development of an irradiated sporozoite vaccine [41] and an anti-parasite toxin vaccine that targets the parasite toxins which contribute to the disease, such as the glycosylphosphatidylinositol (GPI) anchor. Recent promising results obtained with knock-out sporozoites in mice will likely lead to further developments over the coming years [21,42,43].

4.2. Pre-erythrocytic vaccines

These vaccines aim to protect against malaria infection [44] and ideally should provide sterilizing humoral immunity by eliciting antibodies that target the invading sporozoites and prevent them from invading the liver. Additionally, or as an alternative, the vaccine should induce a cell-mediated immune response able to inhibit the sporozoite maturation cycle once they have invaded hepatocytes [45]. CD4+ and CD8+ cytotoxic T cells (CTL), NK T cells, as well as γδ T cells have been implicated in such a control [46–51].

4.2.1. CSP vaccines

The most advanced and well-documented pre-erythrocytic (liver-stage) vaccine candidate is derived from the circumsporozoite protein (CSP) that is found at the surface of the sporozoite and of the infected hepatocyte. This protein contains two major B cell epitopes consisting of tandem repeats (Asn-Ala-Asn-Pro and Asn-Val-Asp-Pro). The candidate vaccine which was developed by Glaxo Smith Kline (GSK) in collaboration with the Walter Reed Army Institute of Research (WRAIR), RTS,S, comprises the C-terminus (amino acids 207–395) of the CSP from *P. falciparum* fused to the hepatitis B surface antigen and expressed in the form of virus-like particles (VLPs) in *Saccharomyces cerevisiae* [52,53]. Initial Phase I clinical trials of RTS,S formulated with GSK AS02 adjuvant (containing MPL, QS21 and an oil-in-water emulsion) showed protection against malaria challenge in six out of seven volunteers. A dose-range Phase I/II study showed levels of efficacy from 30% (single dose) to 55% (three doses), with an overall protective efficacy of 40%, but which, however, was not long-lived. Further trials in The Gambia demonstrated a 70% protection efficacy against infection for the first 9 weeks, with efficacy waning rapidly thereafter [54]. Volunteers who received a fourth dose of the vaccine the following year, prior to the onset of the malaria season, again exhibited a 47% protection over a 9-week follow-up period [53,55,56].

Clinical development of the RTS,S candidate vaccine has continued in partnership with PATH MVI. In a proof-of-

concept trial on 2022 children in Mozambique, efficacy of three doses of RTS,S/AS02 at preventing a first malaria attack in 1–4 years old children was about 30% and, most significantly, overall incidence of severe disease was decreased 58% over a 6-month follow-up period [13]. Vaccine efficacy over a 18-month follow-up period was recently reported to be 35.3% against time to first or only episode of malaria and 48.6% against severe disease [14,57]. Other clinical studies of the RTS,S vaccine are underway, including one aimed at combining RTS,S with the blood-stage antigen MSP-1 (see below) [58,59]. Heterologous prime-boost immunization with RTS,S/AS02 and a live vaccinia virus (MVA)-CSP recombinant vaccine was well tolerated in human volunteers but yielded only small increments in T cell responses, no increase in antibody response and no improvement in percent protection [60].

The history of development of the RTS,S AS02 vaccine demonstrates the critical importance of appropriate adjuvants and formulations for the efficacy of vaccines based on purified antigens. Thus, the same malaria antigen formulated with aluminium hydroxide, with MPL, or as an oil-in-water emulsion failed to elicit a protective immune response, which was only obtained with the AS02 formulation, an oil-in-water emulsion plus MPL and QS21 [52]. The majority of other malaria recombinant antigens that have been clinically evaluated so far were formulated with either aluminium, QS21, or water-in-oil emulsions such as Montanide ISA 720, adjuvants which are less potent than the proprietary AS02 adjuvant and which may have been inadequate to induce protective responses in those studies.

Another CSP-based candidate vaccine was based on a 102-amino acid synthetic peptide, CS102, that represents the C-terminus of the CSP of *P. falciparum* formulated with Montanide ISA 720 [61]. The formulation, which was developed by Dictagen, Inc., in collaboration with the University of Lausanne, was shown to be safe in human volunteers and to elicit both an antibody and a cellular immune response including CD8+ CTLs and secretion of IFN-γ [62]. The vaccine now has undergone further Phase I and Phase IIa clinical trials, but has failed to show protection against experimental malaria challenge.

An alternative approach to synthetic vaccines was to incorporate several copies of a protective CSP epitope into a multiple antigenic peptide (MAP). As expected, the resulting vaccine was found to elicit an immune response only in the volunteers with cognate HLA haplotypes [63]. In an effort to bypass MHC restriction, the peptide was linked to a "universal" T-cell epitope. This construct was shown to elicit robust immune responses in humans with diverse genetic background [64]. The B- and T-cell epitopes from the MAP trials also have been incorporated into a recombinant VLP based on hepatitis B core particles. This CSP-HBc particle vaccine, known as ICC-1132, was developed by Apovia in the USA with the help of MVI. Although this candidate vaccine elicited both humoral and cellular immune responses, no evidence of protection was found in a Phase II study [65] and this approach was discontinued.

4.2.2. DNA vaccines and live recombinant vaccines

Other vaccines based on the CSP antigen include plasmid DNA vaccines and live recombinant vaccines that use the attenuated modified vaccinia Ankara (MVA) strain, fowlpoxvirus (FPV), Adenovirus, Sindbis virus, yellow fever virus or a cold-adapted attenuated influenza virus strain as a vector. Some of these vaccines were tested together in prime-boost combinations [66,67]. A series of trials using DNA and recombinant fowlpoxvirus or MVA vaccines encoding the *P. falciparum* CSP in various prime-boost combinations unfortunately did not show evidence of protective efficacy against a sporozoite challenge [68]. Both the MVA and fowlpoxvirus recombinant vaccines were nevertheless found to be safe and suitable for large scale studies of children in Africa [69,70].

In spite of promising humoral and cellular immune responses in mouse models, plasmid DNA vaccines injected through various routes did not translate into similar immunogenicity and protective efficacy in humans. The US Department of Defense (DOD), in collaboration with Vical, Inc., has developed candidate DNA vaccines for malaria (the Multi-Stage DNA Operation, MuStDO), including a liver-stage DNA vaccine that encodes the CSP of *P. falciparum*. This DNA vaccine was tested in a "proof-of-concept" Phase I study carried out by the US Navy Malaria Program. The vaccine elicited cell-mediated immune responses but only modest antibody responses and no protection against experimental challenge in human volunteers [71].

A multiple-antigen DNA vaccine, MuStDO-5, has been designed to encode five different liver-stage antigens: CSP, liver stage antigens 1 and 3 (LSA-1 and -3), exported protein 1 (EXP1), and the sporozoite surface protein 2 (SSP2, also known as thrombospondin-related adhesive protein, TRAP). Various studies conducted in endemic areas have linked LSA-1 and LSA-3 with protective immunity. MuStDo-5 was manufactured as a combination of five separate plasmids. The vaccine, administered with GM-CSF DNA as an adjuvant, was safe and well tolerated in mice and rabbits [72], but showed only weak immunogenicity in primates and no evidence of protection was obtained in Phase IIa challenge trials [73–75].

The Oxford University Malaria Vaccine Clinical Trials Group conducted studies of a DNA, a fowlpoxvirus (FPV), and a MVA-based vaccines expressing TRAP fused to a polyepitopic construct, demonstrating strong correlation between the induction of IFN-γ-secreting CD4+ and CD8+ T-cell responses and protection against malaria in a mouse model [76]. The DNA and MVA candidate vaccines were combined in a prime-boost immunization trial in human volunteers in The Gambia [77]. No protection was observed against occurrence of disease, but malaria-related mortality was reduced [75,77,78]. The substitution of plasmid DNA as the priming vector with a specific recombinant fowlpoxvirus vaccine was found to elicit sterile protection in two of five

volunteers, and in one of them protection lasted for 20 months and was associated with persisting memory T cell responses [79]. The same recombinant FPV-TRAP/MVA-TRAP prime-boost immunization regimen was tested in Kenya. Strong T cell responses were elicited in children by half-dose FPV priming followed by MVA boosting [80], but the partial cross-reactivity between the two poxvirus vectors still might impair full immunogenicity of the MVA vaccine. A new efficacy trial is currently ongoing in Kenya.

Additional antigens that have been targeted for pre-erythrocytic vaccine development include the liver stage antigens 1 and 3 (LSA-1 and -3), the sporozoite and liver stage antigen (SALSA) and the sporozoite threonine and asparagine rich protein (STARP). LSA-3, a highly conserved pre-erythrocytic antigen, has been demonstrated to induce protective immunity against *P. falciparum* sporozoite challenge in chimpanzees and *Aotus* monkeys [81–84]. Various LSA-3 formulations, including a LSA-3 lipopeptide candidate are planned to presently enter Phase I trials.

4.3. Asexual blood-stage vaccines

These vaccines, also referred to as erythrocytic stage vaccines, are aimed to primarily protect against severe malaria disease, and not against infection, on the assumption that inhibition of parasite invasion cycles will lead to reduced parasite burden and decreased morbidity and mortality. Despite encouraging progress, the lack of immune correlates of protection and that of predictive animal models, together with the polymorphism and strain variability of many asexual stage antigens constitute major challenges to the development effort of asexual stage vaccines. In contrast with pre-erythrocytic vaccine candidates, asexual stage vaccine candidates also currently lack a human artificial challenge model and have to rely on natural challenge in field trials to provide proof-of-concept. Their development is therefore slower and necessitates major commitment, intensive collaborations as well as high-level coordination supported by adequate funding [22].

The first asexual blood-stage malaria vaccine to be developed was the SPf66 vaccine candidate that was developed in Colombia as a synthetic, multiepitope, multi-stage peptide vaccine mixed with alum as an adjuvant [85]. The vaccine was tested in several Phase III field trials involving thousands of volunteers, but its reported efficacy was too low to warrant further development [86–89], although one may suspect that the vaccine would have fared better with more potent adjuvants [90].

The most advanced asexual blood-stage vaccines at this time are based on the use of merozoite surface proteins 1 (MSP-1), 2 (MSP-2) and 3 (MSP-3), the apical membrane antigen 1 (AMA-1) and the glutamate-rich protein (GLURP). Antibodies to MSP-1 have been shown to block parasite invasion of RBCs *in vitro* [91]. AMA-1 is a natural target of protective responses *in vivo*. Both AMA-1 and MSP-1 have their 3D conformation stabilized by intramolecular disulphide bonds which are critical for optimal immunogenicity of the molecule. MSP-1 contains two cysteine-rich epidermal growth factor (EGF)-like domains that generate protective antibodies and are conserved across all species of *Plasmodium*.

4.3.1. MSP-1, MSP-2 and RESA combination

Certainly the furthest along the vaccine development pathway of blood-stage malaria vaccine candidates is the "Combination B" vaccine, which resulted from a collaborative effort by the Papua New Guinea Institute for Medical Research along with the Australian Cooperative Research Center for Vaccine Technology in Queensland, The Walter and Eliza Hall Research Institute and the Swiss Tropical Institute. This vaccine combined the merozoite surface proteins MSP-1 and MSP-2 with *P. falciparum* ring-stage infected-erythrocyte surface antigen (RESA) in a Montanide adjuvant formulation. A Phase I/IIb trial in 120 5–9 years old children in Papua New Guinea showed a 62% reduction in parasite density in vaccinees [92]. However, the vaccine preparation contained only the 3D7 allelic form of MSP-2. Analysis of the genotype of breakthrough parasites showed a significant increase in parasites with the FC27 allele genotype, the opposite dimorphic form of MSP-2 [93]. A new version of the vaccine is being developed using both variants of MSP-2 in order to target both genotypes.

4.3.2. MSP-1 and AMA-1

Much work has concentrated on the entire MSP-1 molecule, its 42 kDa C-terminal moiety, or a further-processed 19 kDa fragment [94]. These were expressed either as such or as part of fusion molecules using baculovirus, *Escherichia coli*, or yeast (*Saccharomyces* or *Pichia*). Recombinant MSP-1 42 kDa and 19 kDa fragments have been shown to protect both mice and *Aotus* monkeys against lethal parasite challenge [95–98], but a Phase I trial of the 19 kDa fragment carried out at Baylor University (USA) demonstrated that it was poorly immunogenic and had unacceptable side-effects. The MSP-1 42 kDa fragment formulated in AS02 adjuvant by GSK in collaboration with the Walter Reed Army Institute (WRAIR), USA, and MVI was found to be safe and very immunogenic in human volunteers in the USA, Kenya and Mali [99,100]. The vaccine currently is being tested in pediatric efficacy trials in Kenya. A fragment of the MSP-1 from *P. vivax*, termed Pv200L, which shows significant sequence homology with its *P. falciparum* equivalent, was found to provide partial protection against challenge with *P. vivax* in mice and *Aotus* monkeys [101].

Another vaccine studied in collaboration between WRAIR and GSK is based on the AMA-1 protein formulated in the AS02 adjuvant. Like MSP-1, the vaccine potential of AMA-1 is supported by the observation that antibodies to the protein inhibit invasion of RBCs *in vitro* and that recombinant AMA-1 protein can induce species-specific protection against malaria in rodent and non-human primate models

[102,103]. The high polymorphism of the AMA-1 antigen is however a matter of concern [104–107]. An AMA-1 vaccine was studied in a Phase I study in the USA and now is in Phase I studies in Mali and Kenya [108,109]. A similar vaccine formulated in Montanide ISA 720 adjuvant was tested in a Phase I trial in Australia but only showed moderate immunogenicity [110].

A MSP-1/AMA-1 fusion antigen made of the C terminal region of AMA-1 and the 19 kDa fragment of MSP-1 [111] was produced in Shanghaï using *Pichia pastoris*. The vaccine (PfCP-2.9), which was formulated with Montanide ISA 720 as an adjuvant, showed good immunogenicity in rabbits and non-human primates. WHO sponsored and coordinated the first Phase I clinical testing of the PfCP-2.9 vaccine in China in collaboration with the Second Military Medical University in Shanghai. The vaccine was found to be safe and immunogenic. Plans are underway for further clinical development through an agreement with MVI. The combination of the PfCP-2.9 vaccine with a fragment of the PfEBA-175 antigen expressed in *P. pastoris* yeast elicited in both rabbits and monkeys antibodies that inhibited parasite growth *in vitro* [112].

4.3.3. MSP-3, GLURP and SERA

The merozoite surface protein 3 (MSP-3), which was discovered by a reverse vaccinology approach and found to be a target of protective antibodies from immune adults, has been developed as a long synthetic peptide by the Pasteur Institute in Paris and the European Malaria Vaccine Initiative (EMVI). The vaccine construct contains B and T cell epitopes that were selected based on their targeting by cytophilic antibodies that interact with monocytes in the antibody-dependent cellular inhibition (ADCI) assay [113–116]. The African Malaria Network Trust (AMANET), in collaboration with EMVI, sponsored and coordinated a Phase I study of the vaccine in Burkina Faso, where the vaccine was shown to be safe and to induce long-lasting antibodies that display ADCI activity *in vitro* as well as *in vivo* in a new mouse model of *P. falciparum* malaria [117,118].

Another long synthetic peptide vaccine whose rationale is based on the induction of antibodies with ADCI activity is based on the glutamate-rich protein (GLURP), also a target of protective antibodies from immune adults [119–122]. The GLURP vaccine, which is developed by the Staten Serum Institute in Denmark in collaboration with EMVI, was formulated either in alum or Montanide ISA 720 and tested in a Phase I clinical trial [123].

Yet another candidate being developed in an Asian–African collaboration is based on the serine repeat antigen (SERA), also known as P126 antigen. This is the largest parasite protein, it accumulates in the parasitophorous vacuole of trophozoites and schizonts and is processed into three fragments (18 kDa, 47 kDa, 50 kDa) [124]. Cross-sectional studies conducted in the Solomon Islands, Brazil and Uganda [125] have shown a significant association between responses to SERA and lower parasitemia [126]. Various SERA-based constructs have been tested and shown to induce *in vitro* parasite killing activity in a dose dependent manner through both complement-mediated inhibition and ADCI [127,128]. Protective efficacy of a subunit SERA vaccine against challenge in *Aotus* and squirrel monkeys also has been demonstrated [129,130]. A candidate SERA-based vaccine currently is in Phase I studies in Japan.

4.3.4. Other antigens

Additional merozoite surface antigens under development as vaccine candidates include MSP-4, -5, -8 and -9. These molecules contain one or more of the hallmark EGF-like domains present in MSP-1 [131,132]. MSP-5 is of particular interest because it lacks the sequence variation between different isolates of *P. falciparum* from different geographical locations that is typically seen with most of the MSPs. The erythrocyte-binding antigen (EBA-175), which binds glycophorin A [133], and its paralog in *P. vivax*, the Duffy binding antigen (DBA), also are currently developed as recombinant vaccine candidates expressed either in *E. coli* or in *P. pastoris* or as a DNA vaccine in prime-boost regimens [134,135].

With regards to pregnancy-associated malaria, the erythrocyte protein 1 (PfEMP1) antigen, a highly variable *P. falciparum* protein, was identified as the ligand that allows infected erythrocytes to adhere to the chondroitin sulfate receptor. A candidate vaccine has been developed based on that antigen [136–138] but initial studies in *Aotus* monkeys failed to show protective efficacy [23].

4.4. Transmission-blocking vaccines

These vaccines are aimed to induce antibodies against the sexual stage antigens in order to prevent the development of infectious sporozoites in the salivary glands of the *Anopheles* mosquitoes [139]. They are intended to protect communities from infection, not individuals from disease and therefore face the difficult challenge of conducting Phase III trials to assess their impact on malaria transmission in the field. The leading candidate vaccines contain the *P. falciparum* ookinete surface antigens Pfs25 and Pfs28 or their *P. vivax* homologues Pvs25 and Pvs28 [135,140,141]. These vaccines are currently being developed at the National Institutes of Health (NIH) in the USA as recombinant yeast-secreted proteins (*S. cerevisiae*) [142,143]. Initial human Phase I trials that were conducted for Pvs25 demonstrated safety and a modest immunogenicity [144,145]. Other sexual stage-specific antigens that are being developed as transmission-blocking vaccines are Pfs48/45 and Pfs230 [146].

4.5. Other approaches

Various groups such as the Centers for Disease Control (CDC), Navy Medical Research Center (NMRC) and WRAIR are developing multi-antigens, multi-stage vaccine concepts [4]. Advantages of a combination vaccine include

the potential to address the problems of antigenic variation, of inducing immunity in genetically heterogeneous populations and of coping with possible immune escape of the parasite. However, the risk of interference between components of the vaccine and increased reactogenicity of the formulation must be taken into account.

The concept of attenuation and parasite challenge to elicit immunity also has been explored [42]. An attempt to develop an attenuated sporozoite vaccine has been undertaken by Sanaria, Inc., with the support from the Bill and Melinda Gates Foundation and the NIH. Challenges are numerous, including consistent production of large quantities of sporozoites, production quality control and process monitoring, GMP guidelines, stability, delivery and potential cost of the product [37,41] The possibility of using low doses of *P. falciparum*-infected RBCs also has been entertained [147].

Finally, the glycosylphosphatidylinositol (GPI) anchor, which tethers several of the *Plasmodium* antigens to the membrane, has been shown to be highly toxic in mouse models [148]. An anti-toxic vaccine is currently being developed as a carbohydrate anti-GPI vaccine. A proof-of-principle study testing synthetic GPI as a vaccine in rodent models of malaria showed that the candidate vaccine was immunogenic and protected the animals from significant malaria pathology and mortality [149]. Whether further development of malaria toxin neutralization as a vaccine strategy will continue is however far from certain.

5. Concluding remarks

The first stage in designing a malaria vaccine is the identification of the appropriate antigens and their formulation in a presentation that will elicit the desired protective immune responses. The lack of definitive knowledge on the nature of protective host responses to malaria deeply affects the assessment and rational prioritization of the current candidate *Plasmodium* antigens to be used in a malaria vaccine [150]. Since there are as yet no reliable correlates of protection, the selection of candidate antigens has been done by screening parasite extracts or gene libraries against sera or lymphocytes from immune individuals [151,152].

The determination of an antigen's vaccine potential is highly dependent on its performance in biological assays that measure immunogenicity, yet the relevance of these immunogenicity studies often remains unclear. The paucity of standardized assays prevents useful comparisons between results from different laboratories often pursuing different formulations of the same antigen. Efforts to enable consistent intra- and inter-laboratory comparisons and thus to enhance the predictability of assay data are essential to provide confidence in decision-making relative to choice of candidate vaccines. An example of a standardized comparative immunoassay is the growth inhibition assay (GIA), which measures the capacity of antibodies to limit the growth of *P. falciparum* in RBCs in culture [153]. Another comparative assay is the ADCI assay, which initially was developed at the Pasteur Institute in Paris.

The various immune mechanisms of protection against malaria still are basically obscure [154]. Thus, vaccines against the pre-erythrocytic stage of the parasite have been developed with the goal of inducing antibodies that block invasion of hepatocytes by sporozoites, but field studies do not support a major role of these antibodies in protection. Antibody-opsonization of sporozoites, inhibition of intrahepatic parasite development by CD4+ and CD8+ T cell secretion of IFN-γ as well as killing of infected hepatocytes by CTL have been demonstrated to occur in response to the RTS,S vaccine [51] and in the irradiated sporozoite model [155]. Thus, current approaches aim to induce both these types of antibodies and T cell responses but the durability of the response remains a major challenge. Understanding the mechanisms by which CD4+ helper T cells are activated and the requirements for development of a specific and effective T cell memory and immunity is therefore essential in the quest of a malaria vaccine [156].

Regarding vaccines against the asexual (erythrocytic) stage of the parasite, multiple mechanisms have been identified which could underly protective immunity, such as antibodies that interact with the erythrocyte surface receptors and inhibit their invasion by merozoites, cytophilic antibodies that help destroy intra-erythrocytic parasites by monocytes, antibodies directed against merozoite surface antigens that mediate free merozoite agglutination and facilitate their phagocytosis, or antibodies that prevent the binding of infected erythrocytes to vascular endothelia, not to mention cytokines such as IFN-γ, TNF-α or IL-12, which can mediate parasite killing [157]. The major challenge met by vaccines which aim to elicit these types of responses is the high polymorphism and variability of the merozoite antigens [158].

As mentioned above, the absence of understanding regarding the nature of protective immunity adds to the complexity of vaccine development as the formulation of the parasite antigens remains purely empirical and a potentially protective antigen may fail in clinical trials because the formulation used may be inducing an inappropriate or inadequate immune response. The paucity of potent adjuvant systems with demonstrated clinical safety adds to the complexity of the field.

Another difficulty in the development of malaria vaccines stems from the absence of relevant animal models. Rodent models using *P. yoelii*, *P. berghei* or *P. chabaudi*, which lead to lethal infections, are basically different from the chronic infection with *P. falciparum* in humans [159]. The development of a SCID mouse model [160] might provide an interesting model. Non-human primates such as New World (*Aotus* or *Saimiri*) monkeys, rhesus macaques, *Cercopithecus* monkeys or chimpanzees are prime animal models for *P. falciparum* and *P. vivax* vaccine protection

studies, but their cost, scarcity and strong ethical constraints limit their use. Also, the tolerance of high parasitemia and rapid acquisition of immunity following infection in the animals limit the interpretation of efficacy studies [161,162].

The choice of clinical case definitions and end-points in malaria vaccine trials is yet another difficult problem which may have marked influence on reported vaccine efficacy. Clinical case definition is straightforward for sterile protection, as used as an end-point in trials of pre-erythrocytic stage vaccines such as RTS,S [13] but can be most difficult for trials of vaccines which aim to decrease morbidity by reducing parasitemia [163].

All candidate malaria vaccines also meet with the problem of immunological memory. The protective immune status acquired through repeated natural exposure of infected individuals to the parasite over a period of years can readily be lost if continuous antigenic stimulation ceases. This suggests that low-level antigenic persistance is a prerequisite of immunity to malaria, and, therefore, that vaccines will need to simulate such a persistance to induce long-lasting protection [164]. So far, the hope was that boosting due to natural exposure would occur to provide this stimulus. Recent data suggest that alternating vectors in prime-boost vaccination regimens generate a large resting memory T cell population, which provides unprecedented durability of the immune response [79,165].

The major malaria vaccine funding agencies are the NIH in the USA, the Wellcome Trust in the U.K., the European Union, either directly through the European and Developing Countries Clinical Trials Partnership (EDCTP) or through the European Malaria Vaccine Initiative (EMVI), USAID, the Malaria Vaccine Initiative (MVI) at PATH, the Bill and Melinda Gates Foundation and WHO. In addition, the African Malaria Network Trust (AMANET) and the Malaria Clinical Trial Alliance (MCTA) recently were established to build up the capacity to plan and coordinate malaria vaccine trials in Africa. The recent infusion of public and private funding for malaria vaccine development has greatly accelerated the pace at which candidate malaria vaccines are entering the clinic. Increased collaboration and cooperation among key research stakeholders in the malaria vaccine field also has played a role in this trend. A recent international consultative process that engaged vaccine researchers, experts and donors has resulted in a *Roadmap*, which outlines a strategy for the development and licensure of an effective pediatric malaria vaccine by 2015 and a more broadly effective vaccine by 2025. Top priorities were identified where intensified coordination and more focused action could be applied to overcome the significant challenges discussed above. The need for creative strategies, strong partnership between industrialized and developing countries and political vision and leadership on the part of wealthy nations is more than ever critical to successfully continue the development of malaria vaccines and implement their use in an effort to fight the terrible burden of malaria [166].

Acknowledgement

The efficient editorial assistance of Olga Assossou is gratefully ackowledged.

References

[1] World Malaria Report. World Health Organization, 2005.
[2] Subbarao SK, Sharma VP. Anopheline species complexes & malaria control. Indian J Med Res 1997;106:164–73.
[3] Riehle MM, Markianos K, Niare O, Xu J, Li J, Toure AM, et al. Natural malaria infection in *Anopheles gambiae* is regulated by a single genomic control region. Science 2006;312(5773):577–9.
[4] Dubovsky F, Rabinovich NR. Malaria vaccines. In: Plotkin SA, Orenstein WA, editors. Vaccines. 4th ed. Philadelphia: Saunders; 2004. p. 1283–9.
[5] Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature 2002;415(6872):673–9.
[6] Greenwood B. Malaria vaccines. Evaluation and implementation. Acta Trop 2005;95(3):298–304.
[7] Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 2006;440(7086):940–3.
[8] Malkin E, Dubovsky F, Moree M. Progress towards the development of malaria vaccines. Trends Parasitol 2006;22(7):292–5.
[9] Day KP, Marsh K. Naturally acquired immunity to *Plasmodium falciparum*. Immunol Today 1991;12(3):A68–71.
[10] Smith TA, Leuenberger R, Lengeler C. Child mortality and malaria transmission intensity in Africa. Trends Parasitol 2001;17(3):145–9.
[11] Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg 1991;45(3):297–308.
[12] Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. Nature 1967;216(111):160–2.
[13] Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. Lancet 2004;364(9443):1411–20.
[14] Smith PG, Milligan PJ. Malaria vaccine: 3 or 6 months' protection? Lancet 2005;365(9458):472–3.
[15] Targett GA. Malaria vaccines 1985–2005: a full circle? Trends Parasitol 2005;21(11):499–503.
[16] Aguiar JC, LaBaer J, Blair PL, Shamailova VY, Koundinya M, Russell JA, et al. High-throughput generation of *P. falciparum* functional molecules by recombinational cloning. Genome Res 2004;14(10B):2076–82.
[17] Duffy PE, Krzych U, Francis S, Fried M. Malaria vaccines: using models of immunity and functional genomics tools to accelerate the development of vaccines against *Plasmodium falciparum*. Vaccine 2005;23(17/18):2235–42.
[18] Reed ZH, Friede M, Kieny MP. Malaria vaccine development: progress and challenges. Curr Mol Med 2006;6(2):231–45.
[19] Kemp DJ, Coppel RL, Cowman AF, Saint RB, Brown GV, Anders RF. Expression of *Plasmodium falciparum* blood-stage antigens in *Escherichia coli*: detection with antibodies from immune humans. Proc Natl Acad Sci USA 1983;80(12):3787–91.
[20] Ellis J, Ozaki LS, Gwadz RW, Cochrane AH, Nussenzweig V, Nussenzweig RS, et al. Cloning and expression in *E. coli* of the malarial sporozoite surface antigen gene from *Plasmodium knowlesi*. Nature 1983;302(5908):536–8.
[21] Good MF. Vaccine-induced immunity to malaria parasites and the need for novel strategies. Trends Parasitol 2005;21(1):29–34.

[22] Good MF, Kaslow DC, Miller LH. Pathways and strategies for developing a malaria blood-stage vaccine. Annu Rev Immunol 1998;16:57–87.
[23] Makobongo MO, Keegan B, Long CA, Miller LH. Immunization of *Aotus* monkeys with recombinant cysteine-rich interdomain region 1 alpha protects against severe disease during *Plasmodium falciparum* reinfection. J Infect Dis 2006;193(5):731–40.
[24] Sabatinelli G, Ejov M, Joergensen P. Malaria in the WHO European Region (1971–1999). Euro Surveill 2001;6(4):61–5.
[25] Sunstrum J, Elliott LJ, Barat LM, Walker ED, Zucker JR. Probable autochthonous *Plasmodium vivax* malaria transmission in Michigan: case report and epidemiological investigation. Am J Trop Med Hyg 2001;65(6):949–53.
[26] Kovats RS, Bouma MJ, Hajat S, Worrall E, Haines A. El Nino and health. Lancet 2003;362(9394):1481–9.
[27] Lindsay SW, Martens WJ. Malaria in the African highlands: past, present and future. Bull World Health Organ 1998;76(1):33–45.
[28] Maguire JD, Tuti S, Sismadi P, Wiady I, Basri H, Krisin, et al. Endemic coastal malaria in the Thousand Islands District, near Jakarta, Indonesia. Trop Med Int Health 2005;10(5):489–96.
[29] Chen Q, Schlichtherle M, Wahlgren M. Molecular aspects of severe malaria. Clin Microbiol Rev 2000;13(3):439–50.
[30] Murphy SC, Breman JG. Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. Am J Trop Med Hyg 2001;64(1/2 Suppl):57–67.
[31] Guinovart C, Navia MM, Tanner M, Alonso PL. Malaria: burden of disease. Curr Mol Med 2006;6(2):137–40.
[32] Gallup JL, Sachs JD. The economic burden of malaria. Am J Trop Med Hyg 2001;64(1/2 Suppl):85–96.
[33] Sachs J, Malaney P. The economic and social burden of malaria. Nature 2002;415(6872):680–5.
[34] Tuikue Ndam NG, Salanti A, Le-Hesran JY, Cottrell G, Fievet N, Turner L, et al. Dynamics of anti-VAR2CSA immunoglobulin G response in a cohort of senegalese pregnant women. J Infect Dis 2006;193(5):713–20.
[35] Fried M, Domingo GJ, Gowda CD, Mutabingwa TK, Duffy PE. *Plasmodium falciparum*: chondroitin sulfate A is the major receptor for adhesion of parasitized erythrocytes in the placenta. Exp Parasitol 2006;113(1):36–42.
[36] Egan JE, Hoffman SL, Haynes JD, Sadoff JC, Schneider I, Grau GE, et al. Humoral immune responses in volunteers immunized with irradiated *Plasmodium falciparum* sporozoites. Am J Trop Med Hyg 1993;49(2):166–73.
[37] Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, et al. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. J Infect Dis 2002;185(8):1155–64.
[38] Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, et al. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. Am J Trop Med Hyg 1994;51(2):123–37.
[39] Baird JK. Age-dependent characteristics of protection vs. susceptibility to *Plasmodium falciparum*. Ann Trop Med Parasitol 1998;92(4):367–90.
[40] Hill AV. Pre-erythrocytic malaria vaccines: towards greater efficacy. Nat Rev Immunol 2006;6(1):21–32.
[41] Luke TC, Hoffman SL. Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. J Exp Biol 2003;206(Pt 21):3803–8.
[42] Mueller AK, Labaied M, Kappe SH, Matuschewski K. Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine. Nature 2005;433(7022):164–7.
[43] van Dijk MR, Douradinha B, Franke-Fayard B, Heussler V, van Dooren MW, van Schaijk B, et al. Genetically attenuated, P36p-deficient malarial sporozoites induce protective immunity and apoptosis of infected liver cells. Proc Natl Acad Sci USA 2005;102(34):12194–9.
[44] Walther M. Advances in vaccine development against the pre-erythrocytic stage of *Plasmodium falciparum* malaria. Expert Rev Vaccines 2006;5(1):81–93.
[45] Doolan DL, Hoffman SL. The complexity of protective immunity against liver-stage malaria. J Immunol 2000;165(3):1453–62.
[46] Nardin EH, Nussenzweig RS. T cell responses to pre-erythrocytic stages of malaria: role in protection and vaccine development against pre-erythrocytic stages. Annu Rev Immunol 1993;11:687–727.
[47] Tsuji M, Mombaerts P, Lefrancois L, Nussenzweig RS, Zavala F, Tonegawa S. Gamma delta T cells contribute to immunity against the liver stages of malaria in alpha beta T-cell-deficient mice. Proc Natl Acad Sci USA 1994;91(1):345–9.
[48] Good MF, Doolan DL. Immune effector mechanisms in malaria. Curr Opin Immunol 1999;11(4):412–9.
[49] Nardin E, Zavala F, Nussenzweig V, Nussenzweig RS. Pre-erythrocytic malaria vaccine: mechanisms of protective immunity and human vaccine trials. Parassitologia 1999;41(1–3):397–402.
[50] Gonzalez-Aseguinolaza G, de Oliveira C, Tomaska M, Hong S, Bruna-Romero O, Nakayama T, et al. Alpha-galactosylceramide-activated Valpha 14 natural killer T cells mediate protection against murine malaria. Proc Natl Acad Sci USA 2000;97(15):8461–6.
[51] Sun P, Schwenk R, White K, Stoute JA, Cohen J, Ballou WR, et al. Protective immunity induced with malaria vaccine, RTS,S, is linked to *Plasmodium falciparum* circumsporozoite protein-specific CD4+ and CD8+ T cells producing IFN-gamma. J Immunol 2003;171(12):6961–7.
[52] Stoute JA, Slaoui M, Heppner DG, Momin P, Kester KE, Desmons P, et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. N Engl J Med 1997;336(2):86–91.
[53] Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG, Hall T, et al. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. J Infect Dis 2001;183(4):640–7.
[54] Stoute JA, Kester KE, Krzych U, Wellde BT, Hall T, White K, et al. Long-term efficacy and immune responses following immunization with the RTS,S malaria vaccine. J Infect Dis 1998;178(4):1139–44.
[55] Bojang KA, Milligan PJ, Pinder M, Vigneron L, Alloueche A, Kester KE, et al. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. Lancet 2001;358(9297):1927–34.
[56] Bojang KA, Olodude F, Pinder M, Ofori-Anyinam O, Vigneron L, Fitzpatrick S, et al. Safety and immunogenicity of RTS,S/AS02A candidate malaria vaccine in Gambian children. Vaccine 2005;23(32):4148–57.
[57] Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Aide P, et al. Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. Lancet 2005;366(9502):2012–8.
[58] Heppner Jr DG, Kester KE, Ockenhouse CF, Tornieporth N, Ofori O, Lyon JA, et al. Towards an RTS,S-based, multi-stage, multi-antigen vaccine against falciparum malaria: progress at the Walter Reed Army Institute of Research. Vaccine 2005;23(17/18):2243–50.
[59] Alonso PL. Malaria: deploying a candidate vaccine (RTS,S/AS02A) for an old scourge of humankind. Int Microbiol 2006;9(2):83–93.
[60] Dunachie SJ, Walther M, Vuola JM, Webster DP, Keating SM, Berthoud T, et al. A clinical trial of prime-boost immunisation with the candidate malaria vaccines RTS,S/AS02A and MVA-CS. Vaccine 2006;24(15):2850–9.
[61] Roggero MA, Filippi B, Church P, Hoffman SL, Blum-Tirouvanziam U, Lopez JA, et al. Synthesis and immunological characterization of 104-mer and 102-mer peptides corresponding to the N- and C-terminal regions of the *Plasmodium falciparum* CS protein. Mol Immunol 1995;32(17/18):1301–9.

[62] Prato S, Maxwell T, Pinzon-Charry A, Schmidt CW, Corradin G, Lopez JA. MHC class I-restricted exogenous presentation of a synthetic 102-mer malaria vaccine polypeptide. Eur J Immunol 2005;35(3):681–9.
[63] Nardin EH, Oliveira GA, Calvo-Calle JM, Castro ZR, Nussenzweig RS, Schmeckpeper B, et al. Synthetic malaria peptide vaccine elicits high levels of antibodies in vaccinees of defined HLA genotypes. J Infect Dis 2000;182(5):1486–96.
[64] Kublin JG, Lowitt MH, Hamilton RG, Oliveira GA, Nardin EH, Nussenzweig RS, et al. Delayed-type hypersensitivity in volunteers immunized with a synthetic multi-antigen peptide vaccine (PfCS-MAP1NYU) against *Plasmodium falciparum* sporozoites. Vaccine 2002;20(13/14):1853–61.
[65] Walther M, Dunachie S, Keating S, Vuola JM, Berthoud T, Schmidt A, et al. Safety, immunogenicity and efficacy of a pre-erythrocytic malaria candidate vaccine, ICC-1132 formulated in Seppic ISA 720. Vaccine 2005;23(7):857–64.
[66] Imoukhuede EB, Berthoud T, Milligan P, Bojang K, Ismaili J, Keating S, et al. Safety and immunogenicity of the malaria candidate vaccines FP9 CS and MVA CS in adult Gambian men. Vaccine 2006.
[67] Matuschewski K. Vaccine development against malaria. Curr Opin Immunol 2006;18(4):449–57.
[68] Walther M, Thompson FM, Dunachie S, Keating S, Todryk S, Berthoud T, et al. Safety, immunogenicity, and efficacy of prime-boost immunization with recombinant poxvirus FP9 and modified vaccinia virus Ankara encoding the full-length *Plasmodium falciparum* circumsporozoite protein. Infect Immun 2006;74(5):2706–16.
[69] Bejon P, Peshu N, Gilbert SC, Lowe BS, Molyneux CS, Forsdyke J, et al. Safety profile of the viral vectors of attenuated fowlpox strain FP9 and modified vaccinia virus Ankara recombinant for either of 2 preerythrocytic malaria antigens, ME-TRAP or the circumsporozoite protein, in children and adults in Kenya. Clin Infect Dis 2006;42(8):1102–10.
[70] Webster DP, Dunachie S, McConkey S, Poulton I, Moore AC, Walther M, et al. Safety of recombinant fowlpox strain FP9 and modified vaccinia virus Ankara vaccines against liver-stage *P. falciparum* malaria in non-immune volunteers. Vaccine 2006;24(15):3026–34.
[71] Le TP, Coonan KM, Hedstrom RC, Charoenvit Y, Sedegah M, Epstein JE, et al. Safety, tolerability and humoral immune responses after intramuscular administration of a malaria DNA vaccine to healthy adult volunteers. Vaccine 2000;18(18):1893–901.
[72] Parker SE, Monteith D, Horton H, Hof R, Hernandez P, Vilalta A, et al. Safety of a GM-CSF adjuvant-plasmid DNA malaria vaccine. Gene Ther 2001;8(13):1011–23.
[73] Wang R, Doolan DL, Le TP, Hedstrom RC, Coonan KM, Charoenvit Y, et al. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. Science 1998;282(5388):476–80.
[74] Wang R, Epstein J, Baraceros FM, Gorak EJ, Charoenvit Y, Carucci DJ, et al. Induction of CD4(+) T cell-dependent CD8(+) type 1 responses in humans by a malaria DNA vaccine. Proc Natl Acad Sci USA 2001;98(19):10817–22.
[75] Moore AC, Hill AV. Progress in DNA-based heterologous prime-boost immunization strategies for malaria. Immunol Rev 2004;199:126–43.
[76] Hill AV, Reece W, Gothard P, Moorthy V, Roberts M, Flanagan K, et al. DNA-based vaccines for malaria: a heterologous prime-boost immunisation strategy. Dev Biol (Basel) 2000;104:171–9.
[77] Moorthy VS, Imoukhuede EB, Milligan P, Bojang K, Keating S, Kaye P, et al. A randomised, double-blind, controlled vaccine efficacy trial of DNA/MVA ME-TRAP against malaria infection in Gambian adults. PLoS Med 2004;1(2):e33.
[78] Dunachie SJ, Walther M, Epstein JE, Keating S, Berthoud T, Andrews L, et al. A DNA prime-modified vaccinia virus Ankara boost vaccine encoding thrombospondin-related adhesion protein but not circumsporozoite protein partially protects healthy malaria-naive adults against *Plasmodium falciparum* sporozoite challenge. Infect Immun 2006;74(10):5933–42.
[79] Webster DP, Dunachie S, Vuola JM, Berthoud T, Keating S, Laidlaw SM, et al. Enhanced T cell-mediated protection against malaria in human challenges by using the recombinant poxviruses FP9 and modified vaccinia virus Ankara. Proc Natl Acad Sci USA 2005;102(13):4836–41.
[80] Bejon P, Mwacharo J, Kai OK, Todryk S, Keating S, Lang T, et al. Immunogenicity of the candidate malaria vaccines FP9 and modified vaccinia virus Ankara encoding the pre-erythrocytic antigen ME-TRAP in 1–6 year old children in a malaria endemic area. Vaccine 2006;24(22):4709–15.
[81] Benmohamed L, Thomas A, Bossus M, Brahimi K, Wubben J, Gras-Masse H, et al. High immunogenicity in chimpanzees of peptides and lipopeptides derived from four new *Plasmodium falciparum* pre-erythrocytic molecules. Vaccine 2000;18(25):2843–55.
[82] Daubersies P, Thomas AW, Millet P, Brahimi K, Langermans JA, Ollomo B, et al. Protection against *Plasmodium falciparum* malaria in chimpanzees by immunization with the conserved pre-erythrocytic liver-stage antigen 3. Nat Med 2000;6(11):1258–63.
[83] Aidoo M, Lalvani A, Gilbert SC, Hu JT, Daubersies P, Hurt N, et al. Cytotoxic T-lymphocyte epitopes for HLA-B53 and other HLA types in the malaria vaccine candidate liver-stage antigen 3. Infect Immun 2000;68(1):227–32.
[84] Perlaza BL, Zapata C, Valencia AZ, Hurtado S, Quintero G, Sauzet JP, et al. Immunogenicity and protective efficacy of *Plasmodium falciparum* liver-stage Ag-3 in *Aotus lemurinus griseimembra* monkeys. Eur J Immunol 2003;33(5):1321–7.
[85] Patarroyo G, Franco L, Amador R, Murillo LA, Rocha CL, Rojas M, et al. Study of the safety and immunogenicity of the synthetic malaria SPf66 vaccine in children aged 1–14 years. Vaccine 1992;10(3):175–8.
[86] Alonso PL, Smith TA, Armstrong-Schellenberg JR, Kitua AY, Masanja H, Hayes R, et al. Duration of protection and age-dependence of the effects of the SPf66 malaria vaccine in African children exposed to intense transmission of *Plasmodium falciparum*. J Infect Dis 1996;174(2):367–72.
[87] Alonso PL, Smith T, Schellenberg JR, Masanja H, Mwankusye S, Urassa H, et al. Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. Lancet 1994;344(8931):1175–81.
[88] Beck HP, Felger I, Huber W, Steiger S, Smith T, Weiss N, et al. Analysis of multiple *Plasmodium falciparum* infections in Tanzanian children during the phase III trial of the malaria vaccine SPf66. J Infect Dis 1997;175(4):921–6.
[89] Galindo CM, Acosta CJ, Schellenberg D, Aponte JJ, Roca A, Oettli A, et al. Humoral immune responses during a malaria vaccine trial in Tanzanian infants. Parasite Immunol 2000;22(9):437–43.
[90] Rosas JE, Pedraz JL, Hernandez RM, Gascon AR, Igartua M, Guzman F, et al. Remarkably high antibody levels and protection against *P. falciparum* malaria in *Aotus* monkeys after a single immunisation of SPf66 encapsulated in PLGA microspheres. Vaccine 2002;20(13/14):1707–10.
[91] Woehlbier U, Epp C, Kauth CW, Lutz R, Long CA, Coulibaly B, et al. Analysis of antibodies directed against merozoite surface protein 1 of the human malaria parasite *Plasmodium falciparum*. Infect Immun 2006;74(2):1313–22.
[92] Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, et al. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. J Infect Dis 2002;185(6):820–7.
[93] Fluck C, Smith T, Beck HP, Irion A, Betuela I, Alpers MP, et al. Strain-specific humoral response to a polymorphic malaria vaccine. Infect Immun 2004;72(11):6300–5.
[94] Chitarra V, Holm I, Bentley GA, Petres S, Longacre S. The crystal structure of C-terminal merozoite surface protein 1 at 1.8 A resolution, a highly protective malaria vaccine candidate. Mol Cell 1999;3(4):457–64.

[95] Chang SP, Case SE, Gosnell WL, Hashimoto A, Kramer KJ, Tam LQ, et al. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect Immun 1996;64(1):253–61.

[96] Daly TM, Long CA. Humoral response to a carboxyl-terminal region of the merozoite surface protein-1 plays a predominant role in controlling blood-stage infection in rodent malaria. J Immunol 1995;155(1):236–43.

[97] Udhayakumar V, Anyona D, Kariuki S, Shi YP, Bloland PB, Branch OH, et al. Identification of T and B cell epitopes recognized by humans in the C-terminal 42-kDa domain of the *Plasmodium falciparum* merozoite surface protein (MSP)-1. J Immunol 1995;154(11):6022–30.

[98] Sachdeva S, Mohmmed A, Dasaradhi PV, Crabb BS, Katyal A, Malhotra P, et al. Immunogenicity and protective efficacy of *Escherichia coli* expressed *Plasmodium falciparum* merozoite surface protein-1(42) using human compatible adjuvants. Vaccine 2006;24(12):2007–16.

[99] Pichyangkul S, Gettayacamin M, Miller RS, Lyon JA, Angov E, Tongtawe P, et al. Pre-clinical evaluation of the malaria vaccine candidate *P. falciparum* MSP1(42) formulated with novel adjuvants or with alum. Vaccine 2004;22(29–30):3831–40.

[100] Stoute JA, Gombe J, Withers MR, Siangla J, McKinney D, Onyango M, et al. Phase 1 randomized double-blind safety and immunogenicity trial of *Plasmodium falciparum* malaria merozoite surface protein FMP1 vaccine, adjuvanted with AS02A, in adults in western Kenya. Vaccine 2005.

[101] Valderrama-Aguirre A, Quintero G, Gomez A, Castellanos A, Perez Y, Mendez F, et al. Antigenicity, immunogenicity, and protective efficacy of *Plasmodium vivax* MSP1 PV200l: a potential malaria vaccine subunit. Am J Trop Med Hyg 2005;73(5 Suppl):16–24.

[102] Anders RF, Crewther PE, Edwards S, Margetts M, Matthew ML, Pollock B, et al. Immunisation with recombinant AMA-1 protects mice against infection with *Plasmodium chabaudi*. Vaccine 1998;16(2/3):240–7.

[103] Kennedy MC, Wang J, Zhang Y, Miles AP, Chitsaz F, Saul A, et al. In vitro studies with recombinant *Plasmodium falciparum* apical membrane antigen 1 (AMA1): production and activity of an AMA1 vaccine and generation of a multiallelic response. Infect Immun 2002;70(12):6948–60.

[104] Kocken CH, Dubbeld MA, Van Der Wel A, Pronk JT, Waters AP, Langermans JA, et al. High-level expression of *Plasmodium vivax* apical membrane antigen 1 (AMA-1) in *Pichia pastoris*: strong immunogenicity in *Macaca mulatta* immunized with *P. vivax* AMA-1 and adjuvant SBAS2. Infect Immun 1999;67(1):43–9.

[105] Kocken CH, Narum DL, Massougbodji A, Ayivi B, Dubbeld MA, van der Wel A, et al. Molecular characterisation of *Plasmodium reichenowi* apical membrane antigen-1 (AMA-1), comparison with *P. falciparum* AMA-1, and antibody-mediated inhibition of red cell invasion. Mol Biochem Parasitol 2000;109(2):147–56.

[106] Cortes A, Mellombo M, Mueller I, Benet A, Reeder JC, Anders RF. Geographical structure of diversity and differences between symptomatic and asymptomatic infections for *Plasmodium falciparum* vaccine candidate AMA1. Infect Immun 2003;71(3):1416–26.

[107] Polley SD, Conway DJ. Strong diversifying selection on domains of the *Plasmodium falciparum* apical membrane antigen 1 gene. Genetics 2001;158(4):1505–12.

[108] Mitchell GH, Thomas AW, Margos G, Dluzewski AR, Bannister LH. Apical membrane antigen 1, a major malaria vaccine candidate, mediates the close attachment of invasive merozoites to host red blood cells. Infect Immun 2004;72(1):154–8.

[109] Malkin EM, Diemert DJ, McArthur JH, Perreault JR, Miles AP, Giersing BK, et al. Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for *Plasmodium falciparum* malaria. Infect Immun 2005;73(6):3677–85.

[110] Saul A, Lawrence G, Allworth A, Elliott S, Anderson K, Rzepczyk C, et al. A human phase 1 vaccine clinical trial of the *Plasmodium falciparum* malaria vaccine candidate apical membrane antigen 1 in Montanide ISA720 adjuvant. Vaccine 2005;23(23):3076–83.

[111] Pan W, Huang D, Zhang Q, Qu L, Zhang D, Zhang X, et al. Fusion of two malaria vaccine candidate antigens enhances product yield, immunogenicity, and antibody-mediated inhibition of parasite growth in vitro. J Immunol 2004;172(10):6167–74.

[112] Zhang D, Pan W. Evaluation of three *Pichia pastoris*-expressed *Plasmodium falciparum* merozoite proteins as a combination vaccine against infection with blood-stage parasites. Infect Immun 2005;73(10):6530–6.

[113] Bouharoun-Tayoun H, Attanath P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P. Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. J Exp Med 1990;172(6):1633–41.

[114] Bouharoun-Tayoun H, Druilhe P. *Plasmodium falciparum* malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. Infect Immun 1992;60(4):1473–81.

[115] Bouharoun-Tayoun H, Oeuvray C, Lunel F, Druilhe P. Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. J Exp Med 1995;182(2):409–18.

[116] Oeuvray C, Bouharoun-Tayoun H, Gras-Masse H, Bottius E, Kaidoh T, Aikawa M, et al. Merozoite surface protein-3: a malaria protein inducing antibodies that promote *Plasmodium falciparum* killing by cooperation with blood monocytes. Blood 1994;84(5):1594–602.

[117] Audran R, Cachat M, Lurati F, Soe S, Leroy O, Corradin G, et al. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. Infect Immun 2005;73(12):8017–26.

[118] Druilhe P, Spertini F, Soesoe D, Corradin G, Mejia P, Singh S, et al. A malaria vaccine that elicits in humans antibodies able to kill *Plasmodium falciparum*. PLoS Med 2005;2(11):e344.

[119] Dodoo D, Theisen M, Kurtzhals JA, Akanmori BD, Koram KA, Jepsen S, et al. Naturally acquired antibodies to the glutamate-rich protein are associated with protection against *Plasmodium falciparum* malaria. J Infect Dis 2000;181(3):1202–5.

[120] Theisen M, Soe S, Oeuvray C, Thomas AW, Vuust J, Danielsen S, et al. The glutamate-rich protein (GLURP) of *Plasmodium falciparum* is a target for antibody-dependent monocyte-mediated inhibition of parasite growth in vitro. Infect Immun 1998;66(1):11–7.

[121] Soe S, Theisen M, Roussilhon C, Aye KS, Druilhe P. Association between protection against clinical malaria and antibodies to merozoite surface antigens in an area of hyperendemicity in Myanmar: complementarity between responses to merozoite surface protein 3 and the 220-kilodalton glutamate-rich protein. Infect Immun 2004;72(1):247–52.

[122] Theisen M, Dodoo D, Toure-Balde A, Soe S, Corradin G, Koram KK, et al. Selection of glutamate-rich protein long synthetic peptides for vaccine development: antigenicity and relationship with clinical protection and immunogenicity. Infect Immun 2001;69(9):5223–9.

[123] Hermsen CC, Verhage DF, Telgt DS, Teelen K, Bousema JT, Roestenberg M, et al. Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of *Plasmodium falciparum* in a phase 1 malaria vaccine trial. Vaccine 2006.

[124] Li J, Mitamura T, Fox BA, Bzik DJ, Horii T. Differential localization of processed fragments of *Plasmodium falciparum* serine repeat antigen and further processing of its N-terminal 47 kDa fragment. Parasitol Int 2002;51(4):343–52.

[125] Okech BA, Nalunkuma A, Okello D, Pang XL, Suzue K, Li J, et al. Natural human immunoglobulin G subclass responses to *Plasmodium falciparum* serine repeat antigen in Uganda. Am J Trop Med Hyg 2001;65(6):912–7.

[126] Okech B, Mujuzi G, Ogwal A, Shirai H, Horii T, Egwang TG. High titers of IgG antibodies against *Plasmodium falciparum* serine repeat antigen 5 (SERA5) are associated with protection against

severe malaria in Ugandan children. Am J Trop Med Hyg 2006;74(2): 191–7.
[127] Pang XL, Mitamura T, Horii T. Antibodies reactive with the N-terminal domain of *Plasmodium falciparum* serine repeat antigen inhibit cell proliferation by agglutinating merozoites and schizonts. Infect Immun 1999;67(4):1821–7.
[128] Soe S, Singh S, Camus D, Horii T, Druilhe P. *Plasmodium falciparum* serine repeat protein, a new target of monocyte-dependent antibody-mediated parasite killing. Infect Immun 2002;70(12):7182–4.
[129] Inselburg J, Bathurst IC, Kansopon J, Barchfeld GL, Barr PJ, Rossan RN. Protective immunity induced in *Aotus* monkeys by a recombinant SERA protein of *Plasmodium falciparum*: adjuvant effects on induction of protective immunity. Infect Immun 1993;61(5):2041–7.
[130] Inselburg J, Bathurst IC, Kansopon J, Barr PJ, Rossan R. Protective immunity induced in *Aotus* monkeys by a recombinant SERA protein of *Plasmodium falciparum*: further studies using SERA 1 and MF75.2 adjuvant. Infect Immun 1993;61(5):2048–52.
[131] Marshall VM, Silva A, Foley M, Cranmer S, Wang L, McColl DJ, et al. A second merozoite surface protein (MSP-4) of *Plasmodium falciparum* that contains an epidermal growth factor-like domain. Infect Immun 1997;65(11):4460–7.
[132] Morgan WD, Birdsall B, Frenkiel TA, Gradwell MG, Burghaus PA, Syed SE, et al. Solution structure of an EGF module pair from the *Plasmodium falciparum* merozoite surface protein 1. J Mol Biol 1999;289(1):113–22.
[133] Tolia NH, Enemark EJ, Sim BK, Joshua-Tor L. Structural basis for the EBA-175 erythrocyte invasion pathway of the malaria parasite *Plasmodium falciparum*. Cell 2005;122(2):183–93.
[134] Jones TR, Narum DL, Gozalo AS, Aguiar J, Fuhrmann SR, Liang H, et al. Protection of *Aotus* monkeys by *Plasmodium falciparum* EBA-175 region II DNA prime-protein boost immunization regimen. J Infect Dis 2001;183(2):303–12.
[135] Arevalo-Herrera M, Castellanos A, Yazdani SS, Shakri AR, Chitnis CE, Dominik R, et al. Immunogenicity and protective efficacy of recombinant vaccine based on the receptor-binding domain of the *Plasmodium vivax* Duffy binding protein in *Aotus* monkeys. Am J Trop Med Hyg 2005;73(5 Suppl):25–31.
[136] Chen Q, Pettersson F, Vogt AM, Schmidt B, Ahuja S, Liljestrom P, et al. Immunization with PfEMP1-DBL1alpha generates antibodies that disrupt rosettes and protect against the sequestration of *Plasmodium falciparum*-infected erythrocytes. Vaccine 2004;22(21–22):2701–12.
[137] Smith JD, Deitsch KW. Pregnancy-associated malaria and the prospects for syndrome-specific antimalaria vaccines. J Exp Med 2004;200(9):1093–7.
[138] Ahuja S, Pettersson F, Moll K, Jonsson C, Wahlgren M, Chen Q. Induction of cross-reactive immune responses to NTS-DBL-1alpha/x of PfEMP1 and in vivo protection on challenge with *Plasmodium falciparum*. Vaccine 2006.
[139] Carter R, Mendis KN, Miller LH, Molineaux L, Saul A. Malaria transmission-blocking vaccines—how can their development be supported? Nat Med 2000;6(3):241–4.
[140] Hisaeda H, Stowers AW, Tsuboi T, Collins WE, Sattabongkot JS, Suwanabun N, et al. Antibodies to malaria vaccine candidates Pvs25 and Pvs28 completely block the ability of *Plasmodium vivax* to infect mosquitoes. Infect Immun 2000;68(12):6618–23.
[141] Arakawa T, Komesu A, Otsuki H, Sattabongkot J, Udomsangpetch R, Matsumoto Y, et al. Nasal immunization with a malaria transmission-blocking vaccine candidate, Pfs25, induces complete protective immunity in mice against field isolates of *Plasmodium falciparum*. Infect Immun 2005;73(11):7375–80.
[142] Gozar MM, Muratova O, Keister DB, Kensil CR, Price VL, Kaslow DC. *Plasmodium falciparum*: immunogenicity of alum-adsorbed clinical-grade TBV25-28, a yeast-secreted malaria transmission-blocking vaccine candidate. Exp Parasitol 2001;97(2):61–9.
[143] Milek RL, Stunnenberg HG, Konings RN. Assembly and expression of a synthetic gene encoding the antigen Pfs48/45 of the human malaria parasite *Plasmodium falciparum* in yeast. Vaccine 2000;18(14):1402–11.
[144] Malkin EM, Durbin AP, Diemert DJ, Sattabongkot J, Wu Y, Miura K, et al. Phase 1 vaccine trial of Pvs25H: a transmission blocking vaccine for *Plasmodium vivax* malaria. Vaccine 2005;23(24):3131–8.
[145] Collins WE, Sullivan JS, Williams A, Nace D, Williams T, Galland GG, et al. *Aotus nancymaae* as a potential model for the testing of anti-sporozoite and liver stage vaccines against *Plasmodium falciparum*. Am J Trop Med Hyg 2006;74(3):422–4.
[146] Bustamante PJ, Woodruff DC, Oh J, Keister DB, Muratova O, Williamson KC. Differential ability of specific regions of *Plasmodium falciparum* sexual-stage antigen, Pfs230, to induce malaria transmission-blocking immunity. Parasite Immunol 2000; 22(8):373–80.
[147] Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk C, Bryden M, Cloonan N, et al. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. Lancet 2002;360(9333):610–7.
[148] Schofield L, Vivas L, Hackett F, Gerold P, Schwarz RT, Tachado S. Neutralizing monoclonal antibodies to glycosylphosphatidylinositol, the dominant TNF-alpha-inducing toxin of *Plasmodium falciparum*: prospects for the immunotherapy of severe malaria. Ann Trop Med Parasitol 1993;87(6):617–26.
[149] Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. Nature 2002;418(6899):785–9.
[150] Schofield L, Mueller I. Clinical immunity to malaria. Curr Mol Med 2006;6(2):205–21.
[151] Perraut R, Marrama L, Diouf B, Sokhna C, Tall A, Nabeth P, et al. Antibodies to the conserved C-terminal domain of the *Plasmodium falciparum* merozoite surface protein 1 and to the merozoite extract and their relationship with in vitro inhibitory antibodies and protection against clinical malaria in a Senegalese village. J Infect Dis 2005;191(2):264–71.
[152] Pinder M, Sutherland CJ, Sisay-Joof F, Ismaili J, McCall MB, Ord R, et al. Immunoglobulin G antibodies to merozoite surface antigens are associated with recovery from chloroquine-resistant *Plasmodium falciparum* in Gambian children. Infect Immun 2006;74(5):2887–93.
[153] Persson KE, Lee CT, Marsh K, Beeson JG. Development and optimization of high-throughput methods to measure *Plasmodium falciparum*-specific growth inhibitory antibodies. J Clin Microbiol 2006;44(5):1665–73.
[154] Good MF, Xu H, Wykes M, Engwerda CR. Development and regulation of cell-mediated immune responses to the blood stages of malaria: implications for vaccine research. Annu Rev Immunol 2005;23:69–99.
[155] Krzych U, Schwenk J. The dissection of CD8 T cells during liver-stage infection. Curr Top Microbiol Immunol 2005;297:1–24.
[156] Stephens R, Langhorne J. Priming of CD4+ T cells and development of CD4+ T cell memory; lessons for malaria. Parasite Immunol 2006;28(1/2):25–30.
[157] Barrero CA, Delgado G, Sierra AY, Silva Y, Parra-Lopez C, Patarroyo MA. Gamma interferon levels and antibody production induced by two PvMSP-1 recombinant polypeptides are associated with protective immunity against *P. vivax* in *Aotus* monkeys. Vaccine 2005;23(31):4048–53.
[158] Kidgell C, Volkman SK, Daily J, Borevitz JO, Plouffe D, Zhou Y, et al. A systematic map of genetic variation in *Plasmodium falciparum*. PLoS Pathog 2006;2(6):e57.
[159] Druilhe P, Hagan P, Rook GA. The importance of models of infection in the study of disease resistance. Trends Microbiol 2002;10(10 Suppl):S38–46.
[160] Badell E, Oeuvray C, Moreno A, Soe S, van Rooijen N, Bouzidi A, et al. Human malaria in immunocompromised mice: an in vivo model to study defense mechanisms against *Plasmodium falciparum*. J Exp Med 2000;192(11):1653–60.

[161] Stowers AW, Miller LH. Are trials in New World monkeys on the critical path for blood-stage malaria vaccine development? Trends Parasitol 2001;17(9):415–9.
[162] Heppner DG, Cummings JF, Ockenhouse C, Kester KE, Lyon JA, Gordon DM. New World monkey efficacy trials for malaria vaccine development: critical path or detour? Trends Parasitol 2001;17(9):419–25.
[163] Rogers WO, Atuguba F, Oduro AR, Hodgson A, Koram KA. Clinical case definitions and malaria vaccine efficacy. J Infect Dis 2006;193(3):467–73.
[164] Struik SS, Riley EM. Does malaria suffer from lack of memory? Immunol Rev 2004;201:268–90.
[165] Bejon P, Kai OK, Mwacharo J, Keating S, Lang T, Gilbert SC, et al. Alternating vector immunizations encoding pre-erythrocytic malaria antigens enhance memory responses in a malaria endemic area. Eur J Immunol 2006.
[166] Ballou WR. Malaria vaccines in development. Expert Opin Emerg Drugs 2005;10(3):489–503.