SCIENCE AND SOCIETY

Experimental human challenge infections can accelerate clinical malaria vaccine development

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Abstract | Malaria is one of the most frequently occurring infectious diseases worldwide, with almost 1 million deaths and an estimated 243 million clinical cases annually. Several candidate malaria vaccines have reached Phase IIb clinical trials, but results have often been disappointing. As an alternative to these Phase IIb field trials, the efficacy of candidate malaria vaccines can first be assessed through the deliberate exposure of participants to the bites of infectious mosquitoes (sporozoite challenge) or to an inoculum of blood-stage parasites (blood-stage challenge). With an increasing number of malaria vaccine candidates being developed, should human malaria challenge models be more widely used to reduce cost and time investments? This article reviews previous experience with both the sporozoite and blood-stage human malaria challenge models and provides future perspectives for these models in malaria vaccine development.

Half of the world's population (more than 3 billion people) live in malaria-endemic areas, and an estimated 243 million cases of malaria led to nearly 863,000 deaths in 2008 according to the World Health Organization (WHO) World Malaria Report 2009. There are five species of human malaria parasite: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi.* Recent evidence indicates that *P. ovale* is composed of two subspecies[1]. Most infections are caused by *P. falciparum*, which is particularly dominant in sub-Saharan Africa. *P. vivax* is the most widely spread cause of malaria, being responsible for an estimated 80 million to 300 million cases every year, and thus it accounts for a major burden of disease[2]. *Plasmodium* parasites are highly prevalent in Asia and South America, where individuals can be infected with more than one malaria parasite species simultaneously. Infective foci of *P. knowlesi* have been identified in the past decade in Malaysia, where *P. knowlesi* is transmitted from simian hosts to humans.

Plasmodia are transmitted by the bites of infected *Anopheles* mosquitoes. Control strategies are based on the early diagnosis and treatment of uncomplicated infections with artemisinin-based combination therapies, thereby also decreasing transmission[3], combined with preventive measures aimed at vector (mosquito) control.

Artemisinin-based combination therapies — such as artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine or artesunate plus sulphadoxine–pyrimethamine — have been the WHO-recommended treatment for uncomplicated *P. falciparum* malaria since the development of widespread resistance to chloroquine and sulphadoxine–pyrimethamine. Unfortunately, in *P. falciparum*, resistance has been observed to all current antimalarial drugs (amodiaquine, chloroquine, mefloquine, quinine and sulphadoxine-pyrimethamine) and, more recently, also to artemisinin derivatives. For uncomplicated *P. vivax* infection, treatment with chloroquine is recommended in those areas without chloroquine resistance. Artemisinin-based combination therapies can be used as an alternative treatment for chloroquine-resistant *P. vivax*. In these cases, artemether plus sulphadoxine–pyrimethamine is not recommended because *P. vivax* can acquire resistance to sulphadoxine–pyrimethamine. To fully eradicate *P. vivax* infection, primaquine must be administered to prevent relapses. *P. ovale* and *P. malariae* infections are treated similarly to *P. vivax* infections, although there is no need for primaquine treatment in patients who are infected with *P. malariae*, as this species does not form dormant or latent hypnozoites in hepatocytes (see the WHO Guidelines for the Treatment of Malaria).

Vector control is the primary intervention for decreasing malaria transmission at the community level. When universal vector control coverage is achieved by impregnating bed nets and spraying indoor surfaces of houses with insecticides, malaria transmission can be decreased to close to zero. Unfortunately, the increasing resistance of mosquitoes to insecticides such as dichlorodiphenyltrichloroethane (DDT) and pyrethroids, particularly in Africa, poses challenges to current prevention policies (see the WHO World Malaria Report 2009).

In this context, the development of an effective vaccine could make a significant contribution to the fight against malaria. Ambitious goals in this regard have been set by the Malaria Vaccine Technology Roadmap Process, which aims to achieve a licensed first-generation *P. falciparum* malaria vaccine with more than 50% protective efficacy against severe disease and death, lasting for at least 1 year, by the year 2015. Malaria vaccine development has been fuelled by new technology enabling the sequencing of the *P. falciparum*, *P. vivax* and *Anopheles gambiae* genomes and the development of experimentally relevant animal models, combined with significant increases in financial resources from funders such as the Bill & Melinda Gates Foundation, the European Union, the US National Institutes of Allergy and Infectious Diseases and the US Agency for International Development. Currently, there are 38 *P. falciparum* and two *P. vivax* candidate malaria vaccines or

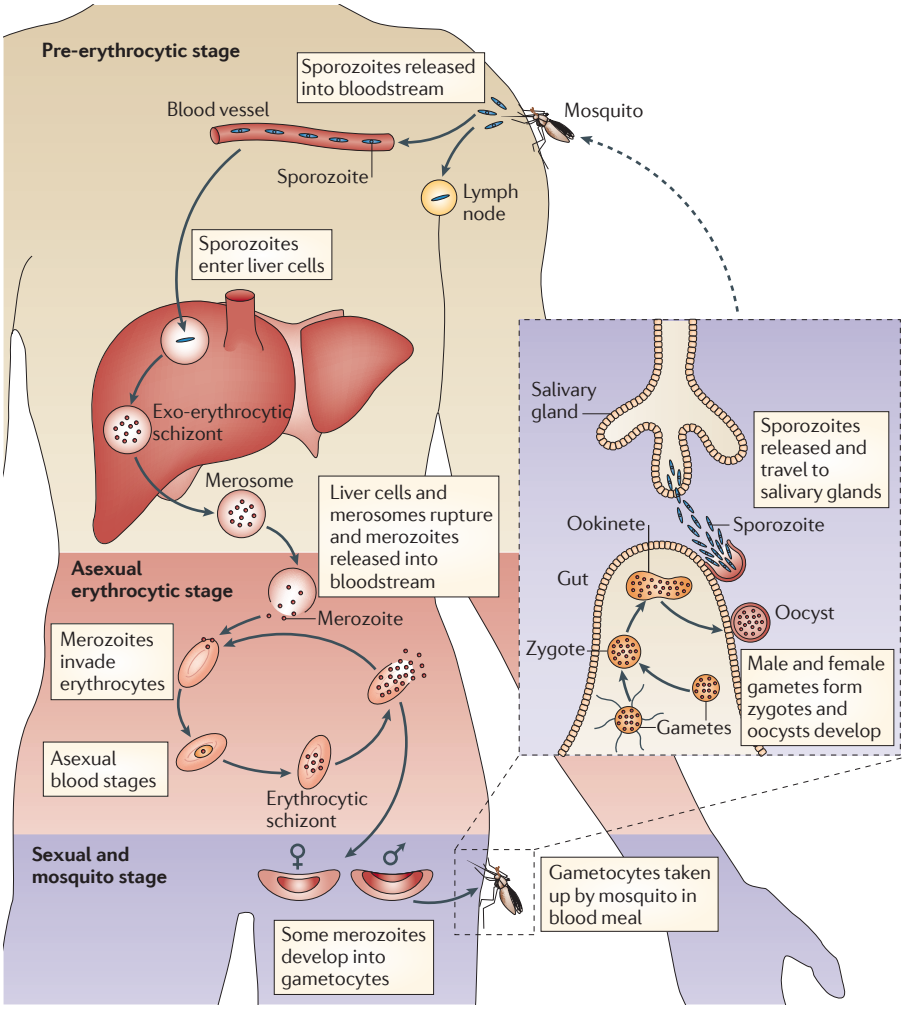

Figure 1 | ***Plasmodium falciparum* life cycle showing the three developmental stages of the parasite that are targeted by malaria vaccine candidates.** Parasites (sporozoite stage) are injected into the skin by a female *Anopheles* spp. mosquito. From the skin, a proportion of sporozoites will travel through the bloodstream to the liver. Some sporozoites will be trapped in regional lymph nodes. In hepatocytes, parasites develop and multiply for 6–7 days before merosomes are budded from the cell and enter the hepatic sinusoids. Merosomes eventually rupture, releasing merozoite-stage parasites that invade erythrocytes for further reproduction. Clinical malaria is caused by the 48-hour cyclical proliferation of asexual-stage parasites in erythrocytes. Malaria mortality is primarily due to organ dysfunction, in particular of the brain, following sequestration of infected erythrocytes in the microvasculature. The development of sexual forms of the parasite (gametocytes) in the blood allows the transmission of parasites to mosquitoes with subsequent bites. Once ingested, the parasite gametocytes taken up in the blood further differentiate into male or female gametes and then fuse in the mosquito gut. This produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host. Image is modified, with permission, from REF. 59 © (2004) Macmillan Publishers Ltd. All rights reserved.

vaccine components in advanced preclinical or clinical development as listed by the WHO Malaria Vaccine Rainbow Tables.

Malaria vaccine candidates are categorized according to the *Plasmodium* life cycle stage at which the targeted antigen is expressed (FIG. 1). Pre-erythrocytic stage vaccines aim to prevent the passage of parasites through the human liver and subsequent blood-stage infection, leading to the induction of sterile immunity. Asexual erythrocytic stage vaccines focus on delaying or decreasing parasite multiplication in red blood cells, thereby decreasing morbidity and preventing mortality. Transmission-blocking vaccines consist of sexual- or mosquito-stage antigens that prevent infection passing from humans to mosquitoes, thereby decreasing the spread of malaria in the population.

Generally, less than 10% of preclinical vaccine projects progress to Phase III clinical evaluation[4]. Clinical development is time consuming and costly. Candidate malaria vaccines are selected downstream of clinical testing on the basis of safety, immunogenicity and, eventually, efficacy profiles. Whereas the first two criteria can generally be assessed in a small initial Phase I trial, field vaccine efficacy can only be assessed in Phase II trials, which require larger study groups in malaria-endemic areas. The sample size of Phase II trials depends on the prevalence of malaria infections in that area and the expected efficacy of the candidate vaccine. Ideally, immunological assays carried out in initial clinical trials should predict potential efficacy in subsequent trials, by analogy with, for example, hepatitis B virus surface antigen (HBsAg)-specific antibody titres for the hepatitis B vaccine. However, with the current lack of unequivocal correlates of immune protection against malaria in either animal models or *in vitro* assays on human samples, there is a continuous need to test field efficacy in time-consuming and costly Phase II trials in malaria-endemic areas. There are only a limited number of competent field trial sites for malaria that can adhere to the good clinical practice guidelines established by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), which describe the monitoring, reporting and archiving responsibilities of all participants in the conduct of clinical trials (see The Malaria Product Pipeline: Planning for the Future). Finally, there seems to be a downward trend in malaria incidence in several endemic areas, most probably as a result of improved policy and adherence to malaria control measures, and this will further increase the size and costs of Phase II field trials[5].

Human experimental sporozoite infections carried out under strictly controlled laboratory and clinical conditions, in which volunteers are exposed to the bites of laboratory-reared *Plasmodium*-infected mosquitoes, are an intermediate step between Phase I and Phase II trials, providing information on preliminary vaccine efficacy. It is common practice to test the efficacy of pre-erythrocytic stage malaria vaccine candidates by experimental sporozoite infection before going into the field. In such cases, a distinction is thus made between Phase IIa trials using experimental infection of volunteers in non-endemic areas and Phase IIb field trials in endemic areas.

Poor preliminary efficacy in Phase IIa trials may subsequently halt progression of the vaccine candidate to Phase IIb trials. By contrast, the efficacy of asexual erythrocytic stage vaccine candidates is generally assessed in field trials only, although blood-stage challenge models have been used.

Here, we present the history of artificial malaria challenge infections, the clinical aspects of *P. falciparum* challenges using sporozoites or blood-stage parasites and experience with *P. vivax* challenges. We discuss the strengths and limitations of both models and provide future perspectives.

Historical perspective

Deliberate infection of humans with malaria was first carried out in 1917 by Wagner von Jauregg[6] as a therapy primarily for patients with neurosyphilis, and he was awarded the Nobel Prize in Medicine for his work in 1927. Thousands of patients have undergone this treatment, which was administered by the bites of infectious mosquitoes or by intravenous or subcutaneous inoculation of dissected *Plasmodium* sporozoites suspended in media. Historically, *P. vivax* was used most frequently, but infections were also carried out with *P. falciparum*, *P. malariae* and *P. ovale*. The objective was to induce a febrile illness that was thought to be beneficial for the prognosis of the disease. This practice stopped with the advent of antibiotics for the treatment of the *Treponema pallidum* infection that causes syphilis.

In the 1960s, experimental human malaria infections were used to assess the effects of anti-malaria treatments on healthy non-immune male inmates in the United States[7]. Following the discovery of protocols for the continuous culture of *P. falciparum* in 1976 (REF. 8) and protocols for the generation of mature *P. falciparum* gametocytes *in vitro* in 1981 (REF. 9), laboratory-reared infectious mosquitoes could be produced[10] and human malaria sporozoite infections could be carried out more routinely.

The first well-documented study of human experimental malaria infection with these laboratory-reared infectious mosquitoes was carried out in 1986 at the US Walter Reed Army Institute of Research (WRAIR), the US Naval Medical Research Institute (NMRI) and the US National Institutes of Health (NIH). Six volunteers were infected with *P. falciparum* sporozoites by the bites of infectious *Anopheles freeborni* and *Anopheles stephensi* mosquitoes[11]. The following year, the efficacies of the first recombinant protein and synthetic peptide *P. falciparum* vaccines were tested in experimentally infected volunteers[12,13]. Since the late 1980s, the number of institutions carrying out experimental infections with *P. falciparum* sporozoites has been growing. In 2007, data were published from a total of 532 volunteers[14]. So far, unpublished analysis shows that a total of 1,343 volunteers have been experimentally infected with *P. falciparum* between 1985 and 2009 (REF. 15); 526 of these volunteers took part in vaccine trials (TABLE 1), and of these, 118 volunteers were protected against infection by the vaccine candidate. The most successful immunogens were RTS,S (a pre-erythrocytic stage vaccine consisting of the *P. falciparum* circumsporozoite protein combined with HBsAg; developed by GlaxoSmithKline in partnership with PATH Malaria Vaccine Initiative) and irradiated whole parasites delivered by mosquito bite.

Comparison with field trials

Differences between natural and experimental infections mean that it is important to validate the results of Phase IIa challenge trials with data from Phase IIb field trials in malaria-endemic areas. Only three candidate vaccines have been assessed by both types of trial, allowing a comparison of the protective outcomes. The best studied candidate vaccine, RTS,S, which is currently in Phase III trials, has repeatedly demonstrated a protective efficacy of ~30–50% in Phase IIa trials with sterile protection as the study end point[16–18]. Interestingly, a similar ~30–50% efficacy of RTS,S was found in Phase IIb trials in the field using time to first clinical malaria episode as the primary study end point[19]. A similar association between the results of Phase IIa and Phase IIb trials was found when testing long-term protection in adults[19,20]. A second pre-erythrocytic stage candidate vaccine, ME-TRAP (a multi-epitope string fused to thrombospondin-related adhesion protein), delivered by a DNA prime and attenuated poxvirus boost, induced complete protection in only a few volunteers (three out of 74) in Phase IIa trials, and no protection was found in adult Phase IIb field studies in the Gambia[21,22]. Artificial blood-stage challenge has been used in a Phase II trial after immunization with Combination B (a combination of merozoite surface protein 1 (MSP1), MSP2 and ring-infected erythrocyte surface antigen (RESA)) in 17 volunteers, which resulted in no decrease in parasite growth rates[23,24]; this is in line with results from a Phase IIb trial of Combination B conducted in Papua New Guinea[25]. These limited data indicate that results obtained in experimental challenges are generally in line with results in the field, but more comparisons are required before definite conclusions can be drawn.

Experimental sporozoite infection

The delivery of sporozoite-stage malaria parasites by mosquito bites has traditionally been used as a model to test pre-erythrocytic stage vaccines. Since the late 1980s, standardization of experimental sporozoite infections has improved and efforts to further increase harmonization are ongoing. Such infections are currently routinely carried out at: the US Military Malaria Vaccine Program; the University of Maryland, USA; Radboud University Nijmegen Medical Centre (RUNMC), the Netherlands; the University of Oxford, UK; and, more recently, Seattle Biomed, USA[15]. All centres use *A. stephensi* mosquitoes that feed on either the chloroquine-sensitive NF54 strain of *P. falciparum* or the 3D7 clone of NF54. In addition, limited numbers of volunteers have been challenged with the 7G8 strain of *P. falciparum*[14].

Approximately 14–21 days after feeding, mosquitoes are checked for infection by microscopic examination of salivary glands. Healthy human volunteers are subsequently exposed to the bites of five infectious mosquitoes for either 5 or 10 minutes (FIG. 2). Almost 100% of volunteers bitten by five infected mosquitoes develop patent parasitaemia, with very rare exceptions[26,27]. Infection rates drop significantly when volunteers are exposed to fewer than five infected mosquitoes[27,28].

After infection, subjects are monitored closely on an outpatient basis. Signs and symptoms such as headache, myalgia and fever are noted, and a physical examination and thick blood smears (a drop of blood on a glass slide) are carried out once, twice or thrice daily. The period before blood-stage parasites can be detected in thick smears by microscopy (the prepatent period) ranges from 7 to 20 days, with a mean of approximately 11 days[7,14,26]. As soon as parasites are microscopically detected, volunteers are treated with a curative regimen of chloroquine, artemether plus lumefantrine, or atovaquone plus proguanil. Nearly all volunteers will develop symptoms of clinical malaria infection; approximately one-fifth of volunteers temporarily develop symptoms graded as severe (symptoms that prevent daily activities), but severe or life-threatening malaria has never occurred[26]. The most common symptoms are fatigue and headache,

Table 1 | **Summary of published Phase IIa sporozoite challenge trials with *Plasmodium falciparum* candidate vaccines**

Vaccine	*Plasmodium* protein	Category	Number of volunteers challenged	Number of volunteers protected	Year of publication	Institution or company	Refs
Irradiated sporozoites	Whole parasite	Pre-erythrocytic	37	20 (54.05%)	1970s–1993	NMRI* and WRAIR*; University of Maryland, USA; University of Sydney, Australia	60–65
Several products	CSP	Pre-erythrocytic	317	94 (29.65%)	1987–2009	University of Maryland; WRAIR*; University of Oxford, UK; Johns Hopkins University School of Hygiene and Public Health, Maryland, USA; NMRI*; University of Lausanne, Switzerland	12,13,16, 18,20,22, 40,45,62, 66–73
Several products	TRAP	Pre-erythrocytic	74	3 (4.05%)	2003–2006	University of Oxford	22,74,75
AMA1 with AS02A or AS01B	AMA1	Asexual erythrocytic	16	0 (0%)	2009	US Military Malaria Vaccine Program	35
LSA1-NRC with AS01 or AS02	LSA1	Pre-erythrocytic	22	0 (0%)	2010	WRAIR*	76
NYVAC-Pf7	CSP, SSP2, LSA1, MSP1, SERA, AMA1, Pfs25	All stages	35	1 (2.86%)	1998	WRAIR*	77
FFM ME-TRAP plus PEV3A	CSP, TRAP and AMA1	Pre-erythrocytic and asexual erythrocytic	24	0 (0%)	2008	University of Oxford	78
SPf(66)30 or SPf(105)20 with Alum	MSP	Asexual erythrocytic	9	0 (0%)‡	1988	Universidad Nacional de Colombia	79
MuStDO 5	CSP, EXP1, SSP2, LSA1, LSA3	Pre-erythrocytic	31	0 (0%)	2005	Naval Medical Research Center*	80
FMP1 with AS02A	MSP1	Asexual erythrocytic	Unknown	0 (0%)	2005	WRAIR*	81

Alum, aluminium hydroxide adjuvant (Alhydrogel; Brenntag biosector); AMA1, apical membrane antigen 1; AS01, GlaxoSmithKline adjuvant system 01; CSP, circumsporozoite protein; EXP1, exported protein 1; FFM ME-TRAP, multi-epitope string fused to TRAP that is delivered in fowlpox virus strain FP9 and modified vaccinia virus Ankara vectors in prime–boost combinations; FMP1, carboxy-terminal region of MSP1; LSA, liver-stage antigen; LSA1-NRC, full-length carboxy- and amino-terminal flanking domains and two of the 17 amino acid repeats from the central repeat region of LSA1; MSP, merozoite surface protein; MuStDO 5, multi-stage DNA vaccine operation 5 antigens; NMRI, Naval Medical Research Institute, USA; NYVAC-Pf7, a highly attenuated vaccinia virus with seven *P. falciparum* genes inserted into its genome; PEV3A, virosomal formulation of CSP and AMA1; Pfs25, 25kDa ookinete surface antigen; SERA, serine-repeat antigen protein; SSP2, sporozoite surface protein 2; SPf, synthetic *P. falciparum* peptides of MSP; TRAP, thrombospondin-related adhesion protein; WRAIR, Walter Reed Army Institute of Research, USA. *Currently the US Military Malaria Vaccine Program. ‡Three of five volunteers immunized with SPf(66)30 eventually cleared parasitaemia after they experienced asexual parasitaemia that was detectable by microscopy.

and severe symptoms can include headache, fatigue, malaise, chills, myalgia, rigors, nausea and vomiting. Clinical symptoms generally coincide with the detection of blood-stage parasites at densities of 10–20 parasites per µl of blood by microscopy of thick blood smears[26]. This corresponds to a parasitaemia of approximately 0.0004%. Severe malaria is generally diagnosed when parasitaemia is 3 to 4 logs greater than the peak parasitaemia in challenge trials. After the start of malaria treatment, symptoms can temporarily increase in severity but subside quickly with an average duration of approximately 2–3 days.

Routine laboratory checks generally show a moderate decrease in leukocyte and platelet numbers during infection, with no change in haemoglobin concentration[27]. Bleeding or thrombogenic complications have never been described[26,27]. Abnormalities of liver enzymes have been observed, but these abnormalities did not result in clinical manifestations and they resolved after a few days[26,27].

Immediate treatment of volunteers at the earliest phase of microscopically detectable blood-stage infection ensures that the potential risks of complications associated with severe malaria are minimized to the greatest extent possible. Indeed, human malaria challenge infections have been shown to be safe in the 1,343 volunteers challenged so far[14,26,27]. Recently, safety concerns were raised because of a cardiac event in a young volunteer shortly after treatment for diagnosed malaria, although a definite relationship between the cardiac event and the experimental malaria infection was not established[29]. Nevertheless, it has been generally agreed that volunteers with an increased risk of cardiac disease should be excluded from such trials.

In addition to the clinical manifestations, participation in an experimental sporozoite infection trial has a major impact on the daily life of volunteers, particularly because of the intense follow-up with blood sampling several times daily. Volunteers' perception of their participation in such a trial depends mainly on whether they have realistic expectations of trial procedures and the severity of symptoms, indicating the importance of providing accurate and sufficient information to volunteers before the onset of the trial.

Measurement of parasitaemia

A real-time quantitative PCR assay based on 18S ribosomal RNA gene transcripts has been developed for tracking the kinetics of developing parasitaemia before a positive diagnosis of infection can be made from a thick blood smear using microscopy[30]. This

assay is becoming increasingly important for assessing very low parasite densities and incremental changes in density in small-scale Phase IIa trials[31]. The detection of parasites below microscopy thresholds by PCR allows for a detailed analysis of cyclical parasite growth in the blood, albeit for a short time window of 2–3 days between liver-stage infection and microscopic detection[30]. Several statistical models have been developed to further analyse profiles of parasitaemia and partial protection in vaccine trials[32–34]. For example, these models allow a separate estimation of liver-stage and blood-stage parasite development. From the first wave of parasite DNA that is detected in the blood, an estimation can be made of the number of merozoites released from the liver, making it possible to approximate the extent of pre-erythrocytic stage inhibition resulting from a vaccine (simulated in FIG. 3a). Similarly, the ratio of parasite DNA between the second and first replication cycles in the blood reflects the growth rate of blood-stage parasites. Comparing these ratios between test subjects and controls can indicate inhibitory effects of a vaccine candidate on the growth of blood-stage parasites (simulated in FIG. 3b). Such analyses could be of particular interest in trials of multi-stage vaccines (combining both liver- and blood-stage antigens) to assess stage-specific protective immunity. For example, statistical modelling of parasitaemia from a recent Phase IIa trial with the vaccine candidate apical membrane antigen 1 (AMA1), a protein that is mainly expressed by blood-stage parasites, indicates inhibition of pre-erythrocytic parasite stages[35], which highlights the possible role of sporozoite-expressed AMA1 in disease progression[36].

Experimental blood-stage infection

The evaluation of asexual erythrocytic stage vaccine candidates requires follow-up of blood-stage parasitaemia over a sufficiently lengthy period of time to determine parasite growth rates. This requirement could compromise the safety of volunteers, as blood-stage parasitaemia is responsible for malaria morbidity and even mortality. In currently accepted protocols, the appearance of *Plasmodium*-infected erythrocytes in thick blood smears examined microscopically leads to immediate initiation of treatment with curative anti-malarial drugs. Harbouring higher numbers of parasites in the bloodstream increases the risks to volunteer safety, so the length of the observation period for parasite multiplication in erythrocytes is limited.

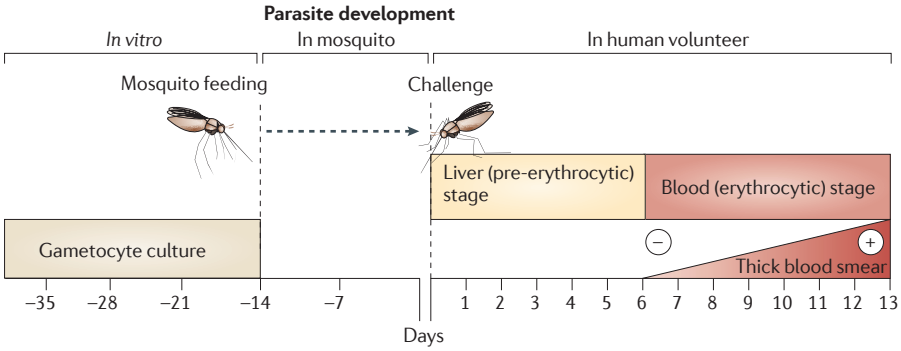

Figure 2 | **Timeline of *Plasmodium falciparum* sporozoite challenge infection in humans.** Gametocytes are derived from *in vitro* parasite culture in donor blood and are fed to laboratory-reared *Anopheles stephensi* mosquitoes. After 14–21 days, five infectious mosquitoes are allowed to feed on malaria-naive human volunteers for 5–10 minutes. Subsequent development of liver-stage parasites is subclinical and takes approximately 6 days. Parasites can be detected in the blood of unprotected volunteers by microscopy (using a thick blood smear) on average 11 days (range 7–15 days) after challenge.

A possible solution is the intravenous inoculation of very small numbers of infected erythrocytes, based on the idea that such a low level of sub-microscopic parasitaemia will not result in clinical risks and will allow extended follow-up of parasite replication in erythrocytes. The number of parasites that are inoculated is approximately 1,000 times lower than the estimated number of merozoites released from the liver following a standard experimental sporozoite challenge with bites from five infected mosquitoes. This allows for an extended blood-stage follow-up of approximately three more replication cycles (6 days) before thick blood smear detection thresholds are reached, with obligatory treatment.

A master cell bank of infected erythrocytes for human clinical use has been generated by storing infected erythrocytes from two parasitaemic volunteers who were infected by mosquito bites, in compliance with blood bank safety criteria[37]. Since the 1990s, approximately 50 humans have been infected by direct inoculation of infected erythrocytes from this master cell bank. The length of the prepatent period — the interval from inoculation until infected erythrocytes are microscopically detectable — correlates with the number of inoculated parasites[7]. The sensitivity of the model has been further increased by the administration of very small inoculae of infected erythrocytes, combined with the introduction of the quantitative real-time PCR assay for measuring parasite growth rates during this sub-microscopic period[37,38]. With inoculae as small as 300 infected erythrocytes, parasite growth curves were generated over a 7–9-day period before initiation of treatment was required [37].

The blood-stage challenge model has several potential shortcomings. The viability of the injected parasites can only be determined retrospectively by culture, so it is difficult to standardize the exact number of viable injected parasites. Differences of a factor of ten in terms of the number of viable parasites have been described between inoculae[38,39]. Furthermore, although the small number of inoculated parasites allows for a long window of observation, it may also boost the immune response, and low-level blood-stage infections are very efficient at inducing complete protection[40]. Finally, the liver stage of parasite development is circumvented by this model, bypassing potential immune effects induced by the vaccine on liver-stage parasites. This may be important, as some asexual erythrocytic stage vaccine candidate antigens can also be expressed during the liver stage[36]. However, irrespective of these disadvantages, low-dose blood-stage challenges allow sufficient time to monitor several parasite multiplication cycles. With further validation, they might function as a crucial decision point for progress to Phase IIb trials, thereby saving time and money, and decreasing the requirement for Phase IIb trial subjects. So far, only one asexual erythrocytic stage vaccine has been tested by blood-stage challenge[24]. The results of a second trial with the vaccine AMA1 carried out at the University of Oxford, UK, will soon be reported (ClinicalTrials.gov identifier: NCT00984763). A direct comparison between blood-stage and sporozoite-stage challenge infections will be helpful to determine the most suitable model to test such asexual erythrocytic stage vaccines.

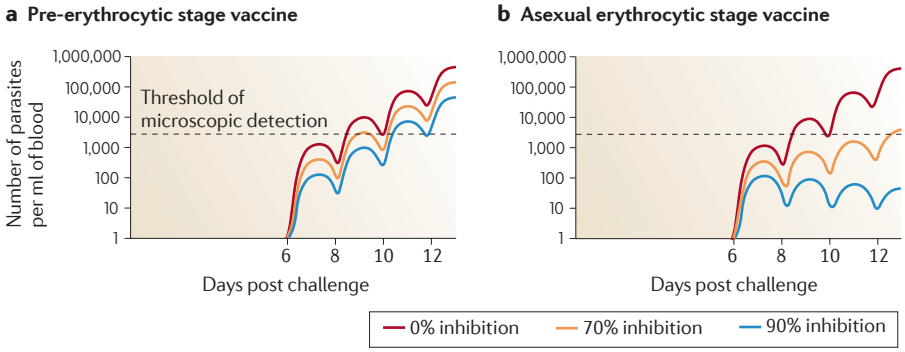

Figure 3 | **Simulated effects of immunization on parasite growth in the peripheral blood of volunteers after *Plasmodium falciparum* sporozoite challenge, based on statistical modelling.** The kinetics of parasite growth after immunization depend on which parasite stage the vaccine targets. We simulated the effect of a pre-erythrocytic (**a**) or asexual erythrocytic (**b**) stage vaccine on cyclical parasite growth in peripheral blood. The effects of 0%, 70% or 90% inhibition on parasite numbers are shown. After sporozoite challenge, the kinetics of blood-stage parasitaemia can be evaluated by quantitative real-time PCR up to the threshold of detection of parasites in the blood by microscopy (thick blood smear); this threshold is approximately 4×10^3 parasites per ml of blood. A careful comparison of blood-stage parasitaemia between immunized and control volunteers in a sporozoite challenge trial will allow investigators to distinguish between pre-erythrocytic or asexual erythrocytic stage inhibition. Furthermore, the percentage inhibition can be estimated. Image is modified, with permission, from REF. 32 © (2004) The American Society of Tropical Medicine and Hygiene.

Plasmodium vivax infection

Although the first experimental human malaria infections were carried out with *P. vivax*[41], the standardization of *P. vivax* challenge for routine use has proven to be much more difficult than for *P. falciparum* challenge. A major hurdle is the absolute requirement for reticulocytes or young erythrocytes to obtain long-term *in vitro* growth of *P. vivax*.

Nevertheless, promising results have been obtained through an alternative approach in which experimental infections are initiated using wild-type *P. vivax* parasites obtained from infected humans in Colombia. Blood from *P. vivax*-infected patients was assessed using routine blood bank procedures to exclude the presence of other transmissible agents (such as *T. pallidum*, hepatitis B virus and hepatitis C virus) and was subsequently fed to laboratory-reared *Anopheles albimanus* mosquitoes. After 14–15 days, mosquitoes were allowed to feed for 10–15 minutes on the forearms of healthy human volunteers. A total of 40 non-immune volunteers took part in two different trials, and data from 17 volunteers have been published so far[15,42]. After microscopic detection of parasites by thick blood smear, all participants were treated with a combination of chloroquine and primaquine. Because a proportion of *P. vivax* parasites can lay dormant as hypnozoites in the liver or develop slowly in humans, resulting in long prepatent periods[23], primaquine is prescribed to ensure clearance of all liver-stage parasites. This complicated protocol may be further compromised by drug resistance of some *P. vivax* strains, as is commonly observed in Southeast Asia.

The most frequently reported symptoms were myalgia, headache and malaise, without the occurrence of severe or serious adverse events. The prepatent period was 9–13 days[42]. The *P. vivax* challenge model has been further developed in the US Military Malaria Vaccine Program by the transportation of freshly infected *Anopheles dirus* mosquitoes from the Thai–Burmese border to infect malaria-naive volunteers in the United States (ClinicalTrials.gov identifier: NCT00935623). Currently, the first *P. vivax* vaccine candidate, based on the *P. vivax* circumsporozoite protein VMP001, is being tested by such challenge studies in the United States (ClinicalTrials.gov identifier: NCT01157897). Quantitative real-time PCR detection of *P. vivax* parasite load and genotyping of wild-type parasites will further improve the *P. vivax* challenge model[15]. Hopefully, the future development of new laboratory tools, including the use of stem cells as a source for young erythrocytes, will facilitate the long-term *in vitro* culture of *P. vivax*.

Strengths and limitations

Experimental human challenges aim to predict the potential efficacy of vaccine candidates against natural infections in the field. A major strength of the sporozoite infection model is the use of infectious mosquitoes, mimicking the natural route of infection. Moreover, human experimental challenges are carried out in a controlled environment, allowing detailed evaluation of parasite growth and immunological determinants. In addition to the evaluation of vaccine efficacy, experimental challenges provide the opportunity to study correlates and mechanisms of protection. An example is the induction of immunity using whole parasites, by exposure of malaria-naive volunteers to infectious mosquito bites while using chloroquine prophylaxis[43]. Chloroquine kills blood-stage parasites but leaves liver-stage parasites unaffected, thereby exposing the liver-stage and early blood-stage antigens to the immune system. Subsequent challenge showed that volunteers were completely protected from infection, and this was associated with multi-functional memory T cell responses. However, such immunization protocols are not practical for use in the field because parasite inoculation cannot be controlled and chloroquine resistance is widespread.

Several differences between experimental and naturally acquired infections might limit the interpretation of results from experimental challenge models. First, experimental infections are carried out using one parasite strain only, whereas it is well accepted that *P. falciparum* field strains are genetically diverse within and between regions[44]. Genetic diversity of the parasite strains is a major challenge for protein-based vaccines that target strain-specific antigens, and puts limitations on the direct translation of results from Phase IIa trials into the field situation. The availability of a small portfolio of genetically well-characterized *P. falciparum* strains for experimental infections would be a major asset. Trials to test such strains in humans are currently being carried out. Another potential difference is that in an experimental infection the parasite load is delivered almost instantly by five infected mosquitoes. Such a high parasite burden has been considered unnatural and might be an overly stringent test for the protective capacity of the vaccine-induced immune response[45]. However, although the frequency of infectious mosquito bites is generally less than this in malaria-endemic areas, intense transmission can occur. A person may be subjected to 35–96 mosquito bites per night, and in certain areas approximately 10% of mosquitoes are infected with *P. falciparum*[46].

A final potential limitation of current malaria challenge models involving sporozoite infection relates to the uncontrolled number of sporozoites inoculated by biting mosquitoes. This number is generally thought to vary up to a maximum of several thousand sporozoites[47–51]. Use of a well-defined number of inoculated sporozoites will strengthen the power of the model, as the dose probably influences the prepatent period[7,27,52].

In principle, the most accurate way of dosing sporozoites is to inject them directly by needle and syringe, as the number of sporozoites counted in mosquito salivary glands or the number of mosquito bites is a poor predictor of the number of sporozoites injected[49]. Early in the practice of malaria therapy, sporozoites were extracted from mosquito salivary glands (in 1927)[53] and the number of injected sporozoites was determined (in 1937)[54]. However, standardized sporozoite viability assays are not yet available. Recent progress has been made by Sanaria Inc.[55], which has developed technology for the purification and cryopreservation of aseptic sporozoites for use in humans according to the current safety standards. The first results of a human challenge study with aseptic *P. falciparum* sporozoite-infected mosquitoes indicate that these mosquitoes might be very efficient at conveying infection[56]. Experimental human infections are underway to test the infectiousness of these cryopreserved sporozoites by needle injection. However, one must bear in mind that needle and syringe administration of a bolus of sporozoites is clearly different from mosquito bite delivery, which may be an important factor to consider particularly for sporozoite vaccines that aim to induce antibodies to immobilize sporozoites.

Conclusions and perspectives

Experimental human infections provide a crude model to predict malaria vaccine efficacy in future field trials in a well-controlled setting. The experimental malaria challenge model in humans using *P. falciparum*-infected mosquito bites is now well established in several international sites and increasingly used as a crucial check point for the clinical development of pre-erythrocytic stage malaria vaccines. Taking into account the potential limitations, such efficacy data from Phase IIa trials will support the decision-making process by ethical boards and communities in malaria-endemic countries regarding whether to further test a candidate vaccine in Phase IIb trials in susceptible populations. In addition to vaccine safety data, the availability of information on potential efficacy is an important asset for ethical justification to conduct experimental malaria infections in human volunteers. In vaccine research, most risk is borne by study subjects and the benefits accrue mainly to the community in finding safe and protective vaccines[57]. The only candidate malaria vaccine showing protective efficacy in Phase IIb field trials so far is RTS,S. This candidate vaccine would almost certainly never have been developed without optimization in a series of Phase IIa trials. As is true for any type of clinical research, risks must be minimized and scientific benefits maximized. We believe that the benefits of Phase IIa trials outweigh the potential risks in well-designed studies and will be essential to the development of an effective malaria vaccine, provided that all safeguards are in place for the safety of volunteers[58].

The more recent introduction of a sensitive PCR assay for parasite detection has enhanced the reproducibility and statistical power of human challenge infections. Statistical models will be applied to further improve the discriminative power between control and test groups as well as to provide biological information about the parasite life cycle (including the duration of liver-stage maturation, number of infected hepatocytes, duration of blood-stage trophozoite maturation and multiplication rates). Initiatives are underway to further strengthen and harmonize the human challenge model, with possible applications for testing asexual erythrocytic stage vaccines and for *P. vivax* vaccine research[15]. As there is substantial variation in the numbers of sporozoites that are injected by mosquitoes and this cannot be controlled in the sporozoite challenge model, administration of a known number of sporozoites by needle injection may be a further improvement to the model. In addition, the human challenge model will benefit from the availability of a small portfolio of genetically well-characterized strains to explore immune responses to different strains and heterologous protection.

Such advances will accelerate malaria vaccine development, with the aim of meeting the ambitious goals of the Malaria Vaccine Technology Roadmap by 2015–2025.

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Competing interests statement
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FURTHER INFORMATION
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ICH: http://www.ich.org
Malaria Vaccine Technology Roadmap Process: http://www.malariavaccine.org/files/Malaria_Vaccine_TRM_Exec_Summary_Final_000.pdf
The Malaria Product Pipeline: Planning for the Future: www.policycures.org/downloads/The_malaria_product_pipeline_planning_for_the_future.pdf
WHO Guidelines for the Treatment of Malaria: http://www.who.int/malaria/publications/atoz/9789,241,547,925/en/index.html
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