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Advances and Challenges in Transmission-Blocking Malaria Vaccine Development. A Systematic Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Malaria continues to cause enormous human suffering throughout most of the tropics and subtropics. In sub-Saharan Africa alone, it is estimated that about two million children die each year due to malaria. Vector control and malaria chemotherapy strategies that were previously effective in controlling and treating malaria, respectively, are now largely ineffective owing to the spread of insecticide-resistant mosquitoes and drug-resistant parasites. A vaccine targeting the sexual stages of the parasite and block transmission is needed to reinforce current malaria control and eradication efforts. Here, we review the status of malaria transmission-blocking vaccines. We focused on the efficacy, progress and development challenges of transmission-blocking vaccines. *Pfs25* and *Pfs48/45* are essentially the lead-candidates malaria transmission-blocking vaccine and should be studied further in clinical trials. Our review highlights the need to develop novel malaria elimination interventions, particularly an effective malaria vaccine with transmission-blocking activity.

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1. BACKGROUND INFORMATION

Malaria is caused by unicellular *Plasmodium* protozoan parasites that are injected by female Anopheles mosquitoes. *P. falciparum*, which causes the most severe form of malaria, is a substantial threat today, causing more than 200 million clinical cases and 409,000 deaths in 2019 [1]. While the level of malaria has dropped over the last 10 years, this decline has recently stalled, with some regions experiencing a resurgence in the number of malaria cases [2]. Unfortunately, resistance to artemisinin-based combination therapy (ACT), the current frontline treatment for malaria, is present in Southeast Asia and now spreading in Africa [3]. This highlights the need for novel strategies in malaria prophylaxis and treatment, especially an effective vaccine that can induce a protective antimalaria immune response. Even though the development of a malaria vaccine is still a challenging task, the development of several vaccine candidates is ongoing.

Malaria vaccines can thus be broadly categorised into three groups based on the parasite stage being targeted, namely sporozoite, liver-stage and asexual blood-stage vaccines. Liver-stage and asexual blood-stage malaria vaccines (a transmission-blocking vaccine), unlike an anti-sporozoite vaccine, do not either directly protect a vaccinee against malaria infection, reduce parasitaemia or prevent disease; but rather employs all these four strategies. Anti-sporozoite vaccines would help by preventing malaria-infected travellers from transferring malaria parasite into a malaria-free zone [4]. The malaria parasite is particularly vulnerable to immune intervention during the sexual and sporogony stages because (1) parasites pass through a substantial numerical bottleneck-with about 100 parasites of the billions of asexual blood-stage parasites developing into gametocytes, get ingested by mosquitoes and subsequently developing into oocysts; (2) the sexual and sporogony stages persist extracellularly for hours unlike other parasite stages; (3) many of the candidate antigens tend to be highly immunogenic owing to their low antigenic diversity as a result of no immune selection in host since parasites do not express vaccine candidate antigens while in the human host [5].

Although antibodies targeting gametocytes (sexual stages) are not able to block human infection or disease, they can potentially stop malaria transmission. Recently, there has been increasing interest in the use of gametocyte-specific antigens as candidates for a transmission-blocking vaccine (TBV) [6]. Such a vaccine would induce antibody production that is taken up alongside the gametocytes during a mosquito blood meal and neutralize the parasites in the mosquito midgut upon expression of the antigens. Key sexual-stage antigens that have been considered as candidate TBV include *Pfs48/45, Pfs230, Pfs25,* and *Pfs47* [7,8]. It has been reported that antibodies from malaria-exposed individuals or vaccinated animals against these antigens could block malaria parasite development in mosquitoes [9]. These findings offer an impetus to test human monoclonal antibodies against TBV antigens, such as those generated recently against *Pfs25*, for the ability to block malaria transmission.

2. ROLE OF TRANSMISSION-BLOCKING VACCINE

The main goal of transmission-blocking vaccine development programs is to eradicate malaria parasites using an inexpensively produced, stably formulated, and easily administered vaccine. It is unlikely that a single antigen subunit vaccine will be enough. It noteworthy however that transmission-blocking vaccines will have a great impact on malaria control when they are combined with other vaccines or control modalities. These may include bed nets, protective vaccines, and massive chemotherapy administration, in controlling or even perhaps eradicating malaria in geographically isolated areas e.g. islands or isolated human and mosquito populations) [10]. In instances where malaria is eradicated, long-term control will be necessary to prevent the re-introduction of the parasite. This could be accomplished by enforcement of mandatory TBV vaccination for persons returning from areas where malaria persists and/or demonstrate evidence of prior vaccination with TBV.

The adoption of TBV combined with other malaria control strategies may boost the effectiveness of existing malaria control efforts. Firstly, a combination of TBV and multistage malaria vaccines could contribute substantially in curtailing the spread of' mutant parasites that may be resistant against the protective components of the vaccines by reducing malaria

transmission to a rate that would make an otherwise partially effective protective component highly effective [11]. Secondly, co-administration of TBV with effective anti-malarial drugs will help prolong malaria drug efficacy and delay the emergence or spread of drug-resistant parasites. Finally, TBV could reduce morbidity/mortality caused by the spread of the virulent strains of the parasite. Previous studies have demonstrated that the more an individual is exposed to new strains of parasites, the more likely they are to develop severe clinical disease [12–14]. However, it is still unclear whether this phenomenon is caused by a new "virulent" parasite strain that is biologically different (e.g., has a different cytoadherence profile) from other strains circulating in a geographical area [13] or it is simply caused by an immunologically new strain to the host.

3. CANDIDATE VACCINES

Prior fertilization, extracellular gamete (male and female)-specific antigens are the earliest point during sexual development to which an antibody-mediated transmission-blocking effect has been described. The primary function of gametes is to find and fuse with a gamete of the opposite sex, fertilization, in a process that occurs within minutes after a mosquito ingests the sexual stages [15]. Since the components of the complement cascade are present in the blood meal are still active for minutes to hours after ingestion, the parasite has developed protective mechanisms against complement-mediated lysis [16]. It is therefore not surprising that the two major mechanisms implicated in antibody-mediated transmission blockade are directed towards gamete surface proteins that interfere with fertilization and sensitization of gametes to complement-mediated lysis [17]. Other mechanisms that contribute to transmission blockage may involve a cellular component, such as antibody-dependent cell cytotoxicity (ADCC) and opsonization have not yet been described, but may occur as well.

The major advantage of transmission-blocking vaccines that target pre-fertilization antigens is that immune responses are boosted by each subsequent natural infection. Whether boost will increase the titer of transmission-blocking antibodies or extend the longevity of transmission-blocking immunity is yet to be determined. Furthermore, antigen immunogenicity also needs to be studied since seroepidemiological studies indicate that less than 50% of humans carrying gametocytes contain detectable antibodies to either of the two lead pre-fertilization vaccine candidates, namely *Pfs230* and *Pfs48/45* [17]. Other pre-fertilization vaccine candidates are *Pfs2400*, *Pfs40* and *Pfg27/25* [18].

3.1 Immune Response To *Pfs25*

Malaria vaccines that target both human and mosquito infections have the potential to impact malaria control. The importance of developing such a vaccine is underscored in a WHO World Malaria Report, which indicates stagnation in the progress towards reducing global malaria cases since 2015 [19]. The malaria vaccine RTS, S, which targets human infections as indicated by its impact on clinical disease, has completed phase 3 testing and is in pilot implementation studies in three African countries, namely Kenya, Ghana and Malawi [20,21]. Efforts to develop a vaccine that disrupts mosquito infection, known as a malaria transmission-blocking vaccine (TBV), have been ongoing since the reporting of induced TBV-induced immunity in chickens against *Plasmodium gallinaceum* in 1976 [22]. The pace of malaria TBV development, until recently, has been hindered by the lack of capacity to produce candidate antigens for clinical testing [22].

Currently, clinical testing is are limited to *Pfs25*, a *P. falciparum*-specific antigen. *Pfs25* is a 25 kDa sexual-stage protein present on the *P. falciparum* zygote and ookinete surface in the mosquito midgut. The leading *Pfs25* TBV is a chemically conjugated vaccine comprising of a *Pichia pastoris* expressed *Pfs25* and the carrier protein ExoProtein A (EPA) or a recombinant detoxified form of *Pseudomonas aeruginosa* ExoToxin A9. The *Pfs25*-EPA conjugate has the biophysical characteristics of a nanoparticle with a size similar to the hepatitis B virus-like-particle used in RTS, S [23]. *ex vivo* standard membrane feeding assays (SMFA) during phase 1 trials of *Pfs25*-EPA conjugates formulated with Alhydrogel™, an aluminium-based adjuvant conducted in the United States of America, and Mali revealed that four doses are required to generate antibody titers that significantly reduce parasite transmission [22]. However, poor clinical trial results have halted the development of *Pfs25*-EPA as a stand-alone TBV. Which can probably be tackled by using the correct carrier protein.

3.2 Immune Response To *Pfs230* and *Pfs48/45*

Another family of sexual-stage proteins with cysteine-rich domains includes the antigens *Pfs230* and *Pfs48/45* that have been targeted for TBV development. However, until recently, there was no recombinant antigen preparation available with the identity, purity, and quality necessary for human clinical trials [24]. Of the two, *Pfs230* cysteine-rich domain was the first antigen to be produced. It consists of a 230 kDa sexual-stage protein that is composed of fourteen 6-cysteine-rich domains that suitable for human clinical testing [25]. Recombinant *Pfs230* domain 1 (*Pfs230*D1M) is well-characterized and has shown to induce transmission-blocking antibodies in small animals using the SMFA16 [24]. Parasite-derived antigen (Pfs23016A) has been shown to induce the production of transmission-blocking monoclonal antibodies (4F12). A phase 1 safety and immunogenicity study evaluating *Pfs230* D1-EPA nanoparticles formulated on Alhydrogel™ has been completed [24]. Pfs230D1M is conjugated chemically to EPA forming nanoparticles with a similar size as hepatitis B virus10.

3.3 Post Fertilization Target Antigens

Several post-fertilization target antigens have been described, including *Pfs28* (D&y& Kaslow. submitted), *Pfs25*. chitinase and mosquito midgut late trypsin [26,27]. The two major mechanisms mediating blockade post-fertilization involve arresting the morphological transformation of the round, sedentary zygote to the oblong, motile ookinete and preventing the egress of the ookinete from the blood meal to the midgut epithelium basal lamina [11]. Preliminary evidence suggests that transmission-blocking antibodies may also interfere with normal sporozoite development. However, the exact mechanism(s) by which this occurs is not yet known though there is increased gametocyte clearance and impaired development in later stages [28]. The major advantages of post-fertilization target antigens are immunogenicity and limited pre-existing antigenic diversity, unequivocally reflecting the lack of prior immune selection on these antigens. Since these target antigens are expressed late in sexual sporogonic development, high antibody titers may be required to account for antibody degradation from proteolysis in the blood meal and boosting after a natural infection may not occur.

4. FIRST GENERATION TRANSMISSION-BLOCKING VACCINE, Tbv25h

One of the most advanced candidate transmission-blocking vaccine in development is a 6 histidine-tagged protein TBV25H (Transmission-Blocking Vaccine based on *Pfs25* with a Histidine tag) secreted from recombinant *Succharomvces cerevisiae* [29]. A modified fed-batch fermentation protocol was developed that optimizes recombinant protein production using the simplest and least expensive fermentors available. The 6 histidine-tag is used to purify the protein with ease from the yeast culture supernatant by affinity chromatography with nickel-NTA agarose. The protein is further purified by simple gel filtration chromatography and then adsorbed to alum. Thus, in keeping with the goal of technology transfer to newly industrialized countries, a simple fermentation and post-fermentation process was developed that should allow the ultimate end-users to produce the vaccine themselves [30]. However, TBV25H/alum is not an ideal transmission-blocking vaccine for three reasons; it may require refrigeration, *in vivo* studies have demonstrated that multiple injections may be required to induce the production of transmission-blocking antibodies and immunity is relatively short-lived. In its current formulation, TBV25H will be invaluable tool for determining the safety of TBV25H/alum and help improve our understanding on how antibodies work to block infectivity *in vitro* and how it can be elicited in humans. A series of human clinical trials will be required [5].

Assessing the efficacy and the impact of a TBV poses some unique challenges [11]. The lack of reliable *in vitro* correlates of protective efficacy has limited the early phases of protective malaria vaccine development; the latter field-testing phases of protective vaccine development are straightforward. In contrast, the early phases of development of a TBV have the advantage of a reliable (but tedious) *in vitro* assay for assessing transmission-blocking activity, the membrane feeding assay in which *in vitro* cultured gametocytes are mixed with test serum and fed through an artificial membrane to starved mosquitoes. The degree to which the test serum inhibits infectivity is determined 1 week after an infectious blood meal by scoring mercurochrome-stained mosquito midguts for oocysts [31]. Nevertheless, *in vivo* efficacy for transmission-blocking vaccines will require labour-intensive, expensive field testing [21].

The assessment of the actual efficacy of a TBV requires field testing unlike any vaccine tested to date, malaria or otherwise. Although a Phase I trial designed to establish safety and immunogenicity has been used to assess efficacy in vitro (using the membrane feeding assay) and a Phase IIb (naturally acquired parasite infection rather than experimental parasite challenge) can be used to assess in vivo efficacy by feeding laboratory-reared mosquitoes directly on infected volunteers, designing and executing robust Phase III trials (i.e. testing efficacy in the field) that determines the impact of the vaccine on natural transmission and on preventing morbidity and mortality is difficult [8]. This is because, in Phase III trials, the statistical or study unit is not individual-based but requires a well-defined and, at least partially, isolated population of sufficient size in a malaria-endemic or seasonal malaria area. The setting must be such that the rate of transmission is not so high that a possible positive effect is missed, but not so low that the observed effect would have little consequence as a public health measure. The control and test study units, or communities, must have little interchange between vaccinated and unvaccinated populations but must be similar to allow comparative analyses between among sites [22]. Depending on the rate of transmission, the endpoints that are chosen, the similarity in malaria transmission and endemicity, the existence of a malaria transmission control programme (e.g., the presence of *P. vivax* transmission), and the cooperation of all individuals in each community, as few as one or two test villages and an equal or greater number of control villages may be all that is necessary to preliminarily evaluate efficacy [28].

Several ethical issues, however, must be adequately addressed before undertaking these human clinical trials. Despite the enormity of the health problems that malaria poses worldwide. an effective malaria vaccine has eluded us. Lack of understanding as to what the mechanism of protective immunity is, limited research funding, and perhaps lack of the appropriate technologies necessary to develop an effective vaccine has probably contributed to the delayed development of effective malaria vaccines. Novel approaches and technologies may be required such as TBVs.

4.1 Sequence Polymorphisms of Vaccine Candidates

Considering that amino acid sequence polymorphisms present a plausible bottleneck for malaria subunit vaccine development [32], there is a major concern that B-cell epitopes targeted by transmission antibodies might be highly variable [13]. To assess whether any of the polymorphisms affect the antibody affinity towards Pfs48/45, serum samples from rats immunized with R0.10C were tested in the SMFA with three distinct *P. falciparum* isolates of Asian and African origin, i.e. NF54, NF166 and NF135 [33]. Taken together, available data from sequencing and antibody binding studies of field isolates suggest that parasite diversity is unlikely to become an obstacle for the clinical development of Pfs48/45-based vaccines [13].

Expression of a recombinant vaccine has been hindered by the inability to produce sufficiently high yield recombinant proteins that refolds into the native structure required for the induction of TBV antibody production [34]. Proper folding of many cysteine-rich proteins, including Pfs48/45, depends on the correct formation of disulphide bridges [35]. In eukaryotes, the oxidizing environment of the endoplasmic reticulum (ER) provides a milieu for disulphide bonds formation, a process that is mediated by ER oxidoreductase 1 (Ero1) and protein disulfide isomerase (PDI) [36]. *P. falciparum* possesses four protein disulfide isomerases (PfPDIs) of which, PfPDI-8 and PfPDI-11 have been expressed in gametocytes [37]. *Pfs48/45* and *Pfs25* have been expressed in different eukaryotic expression systems possessing Ero1 and PDI analogous but some systems have a track record for production of recombinant malaria antigens the reported yields of properly folded recombinant Pfs48/45 have been disappointingly low [38,39].

4.2 Preclinical Studies

Numerous animal studies have shown that *Pfs48/45*, *Pfs25* and other candidates vaccination elicits antibodies with the capacity to inhibit parasite fertilization in mosquitoes as determined in the SMFA [28]. In general, a recombinant protein which assumes a proper fold, as determined by the reactivity with mAbs against conformational epitopes, elicits high levels of transmission-blocking antibodies [21]. In contrast, miss-folded protein does not elicit transmission-blocking antibodies suggesting that correct folding of critical epitopes involved in transmission-blocking immunity is essential for an efficacious TBV [21]. Adjuvants are vital for an efficacious subunit-vaccine by enhancing seroconversion rates and concentrations of functional antibodies [40]. In the preclinical

adjuvant selection, it is important to focus on those with a human clinical development path with records of both safety and manufacturability [28]. Whether this level of vaccine-specific antibodies is comparable to naturally occurring antibodies against Pfs48/45 is unknown. This is mainly because most studies of naturally acquired immunity have reported antibody data as categorical variables (either positive or negative) rather than continuous variable. The availability of new recombinant proteins will help future studies to estimate the level of vaccine-specific as well as naturally acquired antibodies. The ability of newly designed adjuvants to enhance levels of functional antibodies against Pfs48/45 has been investigated in small rodents using recombinant proteins produced in the *L. lactis* expression system [6]. Formulations containing the synthetic TLR4 agonist glucopyranosyl lipid adjuvant (GLA) or a combination of synthetic lipid adjuvant (SLA) and saponin containing QS21 in a liposome formulation (SLA-LSQ) induce the highest titers of antibodies to sexual stage antigens. Both the GLA and SLA agonists have been assessed in human clinical trials as a part of vaccines against several infectious diseases suggesting they might be useful for the development of a TBV[35].

Viral vectors are considered an attractive alternative to protein-based vaccines because they circumvent the need for production and purification of properly folded protein and because of their ability to effectively induce both humoral and cell-mediated immune responses [9]. In recent years this approach has been explored extensively [41]. For instance, *Pfs48/45* has been tested in a ChAd63-MVA heterologous prime-boost regime as a glycosylated and non-glycosylated recombinant protein [42]. Mouse IgG against non-glycosylated *Pfs48/45* shows transmission-blocking activity in SMFA using homologous NF54 parasites and to a lesser extent also in the direct membrane feeding assay (DMFA) using *P. falciparum* parasites collected from naturally exposed gametocyte donors [6]. Collectively, these findings suggest that *Pfs25* and *Pfs48/45*-based subunit vaccines have the capacity to elicit functional antibodies in humans.

5. CONCLUSION

This review highlights the need to develop novel malaria elimination interventions in particular an effective malaria vaccine with transmission-blocking activity. *Pfs25* and *Pfs48/45* are essential lead-candidates for a transmission-blocking vaccine and should be explored further in clinical trials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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