REVIEW

Development of malaria vaccines that block transmission of parasites by mosquito vectors

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Abstract : Malaria is still one of the infectious diseases urgently requiring control and causes socioeconomic burdens on people residing in developing countries. Malaria vaccines are expected to control the disease. However, there is no effective vaccine available despite the intense efforts of malaria scientists. One strategy for a malaria vaccine is to prevent parasite spread by means of interfering with parasite development in mosquito vectors, which is the so-called transmission-blocking vaccine (TBV). We will here review the current progress of TBV. J. Med. Invest. 49 : 118-123, 2002

Keywords **:** *malaria vaccine, transmission-blocking vaccine, P. falciparum, P. vivax*

INTRODUCTION

Malaria is a disease caused by infection with protozoan parasite genus *Plasmodium*, of which four species infect humans. It produces up to 500 million new infections and 2 million deaths every year (1). Chemotherapy and vector control have been insufficient to control the disease because of the emergence and spread of parasites and their vectors resistant to these o ld chemical based control methods (2, 3). Thus, malaria vaccines are urgently needed to control malaria especially caused by two major species, *P. falciparum* and *P. vivax*. The malaria parasite has a complicated life cycle (Fig. 1), which makes difficult to develop a universal, effective and long lasting vaccine. However, several studies suggested that vaccines against each parasite stage are feasible as part of a control strategy.

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LIFE CYCLE OF MALARIA PARASITE AND VACCINE TARGETS

Infection is initiated by inoculation of sporozoites, an infectious form of the parasite, through mosquito bites. Once inoculated, sporozoites spend less than 30 minutes in the blood before entering heptocytes or being eradicated. In heptocytes, a single sporozoite develops into 30,000-40,000 merozoites, each of which, when released into the bloodstream, continues its life cycle in red blood cells. Specific antibodies to sporozoites can inhibit entry to hepatocytes (4). During hepatic development, a variety of liver-stage-specific antigens are synthesized by the parasite and are presented in context with MHC class I molecules and recognized by $CD8^+$ T cells (5). Vaccines against parasites before merozoite release called liver-stage vaccine are expected to induce antibodies that inhibit invasion of hepatocytes and cytotoxic T cells that destroy infected liver cells. Successful vaccines can reduce the chance of a person becoming sick because all symptoms appear during the asexual growth stage in the blood cycle.

In the bloodstream each merozoite invades a red blood cell and asexually divides up to 24 merozites in the host cell over a period of 2 to 3 days. Mature parasites rupture the host cell to infect new red

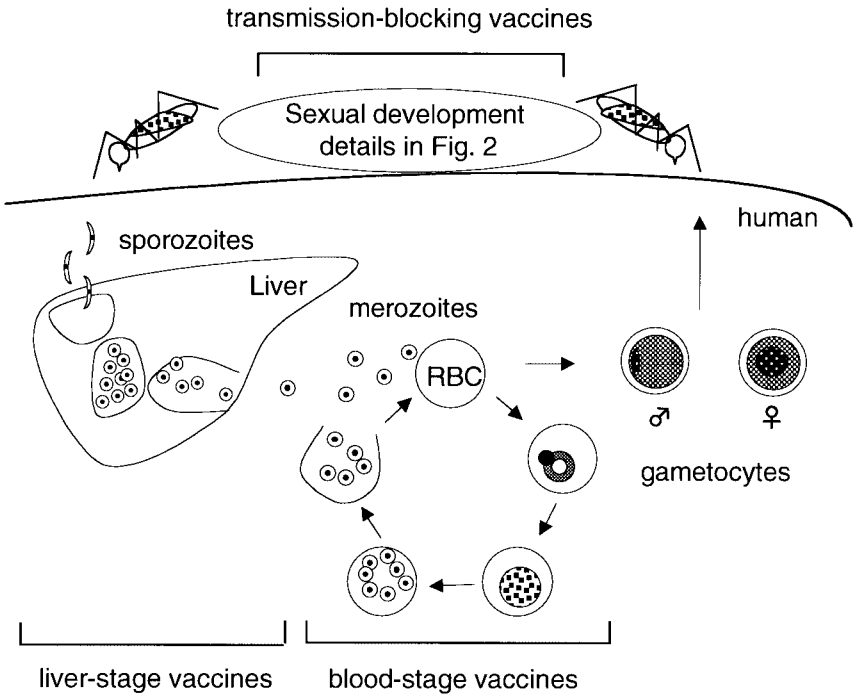

Fig. 1. Life cycle of malaria parasite and vaccines against different stage parasites (from Ref. 6). Liver-stage vaccines target sporozoites and parasite in hepatocytes. Blood-stage vaccines prevent repeating blood cycles. Transmission-blocking vaccines are designed to interfere with parasite development in mosquito vectors.

blood cells, which causes the periodic elevation of body temperature (fever). Antibodies to merozoite can inhibit invasion of red blood cells (6). Antibodies to parasite-derived molecules on the surface of infected red blood cells can prevent adhesion to capillary endothelial cells, responsible for pathogenicity of major complications such as cerebral malaria. Vaccines against parasites undergoing the blood cycle are called blood-stage vaccines. Effective vaccines will reduce disease severity and the risk of death during infection.

A part of the blood-stage parasite develops to a sexual parasite, male and female gametocytes, instead of repeating the asexual cycle. Gametocytes are ingested by mosquitoes during blood sucking and they undergo the sexual process (sporogony). The TBVs focused on here are designed to prevent parasites spread by inhibiting development of parasites within mosquito vectors.

TRANSMISSION-BLOCKING VACCINE AND ITS EFFECT

Gametocytes ingested by susceptible species of mosquito shed the host erythrocyte and the female develops into a macrogamete and the male becomes eight microgametes. Gametes mate, and the fertilized zygotes transform to motile ookinetes that penetrate the mosquito midgut peritrophic matrix and midgut epithelial cells. Then they develop into oocysts on the serous membrane. Matured oocysts release sporozoites that migrate to the salivary glands to prepare the next infection. TBVs are designed to induce antibodies that disrupt at least one of these steps when ingested with parasite-infected blood. Unlike two other malaria vaccines, TBV does not provide direct protection to individuals infected vaccine. However, this vaccine was suggested to be one of the important strategies for integrated malaria control program. Primarily, TBV will reduce the incidence of malaria patients in the community whose residence is vaccinated. In addition, this would control the escape of parasite mutants from vaccines targeted to other stages of the parasites life cycle (7), which would prolong the effective life of other malaria vaccines.

TRANSMISSION-BLOCKING VACCINE CANDIDATES

Target molecules for TBV must be expressed by the sexual stage parasites, gametocytes to ookinetes (Fig. 2). Several candidate molecules expressed by the sexual stage parasites have been reported. They are mostly from *P. falciparum* responsible for nine of ten deaths.

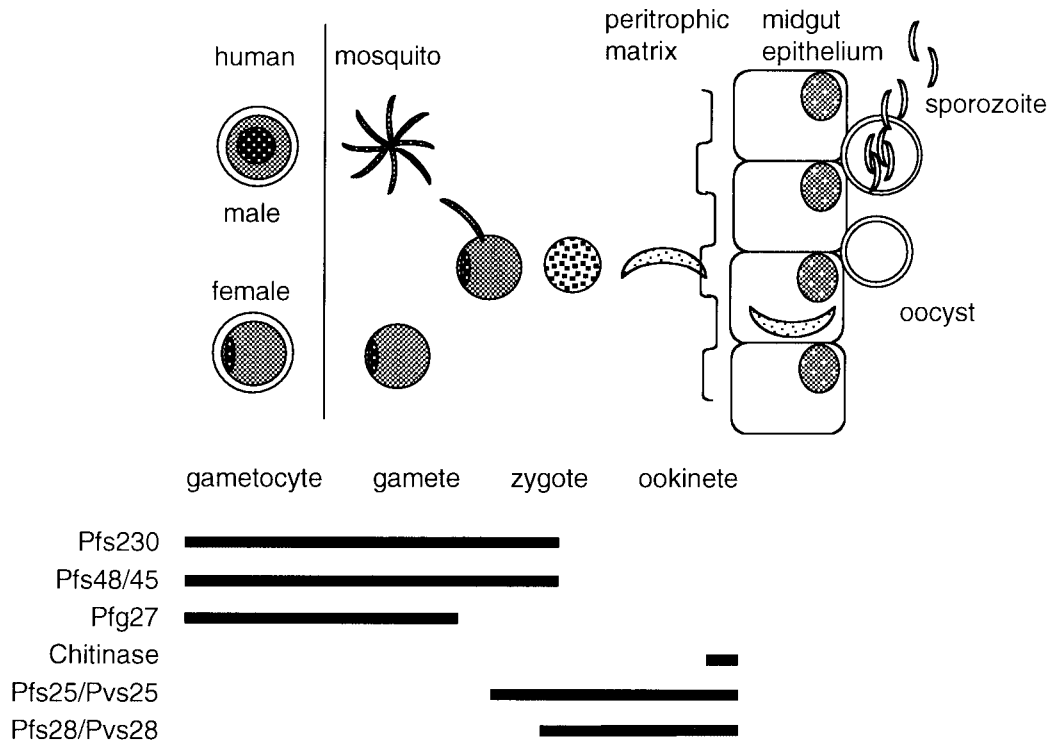

Fig. 2. **Parasite development in mosquito vectors and transmission-blocking vaccine candidates. Gametocytes in human hosts ingested by mosquito vectors become gametes then fertilize to transform zygotes that develop motile ookinetes. TBV induces antibodies against molecules expressed by gametocytes to ookinetes. Six candidates are listed and their expression pattern is indicated with black lines.**

1. Pfs230

Pfs230 is cloned as a molecule expressed by gametocytes of *P. falciprum* and its molecular size is 230 kDa (8). The expression remains after gametes fertilize and transform to zygotes (9). Antibodies against Pfs230 completely block transmission of the parasite to mosquito vectors when ingested with a gametocytemic bloodmeal through a membrane-feeding apparatus (10).

2. Pfs48/45

Pfs48/45 is also a molecule expressed by gametocytes of *P. falciparum* and shows on electric doublet of 48 and 45 kDa on polyacrylamide gel electrophoresis (11). The expression pattern is similar to that of Pfs230. A rodent parasite disrupted gene encoding P48/45 homologue demonstrates that P48/45 family is essential for male gamete fertility (12). Antibodies against this molecule also completely block parasite transmission to mosquitoes (13).

3. Pfg27

Pfg27 is expressed as early as gametocytogenesis (14). *P. falciparum* disrupted gene encoding Pfg27 fails to develop the sexual stage parasites, suggesting that this molecule functions at an early stage of gametocytogenesis in vertebrate hosts (15). This also generates antibodies that block parasite transmission (16).

4. Chitinase

Ookinetes secrete chitinase, an enzyme that hydrolyses chitin, a polymerized form of *N*-acetyl glucosamine (17). Chitin is a major component of mosquito peritrophic matrix and its rigid structure was suggested to function as a physical barrier to parasite invasion. Chitinase is essential to penetrate the peritrophic matrix because a chitinase inhibitor and disruptant of chitinase gene suppresses oocyst formation (18, 19). Thus, this represents a target molecule not only for the transmission-blocking vaccine strategy but also drug development (20).

5. Pfs25/Pvs25, Pfs28/Pvs28

The leading candidates for a transmission-blocking vaccine against *P. falciparum* have been cloned (21). Pfs25 and Pfs28 are expressed on the surface of ookinetes and are composed of four tandem epidermal growth factor-like domains, putatively anchored to the surface membrane by a glycosylphosphatidylinositol-attached motif. Yeast-produced recombinant proteins elicit transmission-blocking antibodies in animal models

(22). Their homologs of *P. vivax*, Pvs25 and Pvs28, were recently cloned (23).

DEVELOPMENT OF TBV AGAINST VIVAX MALARIA BASED ON Pvs25

Among these vaccine candidates, the most advanced is P25 and P28 family proteins of *P. falciparum* and *P. vivax*, Pfs25, 28 and Pvs25, 28. TBV against *P. vivax* based on Pvs25 and Pvs28 were recently developed. Pvs25 and Pvs28 are successfully expressed as recombinant proteins by yeast *Saccharomyces cerevisiae* and the products are highly immunogenic and induce antibodies that block oocyst formation in mosquito when mixed with a gametocytemic blood meal (24). Thus, both proteins are potentially vaccine candidates. Gene knock-out experiments demonstrate that rodent malaria parasites deficient for the P25 and P28 proteins fail to generate oocysts in mosquitoes, suggesting a synergistic role in oocyst formation (25). This suggests that maximum inhibition will be obtained when antibodies to Pvs25 and Pvs28 exist. However, detailed experiments revealed that no synergistic effect of anti-Pvs25 and -Pvs28 antiserum on oocyst formation was observed (26). Together with low yield, the high frequency of variants, and the unresponsiveness in mice in MHC-dependent manner of Pvs28, a single use of Pvs25 as a vaccine was determined as the best candidate for TBV against *P. vivax*. Thereafter, Pvs25 has been tested for immunogenicity in non-human primates (27). A clinical grade Pvs25 is currently being produced and phase I trials will soon be conducted at the Malaria Vaccine Development Unit, NIH (Stowers, personal communication).

OBSTACLES TO BE OVERCOME

The most serious obstacles to developing a malaria vaccine (and other vaccines) are antigenic diversity, lack of *in vitro* assay system, difficulty in conducting human trials, and selection of an adjuvant delivery system. Comparing with two other malaria vaccines, these obstacles are easier to be overcome for the TBV based on Pvs25. Antigenic variation is much less frequent in P25 family proteins (23, 28). The molecules are not exposed to host immune systems because they are expressed only after fertilization in the mosquito. However, this results in another problem. Immune responses to the vaccine would not be boosted during natural infection. Therefore, formulations may need to be developed to extend the effective life of TBV-induced immunity.

The immune mechanism of TBV is antibody-mediated inhibition of parasites' development in mosquitoes. This has enabled an *ex vivo* assay to be established. Mosquitoes were fed with gametocyte-infected blood through a membrane in the presence of immune serum. Thereafter, the number of oocysts is counted. Using this assay, the transmission-blocking activity of serum from human volunteers will be evaluated without requiring experimental infection of each volunteer with malaria. This is a big advantage to design clinical trials. In terms of the adjuvant and delivery systems, we could be optimistic, because alum adjuvant (only adjuvant applicable for human use) combined with Pvs25 could induce high titers of antibody that has strong a transmission-blocking activity in rodents and non-human primates. Moreover, DNA vaccination also induces a high titer of antibodies (29, Kumar, personal communication).

CONCLUDING REMARKS

Although TBV does not provide direct protection to individuals, this is an important strategy for controlling malaria. Since TBV could prevent the spread of escape of mutants from other vaccines, this must be a member of a multi-component malaria vaccine. The biggest problem common to developing malaria vaccine is the relatively low level of commercial interest. This is because the potential market with endemic malaria is mainly in the developing countries who do not have economic support. However, economic support will be obtained in the future (ex. The Bill Gates Foundation for Malaria Vaccine Development was established).

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