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Malaria vaccines 1985–2005: a full circle?

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Few who were actively engaged in malaria vaccine research 20 years ago (including myself) would have imagined that, in 2005, there would still be a prediction of a 10–20-year horizon before vaccines become part of malaria-control strategies. Why is it still proving so challenging to produce effective vaccines?

Prospects

In the debate 'Towards a malaria vaccine', which was published in 1985 in *Parasitology Today,* the conclusions drawn by the authors of the four articles were optimistic: (i) that the tandem-repeat sequences that constitute a substantial and immunodominant part of the circumsporozoite protein (CSP) of *Plasmodium falciparum* provided a promising candidate for vaccine development [1]; (ii) that several different asexual blood-stage antigens or fragments of them could form the basis of an effective vaccine [2]; (iii) that the first phase of clinical testing of a transmission-blocking vaccine could soon be considered [3]; and (iv) that the prospects for practical malaria vaccines had moved into the realm of feasibility in only ten years [4].

Pre-erythrocytic-stage vaccines

By 1985, it had already been shown experimentally and in humans that irradiated sporozoites could confer a strong (sterile) immunity [5]. The subsequent focus became the development of recombinant or peptide vaccines based on the CSP antigen and other sporozoite surface antigens that would reproduce or enhance the level of immunity achieved with the whole attenuated organism. Notably, it was shown that the C-terminal region of the CSP that flanks the naturally immunodominant tandem-repeat region of the molecule contains $CD4^+$ and $CD8^+$ T-cell epitopes and that these were incorporated into vaccine constructs. However, most of the small-scale vaccine trials subsequently conducted obtained disappointing results [6]. Vaccine technologies have advanced considerably since then [7,8] (Box 1), and there is still a range of recombinant and peptide CSP vaccines under investigation [see the World Health Organization portfolio of candidate malaria vaccines (http://www.who.int/vaccine_research/documents/en/malaria_table.pdf)]. As yet, only one construct (RTS,S) has progressed far in clinical trials.

Box 1. Examples of malaria vaccine technologies currently employed in clinical trials

Prime–boost (clinical Phase II)
Priming of the immune response uses antigens expressed as plasmid DNA or in attenuated viral vectors such as fowlpox or adenovirus. Boosting involves use of attenuated viral vectors such as modified vaccinia Ankara (MVA) expressing the same antigen(s) [9].

Virosomes (clinical Phase I)
Spherical unilamellar phospholipids representing liposomes and incorporating antigen in the membrane. The vaccine PEV3A contains peptides of the CSP and of part of AMA-1 of *Plasmodium falciparum* (http://www.pevion.com/downloads/pevion/doc/Pevion%20Phase1%20Malaria_061804_en.pdf).

Virus-like particles (clinical Phase I–II)
A recombinant virus-like particle (VLP) comprising modified hepatitis B core protein with B- and T-cell epitopes of *P. falciparum*. A single dose administered with Montanide ISA 720 adjuvant did not protect subjects [49].

Long synthetic polypeptides (clinical Phase I)
Peptides based on the C terminus of *P. falciparum* CSP combined with alum or Montanide ISA 720 adjuvant-induced antibody, $CD8^+$ T-cell and γ-interferon responses [50]. *Plasmodium vivax* CSP peptides are similarly immunogenic. Long peptides containing B- and T-cell epitopes of MSP-3 induced monocyte-dependent parasite inhibitory Immunoglobulin G responses [9].

Co-expression of recombinant polypeptides (clinical Phase II)
RTS,S consists of a single polypeptide corresponding to substantial parts of *P. falciparum* CSP and the hepatitis B surface antigen, expressed in yeast. An important component is the adjuvant ASO2A, which consists of an oil-in-water emulsion incorporating the immunostimulants monophosphoryl lipid A and the saponin derivative QS21. (See main text for details of results of the trials.)

Subunit recombinant blood-stage antigens (clinical Phase I–II)
The C-terminal region of MSP-1 ($MSP\text{-}1_{42}$) given with ASO2A adjuvant was safe and immunogenic. Challenge studies in adults gave no protection [9]; Phase I and II studies in children in Kenya are in progress. A similar Phase I study with a subunit AMA-1 vaccine and a Phase I trial with an MSP-1–AMA-1 chimaera have been conducted [9].

Recombinant transmission-blocking vaccine (clinical Phase I)
The Pvs25 ookinete antigen of *P. vivax* was expressed in yeast, purified and adsorbed onto Alhydrogel®. Three immunizing doses induced antibodies that blocked transmission significantly [45]. A similar approach with the *P. falciparum* homologue is being tested.

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This vaccine consists of a large segment of the tandem-repeat region of the CSP, in addition to the T-cell epitopes of the flanking region, expressed with the hepatitis B surface antigen in yeast. The vaccine is given with a three-component adjuvant [9]. The results from trials in Gambian adults [10] and Mozambican children [11] are encouraging, with a reported protection in children of 30% in the time to the first clinical episode of malaria, and 58% protection against severe malaria. However, these trials have raised questions of fundamental importance to the development of all vaccines that might partly explain the slow progress in bringing candidate vaccines successfully through clinical trials – namely, is there adequate induction of an appropriate memory T-cell response to provide the long-lived protection that is required? This seems not to be the case in the Gambian study [10], and the Mozambican results are also open to the same interpretation [12]. This must be addressed in the further series of Phase II trials of RTS,S being planned.

Trials of vaccines have (understandably) been carried out in an empirical way, with the focus in the 1980s being on antibodies as the mediators of protection. For the past 15 years, there has been increasing recognition of the need to determine at a cellular level which immune responses must be induced to achieve robust protection (Figure 1). The demonstration that protection induced by irradiated sporozoites involves both antibody and cell-mediated effector mechanisms [5,13] focused attention on liver stages and the induction of both $CD4^+$ and $CD8^+$ T-cell responses that would be effective against multiplicative intrahepatic schizonts.

The development of a prime–boost strategy for vaccination has proved particularly effective in inducing such T-cell responses. The vaccines tested using this approach consist of malaria antigens presented as plasmid DNA or expressed by attenuated viral vectors, given in a sequence that provides strong priming and then boosting of the cellular immune responses [7]. This approach, pioneered in malaria by Hoffman and colleagues at the US Naval Medical Research Institute [14] and by Hill and colleagues [15], has involved the use of complex antigens: for example, the sporozoite surface protein TRAP (thrombospondin-related adhesion protein) fused to a multi-epitope (ME) string of B cell, $CD8^+$ and $CD4^+$ epitopes from sporozoite and liver-stage antigens. Immunization of malaria-naïve volunteers has given full protection in a small number of cases and, where this did not occur, it has provided evidence of a substantial reduction in the number of liver-stage parasites and, hence, the number of merozoites released to invade the blood [16]. Disappointingly, trials in an endemic area following the same regimes have not induced any protection so far, despite greatly enhanced T-cell responses as measured by γ-interferon enzyme-linked immunospot (ELISPOT) assays [17]. Again, this raises the issue of the cellular basis of the immune responses required and, perhaps, those to be avoided. Further clinical trials are in progress.

While the trials with RTS,S and other pre-erythrocytic recombinant and peptide vaccines continue with varying

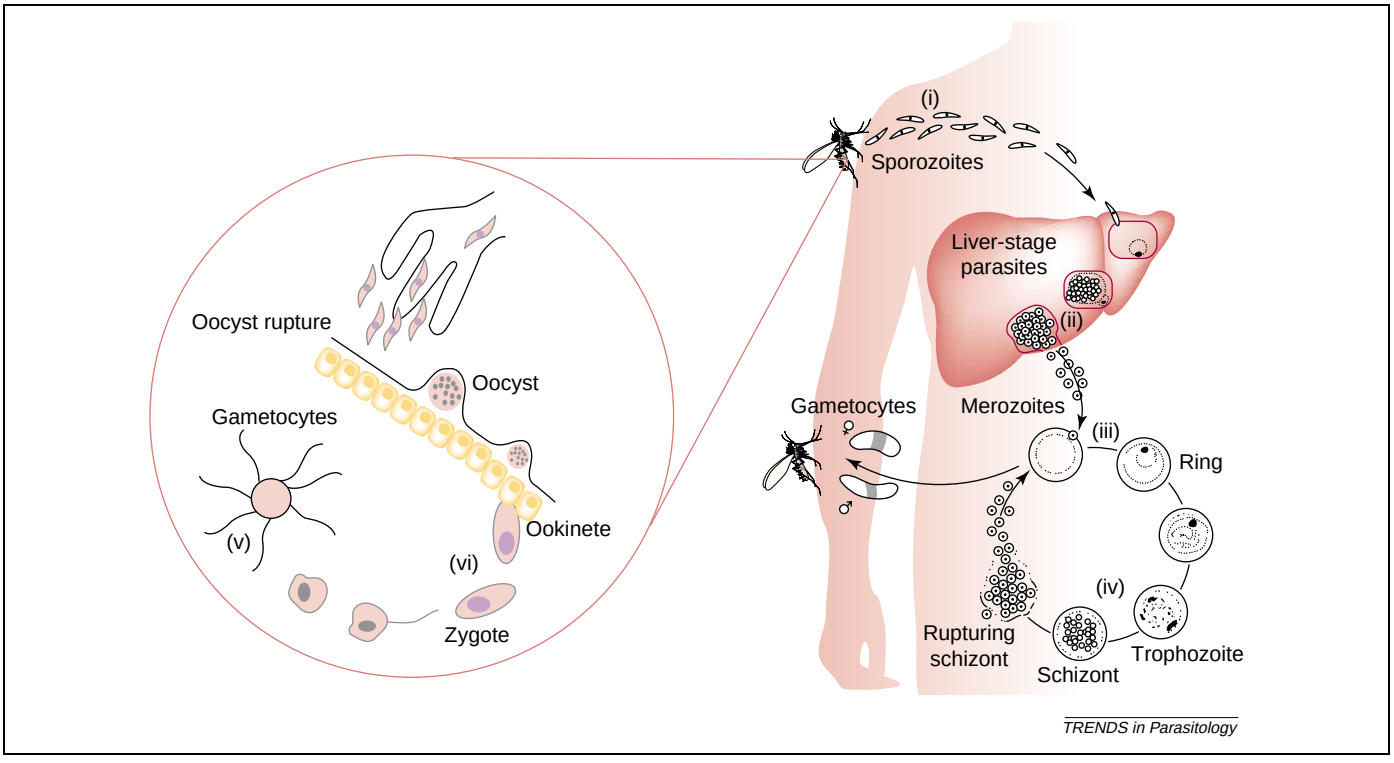

Figure 1. Breaking the malaria life cycle with vaccination. Pre-erythrocytic stages: **(i)** antibodies prevent invasion of hepatocytes by sporozoites. **(ii)** Development of liver-stage schizonts can be stopped by cell-mediated immune responses. Asexual blood stages: **(iii)** invasion of erythrocytes can be blocked by immune responses against the merozoite surface and organellar molecules involved in erythrocyte invasion. **(iv)** Antibodies against selected VSAs expressed on schizont-infected erythrocytes might prevent (*Plasmodium falciparum*) parasite development, probably by preventing cytoadherence. Transmission-blocking: **(v)** antibodies to gamete antigens prevent fertilization from occurring. **(vi)** Antibodies against ookinete antigens prevent further development of the sporogonic cycle in the mosquito. Figure adapted, with permission, from Ref. [51].

schedules, antigen combinations and adjuvants, attempts are being made to exploit the findings from 30 years ago to produce a whole-organism (attenuated sporozoite) vaccine [18]. Of interest is the recent demonstration that irradiated sporozoites enhance the ability of splenic antigen-presenting cells to process and present sporozoite antigens and to prime effector T-cell responses [19]. This might provide a lead as to the type of immune response required for good protection to be achieved [20]. Mueller *et al.* [21] recently described a different approach for producing attenuated sporozoites. They used gene disruption to produce sporozoites deficient in the *uis3* gene, which codes for a protein thought to be involved in sporozoite motility. Immunization with the *uis3*-deficient sporozoites of the rodent malaria parasite *Plasmodium berghei* gave complete protection. The problems associated with the large-scale production of both radiation- and genetically attenuated sporozoites are considerable [22] but the whole-organism approach to vaccination remains a valid alternative to the subunit approaches because it has been shown to provide protection in humans for at least ten months [5].

Asexual-blood-stage vaccines

Although improved vaccine technologies have been central to attempts to develop more-effective pre-erythrocytic-stage vaccines (Box 1), studies of asexual blood stages during the past 20 years have revealed a detailed array of molecules associated with parasite development and pathogenesis, and the natural acquisition of immunity. At the molecular level, the advances have been remarkable during this time and are set to continue with the genomic and proteomic data that are accumulating [23,24]. That said, the asexual-blood-stage target antigen that is undergoing the most intensive investigation is still, as in 1985, the merozoite surface protein MSP-1.

It was already recognized in 1985 that the asexual blood stages are the major target of naturally acquired immune responses, that immunization with merozoite antigens involved in red blood cell invasion (Figure 1) is likely to be complicated by antigenic diversity and that the preferred molecules or epitopes for inclusion in a vaccine might, consequently, be those that are nonvariant [25]. The majority view today would not be very different and it is interesting to consider why the first asexual-blood-stage vaccines are going into clinical trials only now.

Naturally acquired immunity in a highly endemic setting is a state of premunition. Individuals remain 'immune' and asymptomatic because they have low-grade chronic infections [25]. This acquired immunity is induced predominantly by antigens that are polymorphic or that undergo clonal antigenic variation [26]. This contributes to the chronic state of infection by enabling the parasite to evade immune responses. In one of the few clinical trials carried out with asexual-blood-stage antigens, vaccination with MSP-1, MSP-2 and the ring-infected erythrocyte surface antigen (RESA) reduced parasite density significantly, but this was a strain-specific effect [27]. Apical membrane antigen (AMA)-1, another vaccine candidate that was known in 1985, is also highly polymorphic and, similarly, induces strain-specific immunity [28].

A fair conclusion that is drawn frequently from studies of natural immunity is that, for vaccine development, it would be better to focus on cryptic epitopes [24] rather than epitopes of the highly immunogenic polymorphic or clonally variant domains [29]. However, some promising liver- and blood-stage candidate vaccine molecules have been selected after analysis of naturally acquired immune responses [30,31]. Also, the variant surface antigens (VSAs) of parasites that cause severe disease are different from and more immunogenic than those isolated from cases of mild malaria [32]. The possibility of exploiting this as a vaccine strategy is considered later.

Some of the candidate antigens under investigation are poorly immunogenic because they have a limited number of T-cell determinants; hence, they are MHC restricted and induce an immune response in only subsets of the population. There are ways to overcome this that have been known for a long time, notably coupling the relatively small vaccine molecules to carriers containing T-cell epitopes [33]. Other features of the asexual blood stage of infection that could compromise the induction of a strong response to vaccines are that parasitized erythrocytes can suppress maturation of dendritic cells, thus impairing antigen presentation to T cells [34], and can cause apoptosis of malaria-specific T cells and B cells [25]. Infants and young children are the principal target population for malaria vaccines, and maternally derived antibodies might impair the ability of the vaccinated infant to make an adequate antibody response to some candidate vaccines. Vaccine-dosing schedules could overcome this inhibitory effect on antibody production, and $CD8^+$ T-cell responses do not seem to be impaired [35].

These various hurdles pose a considerable problem for subunit vaccine design and probably explain the relative lack of experimental success of blood-stage vaccines, despite the recognition in 1985 that immunization with processed fragments of what is now called MSP- 1 induces protective immune responses [2].

The emphasis on developing candidate asexual-blood-stage vaccines continues to be based on subunit strategies (Box 1) but, stimulated by studies from more than 20 years ago, malaria-naïve volunteers were recently shown to be fully protected against homologous challenge if immunized using extremely low-dose infections by inoculation of ~30 erythrocytes infected with *P. falciparum* on three occasions, with each infection being drug cured eight days after induction. Of particular interest is the observation that protection seemed to be cell mediated rather than antibody mediated [36]. This has the appearance of an innate, cytokine-mediated protection induced early in the infection [37]. However, this re-awakening of the whole-organism approach to vaccination against blood stages requires further investigation to see whether there is a feasible way of exploiting it, perhaps by focusing on antigens that are targets of cell-mediated immunity [38].

In addition, studies of the *P. falciparum* VSAs that are located on the infected erythrocyte surface have revealed a potentially novel approach to vaccination (Figure 1). These variant antigens are responsible for sequestration of the infected red blood cells and for antigenic variation of the parasites. A unique subset of VSAs that binds to

chondroitin sulfate A has been identified on placental isolates of *P. falciparum*. The best-characterized variant is VAR2CSA, which is sex specific and highly transcribed [39,40]. Also, as we have seen, the variant proteins from parasites that cause severe malaria in non-immune patients differ from those expressed by parasites that cause uncomplicated malaria [32,41]. Targeting these small groups of variants offers the intriguing possibility of developing vaccines specifically to protect women in pregnancy and the children most at risk of severe disease.

Transmission-blocking vaccines

It was known more than 20 years ago that immune responses to antigens expressed on macrogametes and microgametes of various *Plasmodium* species modulate transmission to mosquitoes [3] (Figure 1). The Pfs230 and Pfs48/45 gamete antigens of *P. falciparum* have been studied in detail, and antibodies against both block transmission extremely effectively [42,43]. Parallel studies revealed Pfs25 and Pfs28 antigens, and their homologues in *Plasmodium vivax* and other *Plasmodium* species, which are expressed only on the zygote and ookinete stages within the mosquito (Figure 1). Antibodies against these antigens also block sporogonic (oocyst) development in the mosquito extremely effectively [43]. The conformational structure of both protein families has complicated their cloning and expression in an immunogenic form that is suitable for testing as a candidate vaccine. An advantage when testing this type of vaccine is that animals can be immunized with the selected vaccine construct and then their sera can be tested for transmission-blocking activity against the human parasite in a membrane-feeding assay [44]. Phase I clinical trials of recombinant forms of the ookinete antigens of *P. vivax* – Pvs25 and Pvs28 – have started [45] and the homologous *P. falciparum* vaccines will soon be tested. Forthcoming trials will probably focus on series of Phase I safety and immunogenicity trials of different vaccine constructs, but always with the option of testing sera from the volunteers for efficacy in the membrane-feeding assay. Beyond that, transmission-blocking vaccines might be of greatest use in protecting the efficacy of other malaria vaccines by preventing transmission of vaccine-resistant strains of parasite. The recent demonstration [46] that the proteome of male gametocytes contains 36% male-specific proteins and that the proteome of female gametocytes contains 19% female-specific proteins offers the potential to select new targets for sexual-stage-specific vaccines.

Future perspectives

Effective vaccines for malaria must reproduce or, even better, improve naturally acquired immunity. However, the latter, which is directed primarily against asexual blood stages, requires repeated exposure and involves persistence of infection, responses to complex antigenic polymorphisms, immune modulation and immune evasion. On that basis, it has been argued that, to be effective, a vaccine should not induce a sterilizing immunity, certainly against the clinically important phase of infection [47]. The goal for pre-erythrocytic (and transmission-blocking) vaccines remains the prevention of all parasite development, but this is far from being achieved at present. It is doubtful whether any of the vaccines currently scheduled for clinical trials will, on their own, have the efficacy and long-term effectiveness to justify widescale use. This will probably be achieved only with combination, multi-component vaccines.

The first generation of malaria vaccines will probably be used to supplement strategies of vector control and drug treatment for reducing rates of morbidity and mortality [48]. A vaccine that is good enough to be an effective alternative to treatment and vector control remains a more distant goal and might require another 20 years to perfect.

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