Malaria Vaccines: Multiple Targets

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Malaria ranks among the most prevalent, severe infectious diseases of the tropics and is on the rise in many areas. The World Health Organization estimates that 300 to 500 million clinical cases of malaria occur each year, resulting in up to 2.7 million deaths. Most of these cases occur in Africa, but large areas of Asia, Central America, and South America contribute appreciably to these statistics. Each year approximately 1000 cases of malaria are reported in the United States, usually in travelers, migrant workers, and military personnel (*1*). The widespread occurrence of malaria—often caused by drug-resistant parasites (*Plasmodium falciparum* or *P. vivax*) and an insecticide-resistant vector (the mosquito)—has lent particular urgency to the development of a malaria vaccine. Such a vaccine, not yet available, would be a highly cost-effective control measure.

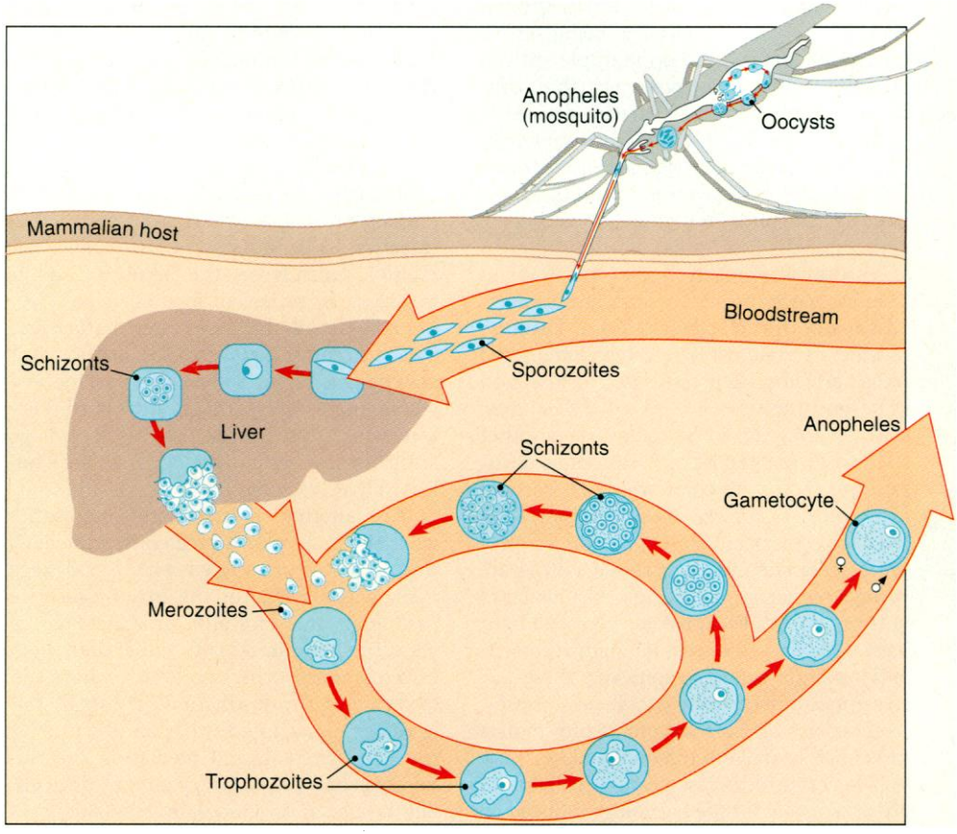
Unlike many acute viral diseases, which produce lifelong resistance to reinfection, malaria only causes immunity after several years of recurring infections and illness. Immunity to malaria acquired in this way is only partially effective and results in milder, sometimes asymptomatic infections, in spite of the persistence of parasites. This immunity is short-lived unless reinforced by frequent reinfection. Thus, in order to be effective a malaria vaccine must do better than the natural immune response and induce extensive, long-lasting, complete protection. In endemic areas, an effective vaccine should protect not only semi-immune persons, but also pregnant women and young children, who most frequently develop severe forms of the disease. A vaccine should also protect individuals from nonendemic areas who become exposed to the parasites and are at risk of developing severe disease.

In humans, the feasibility of inducing complete resistance to malaria by vaccination was first demonstrated in the early 1970s (*2*) and corroborated more recently (*3*). A few human volunteers were vaccinated with irradiated sporozoites (see figure), an immunization procedure that induces full protection in experimental animals (*4*). The vaccine recipients did become resistant to sporozoite challenge, but the unique route of immunization prevents this procedure from being useful for mass immunization: Vaccinees were exposed to multiple bites from irradiated mosquitoes infected with *P. falciparum* or *P. vivax*, the two major species that infect humans.

An in vitro culture system could potentially provide parasites for a vaccine, but only the blood stages of *P. falciparum* have been successfully maintained in continuous culture (*5*). Although the development of this culture system has allowed the characterization of many malarial antigens, the mode of invasion of the parasite, and identification of its erythrocyte receptor (*6*), the culture system is not a source of parasites for mass vaccination; maintenance of *P. falciparum* in vitro requires human erythrocytes and is difficult to scale up for production of very large numbers of parasites.

Current research has therefore focused on antigens produced by recombinant organisms or synthetic peptide chemistry. The choice of potential immunogens is complicated by the multiple stages of parasite development. The sporozoite stage initiates the infection and is then followed by intrahepatocytic stages that, after extensive replication, differentiate into asexual blood stages. Eventually gametocytes develop within some erythrocytes and then differentiate into the sexual stages when taken up by the insect vector (see figure). These distinct developmental forms express stage-specific protective antigens; several of the blood-stage antigens express strain-specific antigenic determinants.

Several approaches have been used to select proteins as vaccine candidates. Some proteins found on the surface of the invasive stages—sporozoites [circumsporozoite (CS) protein] and merozoites [merozoite surface proteins (MSPs)]—and on infected erythrocytes, hepatocytes, or sexual stages have been used. Some antigens have been



Life cycle of the malaria parasite *Plasmodium.* An infected *Anopheles* mosquito bites a vulnerable host and injects sporozoites. Quickly, the sporozoites move through the bloodstream to the liver, where the intrahepatocytic parasites change into schizonts (EEF). They then emerge into the bloodstream and invade, multiply, and rupture red blood cells and burst forth synchronously into the bloodstream, as merozoites seeking new erythrocytes to invade. A few parasites change into the gametocyte form. If picked up by a mosquito, these parasites sexually replicate, differentiate, and multiply. They then migrate as sporozoites to the mosquito's salivary glands and infect the next individual the mosquito bites. [Adapted from (*23*) with permission]

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selected on the basis of their preferential recognition by sera from immune individuals from endemic areas. In some cases the targets of monoclonal antibodies that inhibit *P. falciparum* development in vitro have been used as vaccine candidates. The identification of homologous proteins in simian and rodent malarias has greatly facilitated investigation of potential antigens and their immune mechanisms. However, a biological function has been identified for only a few of these molecules, such as the CS protein, which binds to a hepatocyte receptor and mediates parasite adherence and hepatocyte invasion (*7*).

Most studies of these antigens have been with partial or full-length polypeptides produced in recombinant expression systems. Synthetic peptides have also been used, selected either empirically or on the basis of the amino acid sequence of defined T and B cell epitopes. The first synthetic vaccine to undergo clinical trials in humans contained the immunodominant B cell epitope of the CS protein $(NANP)_3$ and tetanus toxoid as carrier (*8*). Because this synthetic vaccine as well as a recombinant CS vaccine (*9*) were only partially effective, a new generation of CS-based vaccines is being tested. One candidate is based on multiple antigen peptides, a synthetic construct that contains multiple copies of parasite-derived T and B cell epitopes (*10*). MAPs can deliver larger doses of immunogen and incorporate T and B cell epitopes from more than one parasite antigen, including "universal" T cell epitopes.

Another approach is derived from an immune mechanism directed toward infected hepatocytes in rodent malaria. Deletion of $CD8^+$ T cells from sporozoite-immunized mice abrogates their resistance to infection (*11*), and transfer of $CD8^+$ T cell clones, specific for the CS protein or another sporozoite antigen (SSP-2), confers resistance to sporozoite challenge (*12*). Because cytotoxic T cells are most efficiently induced by intracellular expression of proteins, recombinant bacteria and viruses are being engineered to express key malarial antigens or their epitopes. Recombinant *Salmonella* as well as recombinant influenza and vaccinia viruses expressing these CS sequences induce cytotoxic T cells, inhibit liver stages of *P. falciparum*, and induce resistance to malaria infections (*13*).

The erythrocytic stages of the parasite are responsible for the pathology induced by malaria, presenting a particular challenge for vaccine development. Blood-stage parasites are surrounded by several membrane systems and develop within erythrocytes, which lack class I and class II major histocompatibility antigens. However, blood-stage antigens can potentially protect primates against *P. falciparum* challenge. Short peptides from different blood-stage proteins, one being MSP-1, have been linked to NANP sequences from the CS protein. One of these, SPf66, has protected monkeys against blood stages of *P. falciparum* in some studies (*14*).

SPf66 is the first blood-stage vaccine to undergo extensive field trials and has been widely administered in Colombia and some other South American countries. The design of the first clinical trials led to criticism of the claims of efficacy. However, published data have established the safety of this vaccine in adults and children, and a recent double-blind, placebo-controlled study reported a 39% reduction in clinical episodes of malaria (*14*). If these results can be duplicated by others in diverse geographic locations, the feasibility of blood-stage vaccination in humans would be established, providing a basis for attempts to improve its efficacy.

Other blood-stage antigens—MSP-1, SERA (serine repeat antigen), RAP-1 (rhoptry associated protein–1), EBA-175 (erythrocyte binding antigen), and AMA-1 (apical membrane antigen) (*6*, *15*)—have been identified, several of which induce protective immunity in animal models. MSP-1 is the best understood of these (*16*). Affinity-purified native MSP-1 can induce a protective response against malaria challenge in primate and rodent model systems. The conserved carboxyl-terminal region of MSP-1 from the rodent parasite *P. yoelii* can protect mice against an otherwise lethal infection (*17*). Induction of protective immunity depends on the adjuvant and is mediated by serum antibodies, which presumably act as the merozoites mature and escape from the red cells (*18*). Although it may be possible to deliver this vaccine as a recombinant polypeptide, biological vectors, which can encode several blood-stage antigens, are being developed, as are "naked" DNA vaccines.

An alternative to these approaches would be to develop a vaccine that prevents the pathology associated with blood-stage malaria (*19*). One of the major causes of pathology and mortality is adherence of parasitized erythrocytes to the endothelium of cerebral and other capillaries, which can lead to vascular obstruction, frequently followed by coma and death. Identification of the erythrocyte ligand mediating this adherence has proved exceedingly difficult, but has been suggested by some to be a modified version of the host erythrocyte band 3 protein (*20*).

Finally, the sexual parasite stages, which are responsible for disease transmission by the insect vector, are another vaccine target. Such a vaccine would not directly help the immunized individual, but would prevent the spread of disease by blocking mosquito transmission. Transmission might be blocked by nonspecific factors such as cytokines or by antibodies that recognize unique sexual-stage antigens and prevent parasite differentiation in the mosquito midgut. During the past few years the number of malarial proteins identified on gametocytes has increased considerably, and antibodies to some of these antigens block transmission to mosquitoes (*21*). In addition, after the mosquito takes a blood meal, the blood becomes surrounded by a chitinous matrix that the parasite must penetrate to invade the gut wall. Sexual-stage parasites express a chitinase that presumably facilitates penetration of this matrix; this enzyme may be a potential vaccine target (*22*).

The development of an effective vaccine for malaria appears to be in the near future. A highly effective vaccine will likely require a combination of key antigens or epitopes from different developmental stages of the parasite. This approach would permit the interception of plasmodia at successive stages of differentiation. Both humoral and cellular mechanisms of immunity are likely to be required for optimal efficacy. The identification of the most favorable conditions for eliciting selected immune responses may also contribute to vaccine design for other infectious diseases, including AIDS.

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Vaccines for Varicella-Zoster Virus and Cytomegalovirus: Recent Progress

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Despite more than 20 years of research effort, there are no human herpes virus vaccines licensed for use in the United States. Concerns persist about the safety and efficacy of such vaccines, in part because of the complexity of the virus life cycle, which includes latency and reactivation. Human pathogens in this group of DNA viruses include Herpes simplex types 1 and 2, Epstein-Barr virus, cytomegalovirus (CMV), and varicella-zoster virus (VZV), as well as the lesser known herpesviruses 6 and 7. Only CMV and VZV will be discussed here.

Varicella-Zoster Virus

VZV, the causative agent of varicella (chickenpox), is transmitted primarily by aerosolization of droplets from skin lesions. After initial replication in the respiratory tract, the virus moves to the bloodstream and ultimately to the epithelium, where infection is manifested as varicella blisters or vesicles on the skin. The cellular immune system—in particular, T cells and natural killer cells—is critical in suppressing viral replication (*1*). A deficient cellular immune response results in prolonged viral replication in the skin and in the viscera, accompanied by lung and liver disease (Table 1).

Latent VZV infection is established in dorsal root ganglia by ascending infection along sensory nerves from the skin. The site of latency is thought to be the satellite cells around the neurons rather than the neurons themselves (*2*). The latent state is maintained by varicella-specific cellular immunity until the host is immunocompromised by age, disease, or therapy administered for some underlying condition. Under those circumstances, VZV reactivates to cause zoster, a localized varicella restricted to the skin segments innervated by the ganglia in which viral reactivation occurred. In elderly patients, zoster can cause severe and persistent nerve pain.

The greatest progress in prevention of VZV-associated disease has been made not with a high-technology vaccine, but with a classical live attenuated VZV (the Oka strain) that was modified by passage in guinea pig and human cell culture in Japan during the 1970s (*3*). In the United States, a license application for a vaccine made from this virus is now being reviewed by the Food and Drug Administration. The Oka strain has already been licensed for normal (immunocompetent) children in Japan and Korea, where over 2 million have been vaccinated, and it is used on a limited scale in immunosuppressed children in the United States and Europe.

Abundant studies, conducted in thousands of children in the United States by Merck and in Japan by the Biken Institute, have shown that the Oka vaccine induces antiviral antibodies, lymphocyte proliferation responses, and cytotoxic T lymphocyte (CTL) responses (*1*, *3*, *4*). Antibodies persist for at least 8 years and lymphocyte proliferation responses persist for at least 6 years (*5*). Nevertheless, this vaccine does not prevent preschool and school-age children frequently exposed to VZV infection from developing "breakthrough" illness; postvaccination chickenpox occurs at a rate of 1 to 3% per year. Fortunately, the postvaccination illness is almost always mild (*6*). Thus, although 98% of vaccinated children will be protected from severe disease, only 70 to 85% will be completely protected against any form of varicella.

Whether a vaccine is needed to protect normal children against varicella is under debate by pediatricians and public health officials. Although varicella is normally viewed as a mild disease, a small percentage of complications is equivalent to a large number of children, given the millions of cases that occur each year. Furthermore, children who receive modest doses of steroids for diseases such as asthma may die of disseminated varicella. Cost-benefit analyses conducted by the Centers for Disease Control (*7*), as well as purely medical considerations, have convinced both the American Academy of Pediatrics and the

Host status	T cell immunity	Circumstance	Clinical outcome
Immunocompetent			
Susceptible	Normal	Exposure to VZV	Varicella
Immune	Normal	Exposure to VZV	Asymptomatic; boost in T cell immunity
Immune, aged	Poor	Reactivation	Zoster
Immunodeficient			
Susceptible	Poor	Exposure to VZV	Disseminated varicella
Immune	Poor	Reactivation	Disseminated zoster

Table 1. Relation between cellular immunity to VZV and clinical outcome. [Modified from (*1*), copyright 1992, The University of Chicago]

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