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Immunology, pathophysiology, and treatment of malaria

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Despite increasing research and control efforts over the past 20 years, malaria remains one of the world's most significant health problems. As the disease flourishes and drug resistance spreads, the search for vaccines and effective drugs for therapy and prophylaxis becomes ever more important. Recent advances in our understanding of the ultrastructure and biology of *Plasmodia* will aid that quest. For vaccines, work continues on identifying the immune mechanisms and parasite targets responsible for protective immunity and developing methods of constructing the subunit vaccines that will provide such immunity. One promising vaccine, SPf66, is being evaluated in several field trials. Mefloquine, the drug most commonly used for prophylaxis in areas with chloroquine-resistant *Plasmodium falciparum*, remains effective in most areas, except in parts of southeast Asia where high-grade multidrug resistance is prevalent. In prophylactic doses, it has proved to be as safe as chloroquine. For therapy, halofantrine is highly effective in areas with drug resistance. Artemisinin compounds are effective in treating severe malaria caused by multidrug-resistant *P. falciparum*.



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Introduction

Primarily a disease of tropical developing countries, malaria is conservatively estimated to cause 200-300 million new cases and 2 million deaths each year, the majority in sub-Saharan Africa. It also poses a significant threat to travelers. Because of increasing drug resistance and deteriorating economic and social conditions in many malaria-endemic countries, many experts expect the situation to get worse.

The organism's complex life cycle further complicates control efforts. Vaccines and drugs may be effective against one stage of the parasite but have little or no effect against other stages. Infection begins when an anopheline mosquito injects *Plasmodium* spp. sporozoites when taking a blood meal. Within minutes sporozoites invade liver cells, where they multiply. Infected human liver cells rupture after a minimum of 5 days and release tens of thousands of merozoites into the circulation, where the merozoites invade red blood cells (RBCs) and multiply again. After 48-72 h the RBC ruptures, releasing six to 30 new merozoites, which invade other RBCs and start the erythrocytic cycle over again. The liver stage of infection is asymptomatic. It is not until the rupture of RBCs that clinical manifestations occur. Ultimately, some merozoites differentiate into the forms that infect the mosquito, male and female gametocytes. When ingested by an anopheline mosquito, these forms result in the formation of sporozoites, which can then be injected into the host when the mosquito takes a blood meal.

Vaccine development

Vaccine strategies may be directed against any of the above stages. Pre-erythrocytic vaccines focus on inducing antibodies against extracellular sporozoites in the bloodstream, and antibodies, T-cell responses, and cytokines to attack the parasite developing within hepatocytes. Erythrocytic (blood stage) vaccines will be designed to induce antibodies that block extracellular merozoite invasion of erythrocytes, and antibodies and cytokines to attack the infected erythrocyte. Transmission-blocking vaccines may induce antibodies or cytokines that attack gametocytes within erythrocytes, or antibodies that prevent development of the extracellular stages within mosquitoes. A final approach is to limit disease by preventing the release from infected erythrocytes of parasite material that induces the human host to produce cellular products (including cytokines) thought to be important in pathogenesis. It is almost certain that an effective vaccine will have to be multivalent, attacking several parasite stages.

Immunization with irradiated sporozoites induces sterile immunity in humans and laboratory animals, providing the basis for efforts to develop pre-erythrocytic vaccines. Since irradiated sporozoites are not a practical means of immunization, subsequent research has focused on identifying the immune mechanisms and parasite proteins responsible for this protection and producing subunit vaccines. So far subunit vaccines have not proven as efficacious as irradiated sporozoites. Several recent studies

Abbreviations

CSP—circumsporozoite protein; RBC—red blood cell.

have shed light on irradiated sporozoite-induced immunity. Egan *et al.* [1*] demonstrated for the first time that immunization with irradiated sporozoites induces antibodies against regions of the circumsporozoite protein (CSP; the major sporozoite surface protein) outside the immunodominant central repeat region. These nonrepeating (flanking) regions could account for part of the protection resulting from irradiated sporozoites immunization. Rodrigues *et al.* [2*] showed that irradiated sporozoite-induced antibodies alone reduced liver parasites in mice by 47% and that CD4⁺ and CD8⁺ T cells each had strong (and similar) antiparasitic effects. Weiss *et al.* [3**] showed that CD4⁺ T cells were required for protective immunization with irradiated sporozoites, apparently because of their helper functions and not as direct effector cells. By immunizing with a vaccinia virus expressing the *Plasmodium yoelii* CSP and a recombinant influenza virus expressing a *P. yoelii* CSP CD8⁺ T-cell epitope, Li *et al.* [4*] were able to protect 60% of mice; the protection was dependent on CD8⁺ T cells. These studies reinforce the notion that antibodies, CD4⁺ T cells, and CD8⁺ T cells against pre-erythrocytic stages can all be protective independently.

Until now, the longest duration of irradiated sporozoite-induced immunity has been 56 days. Edelman *et al.* [5**] reported a trial in which a patient immunized with irradiated sporozoites was protected when challenged 9 months after his last immunization.

The role of lymphocytes expressing the $\gamma\delta$ T-cell receptor in immunity to malaria was investigated by Tsuji *et al.* [6*]. They found that irradiated sporozoite immunization of $\alpha\beta$ T cell-deficient mice induced an immune response that significantly inhibited development of liver stage parasites, suggesting $\gamma\delta$ T cells may play a role in liver stage immunity.

Identification of specific protective parasite epitopes may be important for subunit vaccine design. Moreno *et al.* [7*] identified a 20 amino acid epitope from the *P. falciparum* CSP that was recognized by CD4⁺ T-cell clones obtained from irradiated sporozoite-immunized volunteers. Most clones recognized variant peptides from different *P. falciparum* strains, suggesting that polymorphism should not be a serious obstacle to inclusion of this epitope in a subunit vaccine. Malik *et al.* [8*] showed that mice immunized with a recombinant *P. falciparum* CSP produced CD8⁺ T cell-dependent cytolytic activity against a 23 amino acid epitope on the CSP. Importantly, adjuvant was not required for this response.

Working in Thailand, Brown *et al.* [9*] studied the safety, immunogenicity, and protective efficacy in humans of R32Tox-A, a recombinant protein derived from the central repeat region of the *P. falciparum* CSP conjugated to toxin A of *Pseudomonas aeruginosa*. Although it was safe and immunogenic, there was no evidence of efficacy despite induction of extremely high levels of antibodies in some volunteers.

Multiple antigen peptide vaccines are synthetic polymers containing multiple B- and T-cell epitopes. Sev-

eral years ago a multiple antigen peptide vaccine based on the *Plasmodium berghei* CSP repeat region was shown to protect up to 80% of mice against sporozoite challenge [10]. Calvo-Calle *et al.* [11*] characterized the immune response in mice to multiple antigen peptides containing the immunodominant B-cell epitope (NANP)₃ and various T-helper epitopes from the *P. falciparum* CSP. The level of response depended on the sequence and stoichiometry of the multiple antigen peptide, and the strain of mice. One of the multiple antigen peptide constructs significantly boosted the anti-sporozoite antibody response in mice immunized with irradiated sporozoites. This opens the possibility of using multiple antigen peptide vaccines to boost immune responses of people living in malaria-endemic areas.

Several blood stage parasite antigens are under investigation. Su *et al.* [12*] reported on the mechanism whereby the major merozoite surface protein-1 binds to erythrocytes. Daly and Long [13**] and Ling *et al.* [14**] reported for the first time that a fragment of merozoite surface protein-1 from *P. yoelii* induced protective immunity in mice. Serine repeat antigen is a *P. falciparum* protein expressed by both liver and blood stage parasites. Tine *et al.* [15*] reported that a recombinant vaccinia virus expressing a serine repeat antigen DNA fragment produced anti-serine repeat antigen antibodies in rabbits and Inselburg *et al.* [16**] demonstrated reduction in parasitemia in *Aotus* monkeys immunized with a serine repeat antigen peptide.

For transmission blocking vaccines, it was demonstrated that antibodies against the mosquito midgut [17*] and the gametocyte protein Pf52400 [18*] inhibited parasite development in the mosquito.

SPf66 is a synthetic malaria vaccine containing three peptides of merozoite origin and one from the CSP of *P. falciparum*. Valero *et al.* [19**] described 1548 volunteers of all ages, half of whom received the vaccine. SPf66 was safe and immunogenic and had a protective efficacy of 39% against *P. falciparum* (34% against first infection). It was most effective in children under 5 years of age and adults over 45 years of age.

Chemotherapy, prophylaxis, and drug resistance

Chloroquine-resistant *P. falciparum* is present in most malarious areas of the world. Resistance to other drugs is common in many areas. Chloroquine-resistant *P. vivax* is now present in Papua New Guinea [20] and Indonesia [21]. This widespread resistance, often to multiple drugs, complicates therapy and chemoprophylaxis of malaria and underscores the need for new and effective drugs.

Since chloroquine is an ideal drug (inexpensive, effective, and well tolerated) in areas where resistance has not developed, work is ongoing to determine the mechanism of chloroquine resistance in an attempt to reverse it [22*].

At present, however, success has been limited and other drugs, generally with greater toxicity, must be used.

Mefloquine is widely used for malaria therapy and prophylaxis. Although highly effective against chloroquine-resistant *P. falciparum* in most areas, there have been concerns about side effects, especially involving the central nervous system. Several recent studies have shown prophylactic doses to be as safe as chloroquine [23[•],24[•],25[•]]. Boudreau *et al.* [23[•]] found that mefloquine caused mild sleep disturbance, increased dream activity, and feelings of depression, which generally decreased over time. Another important issue concerns effective blood levels. In West Africa, Lobel *et al.* [24[•]] suggested that 95% prophylactic efficacy is achieved at whole blood mefloquine concentrations of about 620 ng/ml. Boudreau *et al.* [23[•]] found that protective trough plasma mefloquine concentrations (500–600 ng/ml) were not achieved with 250 mg mefloquine a week until 7 weeks after starting prophylaxis. When a 3-day loading dose (250 mg/day) was used, the mean level was 665 ng/ml after 72 h. The authors suggested a loading dose be considered for short-term adult travelers to areas with chloroquine-resistant *P. falciparum*. Mefloquine resistance has been reported from many areas and is prevalent in some areas of southeast Asia. For therapy, 15 mg/kg suffices in areas where organisms remain sensitive to mefloquine, but for resistant organisms 25 mg/kg is needed. In areas of high-level mefloquine resistance the addition of oral artesunate has proven highly effective [26[•]]. Fortunately, most mefloquine treatment failures respond to quinine and doxycycline [27[•]].

Halofantrine is another drug for therapy of chloroquine-resistant *P. falciparum* infections. Although 24 mg/kg (in three doses at 8-h intervals) is effective for sensitive strains, higher doses are needed for resistant strains and for use in nonimmune patients [28]. In areas of highly drug-resistant malaria in Thailand, high-dose halofantrine was found to be highly effective and better tolerated than both mefloquine and quinine plus doxycycline [29[•],30[•]]. It was especially effective for retreatment of mefloquine failures [29[•]]. When used in higher doses, however, it caused dose-related lengthening of PR and QT intervals in all of 61 patients studied [31[•]]. This arrhythmogenic potential should be considered when using halofantrine.

Artemisinin compounds (qinghaosu) are very promising for the treatment of chloroquine-resistant *P. falciparum*; they decrease parasitemia faster than all other antimalarial drugs with no apparent toxicity [32[•]]. Unfortunately, when compared with quinine for treating patients with cerebral malaria caused by quinine-sensitive parasites, artemisinin compounds have not been shown to reduce mortality. They do, however, decrease the time until the patient regains consciousness [33[•]]. In a study of Rhesus monkeys infected with *Plasmodium coatneyi*, Maeno *et al.* [34[•]] showed for the first time, *in vivo*, that arte-

sunate reduced the rate of parasitized RBC sequestration in cerebral microvessels.

Since anopheline mosquitoes feed at night, bed nets are an important component of malaria prevention efforts. In the Gambia, West Africa [35[•]], permethrin-impregnated bed nets were cost-effective and provided significant protection from mortality, morbidity, and infection in children aged 6 months to 5 years.

Clinical aspects

The pathophysiology of cerebral malaria, the most severe complication of falciparum malaria, is an area of intense research interest. Cerebral malaria is associated with sequestration of parasitized RBCs in the brain microvasculature. Current evidence [36[•]] suggests this phenomenon results from a combination of rosetting (binding of uninfected RBCs and parasitized RBCs) and cytoadherence (adhesion of parasitized RBCs to vascular epithelium). Studies are attempting to define the receptors on the RBC and vascular epithelium as well as the cytokine mediators responsible for this binding. Crandall *et al.* [37[•]] found that two synthetic peptides based on motifs from band 3 protein of human parasitized RBCs inhibited adhesion of *P. falciparum*-infected RBCs *in vitro* and affected sequestration in *Aotus* and *Saimiri* monkeys. Further, monoclonal antibodies against these RBC proteins block cytoadherence. This has important implications for the treatment of cerebral malaria.

Cytokines have been implicated in the pathogenesis of malaria. Tumor necrosis factor, for example, has been associated with severe malaria. Kwiatkowski *et al.* [38[•]] used a murine monoclonal antibody to neutralize tumor necrosis factor in Gambian children with cerebral malaria. Although the therapy did reduce fever, it did not affect mortality.

Malaria is usually diagnosed by observing parasites in Giemsa-stained blood smears. Sensitive diagnosis requires expert interpretation and is time-consuming. Several new methodologies are under investigation, including polymerase chain reaction/DNA probe techniques [39[•]], determination of parasite lactate dehydrogenase in patient serum [40[•]], and a rapid dipstick antigen-capture assay for *P. falciparum* [41[•]]. The dipstick holds special promise because of its ease, speed of use, and high sensitivity and specificity.

General biology

Of particular note in the past year was the discovery by Goonewardene *et al.* [42[•]] of a method for the introduction and transient expression of a foreign gene in a malaria parasite. The ability to transfect malaria parasites with DNA and thereby study the function of malaria

genes will open the way to a better understanding of the basic biology of the parasite. Further work is required to establish methods for the stable transfection of *Plasmodium* parasites.

Plasmodium parasites

Host hemoglobin is a major energy source for intra-erythrocytic *Plasmodium*. Interfering with hemoglobin catabolism is toxic to the parasite. Gluzman *et al.* [43*] reported the isolation and characterization of three proteases that account for the majority of hemoglobin-degrading activity. The proteases were shown to work in an ordered manner, synergistically, and with distinct specificities. This knowledge makes possible the development of drugs that interfere with this essential catabolic pathway.

Antigenic polymorphism is a concern in vaccine development, especially for a complex organism such as *Plasmodium*. If antigenic properties change rapidly then vaccines may only work for a short time. Qari *et al.* [44*] compared present-day CSPs of *P. falciparum*, *P. vivax*, and *P. malariae* with that of organisms collected over the past 50 years and found limited polymorphism. They concluded that such minimal changes should not undermine the use of the CSP as a vaccine antigen.

Other works of interest

A book with nine excellent reviews of molecular immunological considerations in malaria vaccine development [45**] and a review article describing current knowledge of T-cell responses to pre-erythrocytic stages of malaria [46**] were published in 1993.

Conclusion

During the past year there have been a number of extremely important advances that point the way to future work to control malaria. The report from Colombia [19**] that SPf66 provided partial protection in field studies has led to enormous enthusiasm for this vaccine. Studies in progress in Africa, Asia, and South America should establish whether this vaccine is protective in a variety of epidemiologic settings. The report by Daly and Long [13**] and Ling *et al.* [14**] that immunization with purified recombinant *P. yoelii* merozoite surface protein-1 protects mice will undoubtedly provide the foundation for similar studies in humans, and the report by Inselburg *et al.* [16**] showing protection of monkeys with purified recombinant serine repeat antigen should also lead to human trials. Such efforts pro-

vide some of the components for the multicomponent malaria vaccines that most investigators think will be required for effective protection against these parasites.

The major advances in treatment have been to continue to show that artemether and other artemisinin compounds are at least as effective as quinine for treating severe malaria and will probably be as effective in treating quinine-resistant parasites. The work of Crandall *et al.* [37**] demonstrating that band 3 peptides inhibit cytoadherence has the potential to provide a new treatment for severe malaria after extensive additional testing. Because of the rapidity of development of resistance to all antimalarials, and the continued high mortality rates in patients with severe malaria, the development of new antimalarials and adjunct therapies to reduce mortality of severe malaria is still critical.

The finding by Beadle *et al.* [41**] that a dipstick is highly sensitive and specific for diagnosis of *P. falciparum* may revolutionize diagnosis of malaria, particularly by inexperienced technicians in areas where malaria is not transmitted. A similar assay for *P. vivax* must now be developed.

Finally, the demonstration by Goonewardene *et al.* [42**] of transient transfection of *Plasmodium gallinaceum* gametes provides the first step towards an enormously important goal of malarialogists: stable transfection of *Plasmodium* spp. The ability to transfect stably the parasite will revolutionize study of these parasites and must be a major focus of current work.

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