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Malaria Vaccine Development

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THE MALARIA PROBLEM

Malaria is endemic throughout most of the tropical and semitropical world, with transmission occurring in approximately 100 countries where two billion people are exposed to infection. In 1990, the World Health Organization estimated that there were approximately 270 million new infections worldwide each year, resulting in 110 million cases of illness and one to two million deaths, mostly in children (103). Human malaria is caused by one of four parasites, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Two species, *P. falciparum* and *P. vivax*, are the most widely distributed, and both cause a vast amount of human suffering. In the area of vaccine development, considerably more attention has been paid to *P. falciparum* than to *P. vivax*. This is because *P. falciparum* is more virulent and is responsible for more than 95% of the malaria deaths worldwide; until recently, it was also the only human malaria parasite resistant to chloroquine and other antimalarial drugs.

In nonimmune persons, parasitemia is almost always accompanied by illness. While there is no symptom or sign specific to malaria, most patients exhibit some combination of fever, chills, myalgia, headache, nausea, vomiting, and diarrhea. In persons who have acquired, through chronic exposure, some degree of immunity, parasitemia may exist without malaria illness. In general, these immune individuals have complaints similar to those of nonimmune people but of a milder nature. They are also far less likely to die from malaria-related complications.

The number of cases of malaria is increasing yearly. There are several reasons for this, including increasing resistance to insecticides by the vectors, environmental alterations that promote transmission (irrigation and timber clearing), mass movements of people into areas of high transmission, and the increasing inability of developing countries to afford the basic personnel and matériel required for malaria control and treatment. Drugs, the most commonly used tools in the prophylaxis and treatment of malaria, are also becoming increasingly less effective. Resistance to chloroquine, once the drug of choice for the prevention and treatment of all malarias, is common in nearly all areas where *P. falciparum* is transmitted (39, 55). Also of concern is the emergence in the last 3 years of chloroquine-resistant *P. vivax* in Indonesia and New Guinea (2, 62, 79, 102). Resistance to mefloquine, a more recently developed and very promising drug, has already been reported in many parts of the world and approaches 50% in some parts of Thailand (95). The ineffectiveness of malaria control and treatment strategies has resulted in an increase in malaria transmission worldwide, thereby making malaria vaccine development a priority of the first order (65).

BIOLOGICAL BASIS FOR MALARIA SUBUNIT VACCINES

There are several reasons to believe that vaccines to malaria are biologically possible. Persons subjected to long-term exposure to malaria transmission develop a partially protective immune response to malaria. Immune people generally have fewer and less dense parasitemias and are less likely to suffer illness when parasitemic. This immunity also provides signifi-

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cant protection from death (57). This immune protection includes an antibody response that is involved in killing of erythrocytic stage parasites; transfer of immunoglobulin G to naive human volunteers reduces *P. falciparum* parasitemia by as much as 99% (15, 58, 85).

In fact, there is already a way to induce protective immunity to malaria. Immunization of rodents (63, 64) and humans (13, 14, 77, 78) with irradiated sporozoites produces a solid immune protection. In recent studies in humans, nine of ten volunteers were protected from *P. falciparum* when challenged 2 weeks after the immunization regimen was completed, five of six volunteers were protected when challenged 9 months after immunization, and two of two volunteers were protected in a heterologous sporozoite challenge (21, 22, 29, 34). Unfortunately, the irradiated sporozoites must be delivered with the bite of irradiated, infected mosquitoes. This is clearly not a practical method for immunizing more than a few people.

THE PARASITE LIFE CYCLE

The *Plasmodium* life cycle (Fig. 1) is complex but offers several opportunities for interruption. The feeding female anopheline mosquito injects sporozoites into the host. These sporozoites rapidly make their way from the site of injection via the circulation to the liver; the circulation is clear of sporozoites in less than 60 min. After the uninucleate sporozoite has invaded a hepatocyte, it undergoes a huge asexual amplification, producing, in 1 to 2 weeks, as many as 30,000 uninucleate merozoites. When the infected liver cell bursts, the merozoites enter the circulation and recognize, attach to, and invade erythrocytes. In *P. vivax* and *P. ovale*, there is another liver stage form called the hypnozoite, a dormant uninucleate stage responsible for the relapses seen in these two types of malaria. Rupture of the liver schizont and subsequent erythrocyte invasion mark the end of the pre-erythrocytic phase and the beginning of the erythrocytic phase of the parasite life cycle. Once in the erythrocyte, the parasite undergoes another asexual amplification, producing as many as 36 erythrocytic merozoites in a mature schizont; *P. falciparum* produces an average of 16 merozoites per schizont every 48 h. When the schizont ruptures and the merozoites spill into the blood, illness begins. Why illness begins at this point is not known, but it is suspected that parasite components released into the blood induce host responses that induce the symptoms and signs of malaria. Some of the parasitized erythrocytes do not undergo this asexual amplification; instead, they develop into the male and female gametocytes required for species propagation. These gametocytes are taken up in the blood meal of a feeding mosquito, and, after exflagellation, zygote formation occurs in the mosquito midgut. The resulting ookinete penetrates the midgut wall and an oocyst develops. Developing inside this spheroid form are the sporozoites that will ultimately migrate to the salivary glands of the mosquito.

IMMUNE MECHANISMS AND VACCINE STRATEGIES

Both humoral and cellular immune responses play a part in the induction of immunity to malaria. Specific stages of the parasite life cycle are often more vulnerable to one type of immune attack than to others. The first opportunity to stop the parasite is during the brief (<60 min) (25) migration of the sporozoite through the circulation to the liver. It is likely that the only effective immune response is preexisting antibodies directed at surface components of the sporozoites. These antibodies bind to the sporozoite and block effective invasion of and development in the liver cell (44, 59). In one study in which antibodies to the *P. falciparum* circumsporozoite protein were induced by immunization of humans, the antibodies had a half-life of 28 days (3).

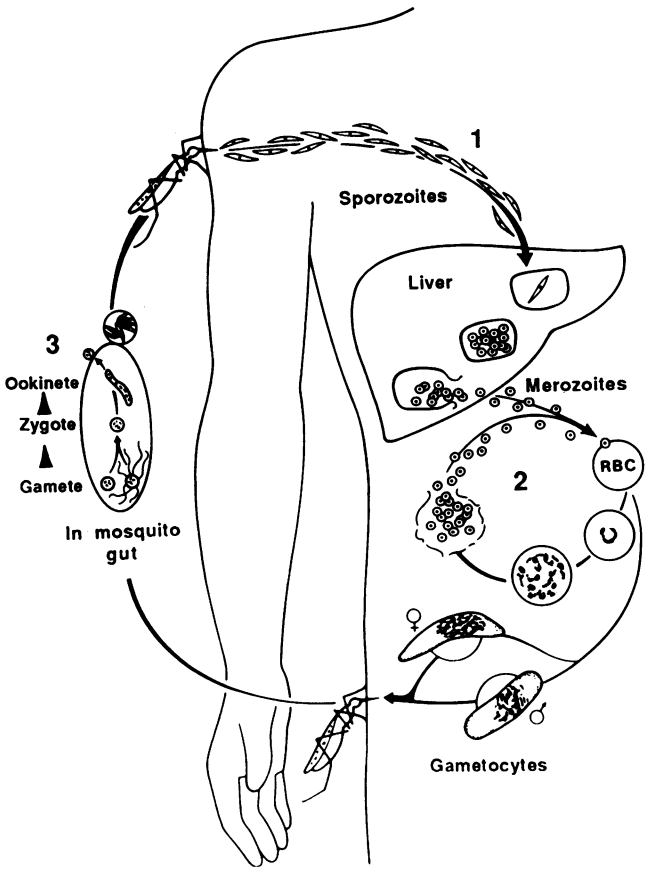

FIG. 1. Life cycle of the malaria parasite, *P. falciparum*. A female anopheline mosquito takes a blood meal during which sporozoites enter the host bloodstream (step 1). The sporozoites invade liver cells and enter a phase of asexual reproduction in which they amplify their number thousands of times by the production of merozoites. The liver cell ruptures and the merozoites spill into the blood, attaching to and invading erythrocytes (RBC), beginning the erythrocytic cycle (step 2). Illness starts when the mature asexual erythrocytic stage, the schizont, ruptures. Other blood stages develop into male and female sexual stage parasites called gametocytes. These gametocytes enter a mosquito as it takes a blood meal. Sexual reproduction occurs in the mosquito midgut (step 3), and new sporozoites migrate to salivary glands, making the mosquito infective.

The liver stage parasite develops within the hepatocyte for at least 5 days, thereby making infected liver cells excellent targets for attack by T cells (41), both $CD8^+$ and $CD4^+$ (75, 101). This type of immune attack is dependent on the expression of parasite antigens on the liver cell surface in conjunction with major histocompatibility complex class I or class II proteins. The mechanisms used by the T cells to destroy infected hepatocytes are not clear but could include (i) necrotic cell death induced by contact between the lymphocyte effector and target cells, (ii) programmed or apoptotic death in which the effector cell induces the target cell to initiate a series of steps that result in its own death, (iii) killing through the release of cytokines such as gamma interferon that induce the hepatocyte to produce nitric oxide (60), (iv) killing via antibody-dependent cellular cytotoxicity, and (v) killing by antibody alone.

Merozoites, like sporozoites, are free in the blood for a short time. They must recognize and attach to an erythrocyte in order to invade and are probably sensitive only to neutralization by circulating antibody which binds to merozoite surface components essential for invasion.

An erythrocytic stage parasite, because it is inside a cell which does not express either class I or class II major histocompatibility complex, is not a target for T-cell attack. Infected erythrocytes are, however, liable to binding by antibody (6, 15, 58, 85). This binding could result in opsonization, thereby making the parasitized erythrocytes more susceptible to clearance by phagocytic cells in the spleen and perhaps to destruction by the fixing of complement (6).

Immune responses against the stages of the parasite just described would, if entirely effective, prevent illness in the host by preventing the development of or by eliminating asexual blood stage parasites. Another approach is to induce immunity to the sexual stages of the parasite, particularly those appearing in the mosquito midgut. Antibody that enters the mosquito when it takes a blood meal is available to disrupt sexual reproduction. Vaccines capable of blocking sexual reproduction of the parasite are called transmission-blocking vaccines and would not offer protection from illness to vaccinated individuals. If enough people in the community were immunized with such a vaccine, however, the cycle of transmission could be broken and everyone could be protected from infection. Another strategy is the development of "anti-disease" vaccines.

Malaria causes much morbidity worldwide. Even a vaccine incapable of eliminating an infection will be useful if it can reduce the incidence or severity of illness. Disease is probably caused by the release into the bloodstream of parasite products that induce responses by the host (cytokine release, for example) that cause the symptoms associated with malaria. Anti-disease vaccines would induce neutralizing antibodies to these parasite products (71). The antibodies would reduce the interaction between the parasite components and reactive immune cells.

P. falciparum has a characteristic which makes it a particularly dangerous parasite. Parasitized erythrocytes can adhere to the endothelium of the microcirculation of the host. This cytoadherence is probably the virulence factor that allows *P. falciparum* to cause cerebral malaria, a form of falciparum malaria in which cerebral blood flow is compromised by parasitized erythrocytes sticking to cerebral vessel walls. Because adults in areas where malaria is endemic have had long-term exposure to malaria and are partially immune, cerebral malaria is most commonly seen in children. This condition uniformly results in death unless aggressively treated. Because cytoadherence requires recognition and attachment, a vaccine that induces antibodies to the components on the parasitized erythrocytes required for endothelial attachment could eliminate the adherence and thereby provide the host with protection from death by cerebral malaria.

VACCINE DESIGN

Rationally designed modern vaccines contain several components, each with a specific function. Synthetic peptide and purified recombinant protein vaccines intended to induce antibodies have one or more B-cell epitopes. T-cell epitopes are also included in such vaccines to induce T-cell help. These epitopes are then presented to the antigen-presenting cells in some form of delivery system. Vaccines intended to raise either a $CD4^+$ or a $CD8^+$ cytotoxic lymphocyte (CTL) response contain one or more cytotoxic T-cell epitopes, perhaps a T-helper lymphocyte epitope, and a delivery system designed to optimize interaction of the vaccine with the antigen-presenting cells. Examples of delivery systems include adjuvants such as aluminum hydroxide, self-assembling lipid spheres such as liposomes, live vectors such as transfected live vaccinia virus, *Mycobacterium bovis* BCG, and salmonellae, and specially designed vaccine structures such as multiple-antigen peptides in which a peptide core is synthesized with B- and T-cell epitope "branches" extending from the core.

MALARIA VACCINE ANTIGENS

A variety of promising parasite antigens from several life cycle stages have already been discovered and are being developed as malaria vaccine components. Those discussed below do not constitute an exhaustive list but are the best characterized.

Pre-Erythrocytic Antigens

CS protein. The circumsporozoite (CS) protein is expressed in large amounts on the surface of the sporozoite (104). Its molecular weight varies depending on the species. For example, *P. berghei* CS protein mass is estimated at 45 to 55 kDa (104); *P. yoelii* CS protein, at 65 to 75 kDa (11); and *P. falciparum* CS protein, at 40 to 66 kDa (19). Immune sera precipitate it (72, 97), and it has a central area of repeated amino acid sequences that are highly immunogenic and are present in all *Plasmodium* species studied to date. Monoclonal antibodies against the repeat regions of *P. berghei* (23, 72), *P. yoelii* (1a, 12), and *P. vivax* (10) all protect when passively transferred to animals prior to challenge with infectious sporozoites. The repetitive amino acid sequence in the *P. falciparum* CS protein is asparagine-alanine-asparagine-proline (NANP) (19). This immunogenic sequence has been used as the immunogen in at least 15 human vaccine trials, some of which have been reported (3, 28, 35). In a recent trial, for example, 2 of 11 vaccinated subjects were fully protected from sporozoite challenge (40). These results have been encouraging enough to justify further investigation. In addition, the passive transfer of cytotoxic $CD8^+$ T cells which recognize the CS protein has protected mice from challenge in both the *P. berghei* (84) and *P. yoelii* (81, 100) murine malaria models. Immunization of mice with synthetic peptides from the *P. yoelii* CS protein induces both $CD8^+$ and $CD4^+$ cytotoxic T cells that specifically eliminated infected liver cells in culture (75, 101). Studies of humans immunized with irradiated *P. falciparum* sporozoites demonstrated the presence of $CD8^+$ and $CD4^+$ cytotoxic T cells that specifically recognize portions of the *P. falciparum* CS protein (56, 61). Collectively, these studies illustrate that cytotoxic T-cell-dependent, antibody-independent protection based on immunity to the CS protein can be induced and suggest that the *P. falciparum* CS protein is an excellent candidate for development as a vaccine that induces both antibody and cell-mediated immunity.

SSP2. Recently, a second sporozoite-associated protein was identified on the surface of the sporozoite of the murine malaria parasite *P. yoelii* (11, 33, 83) and named sporozoite surface protein 2 (SSP2). Transformed mouse cells expressing this protein were used to immunize naive mice, and partial protection from subsequent sporozoite challenge was induced (48). When mice were immunized with both SSP2- and CS protein-expressing cells, all were protected (48). This protection is $CD8^+$ T-cell dependent, and transfer of T-cell clones protects naive animals (49). The *P. falciparum* analog has been characterized (82) and shown to be the previously described

protein known as thrombospondin-related anonymous protein (80).

LSA-1. Liver stage-specific antigen 1 (LSA-1) is a 230-kDa protein first expressed in infected hepatocytes and contains an immunogenic 17-amino-acid repeat (32, 105). Recently, it was discovered that naturally exposed West Africans produced HLA-restricted cytotoxic T cells that recognized a nine-amino-acid peptide from LSA-1 in association with HLA Bw53. The presence of HLA Bw53 correlated with protection from severe malaria, suggesting, but certainly not proving, that immunity to LSA-1 provides some protective immunity against severe malaria (36, 37).

Merozoite and Erythrocytic Antigens

EBA-175. Erythrocyte binding antigen 175 (EBA-175) is a 175-kDa protein expressed on the surface of merozoites that is thought to act as a ligand for the attachment of merozoites to specific receptors on erythrocytes (9). The gene encoding it has been sequenced, and parts of that sequence suspected to encode B-cell epitopes were synthesized and used to immunize rabbits. The hyperimmune serum at a 1:5 dilution reduced merozoite invasion of erythrocytes in vitro by 80% and inhibited the binding of purified native EBA-175 to erythrocytes (90).

MSP-1. Merozoite surface protein 1 (MSP-1), also known as MSA-1, is a 195-kDa protein synthesized during the development of the schizont; it is recognized by serum from immune individuals and is present as a complex of proteolytic fragments on the surface of the merozoite (5, 43). Although some fragments are shed, the 19-kDa fragment ($MSP\text{-}1_{19}$) of this protein is retained during erythrocyte invasion and is present within the newly invaded cell. Monoclonal antibodies to *P. falciparum* and *P. yoelii* $MSP\text{-}1_{19}$ can reduce the efficiency of erythrocyte invasion (5, 7). A homologous protein is also present on the merozoites of *P. vivax* (20). Immunization with *P. falciparum* MSP-1 protects *Saimiri* (24, 69) and *Aotus* (89) monkeys from infection and provides either partial or full protection in the *P. yoelii* murine malaria challenge model (27, 42). In recent studies, a 15-kDa recombinant subunit of *P. yoelii* MSP-1 successfully protected mice from lethal challenge (18).

MSP-2. Merozoite surface protein 2 (MSP-2) is a 45-kDa protein anchored in the merozoite membrane (74, 91). The molecule contains a central repeat region, but analysis of several *P. falciparum* isolates indicates significant sequence variation in about 50% of the protein. Both the N- and C-terminal regions are, however, well conserved (92). *Saimiri* monkeys were immunized with a recombinant vaccinia virus containing the entire gene encoding MSP-2. The animals mounted relatively weak antibody responses which increased after the animals were exposed to merozoites during the challenge. None of the animals was protected from parasitemia (73).

RESA. Ring-infected erythrocyte surface antigen (RESA), also known as Pf155, is a 155-kDa protein synthesized by *P. falciparum* during late schizogony and expressed on the surface of erythrocytes infected with ring stage parasites (17, 68). It is deposited in the membrane of the erythrocyte during invasion. RESA contains tandemly repeated amino acid sequences, and human antibody to it effectively inhibits the invasion of erythrocytes by merozoites in vitro (99). *Aotus* monkeys were immunized with two fusion proteins containing tandem repeats. The monkeys were not protected from infection but were protected from overwhelming parasitemia (16). *Saimiri* monkeys, on the other hand, were immunized with similar constructs, but no evidence of protection was observed (73).

SERA. Serine repeat antigen (SERA), also known as serine protein I and p126, is a protein located within the parasitophorous vacuole. In *P. falciparum*, it weighs 111 to 113 kDa and has a serine content of 11% (8, 51). *Saimiri* monkeys immunized with SERA were protected from subsequent challenge with infectious parasites (70). Two recombinant *Escherichia coli*-expressed hybrid proteins both containing partial sequences of SERA and histidine-rich protein II and one also containing MSP-1 were used to immunize *Aotus* monkeys. These monkeys were protected from subsequent challenge with *P. falciparum* (50). The SERA sequence contained in these recombinant proteins was subsequently shown to be conserved in six *P. falciparum* isolates from various parts of the world (52).

Transmission-Blocking and Pathogenicity Antigens

Pfs25. Pfs25 is one of the most promising transmission-blocking vaccine immunogens. It is a 25-kDa protein found on the surface of *P. falciparum* zygotes and ookinetes. Monoclonal antibody to it completely blocks transmission by interfering with the development of the sexual stages of the parasite within the mosquito (98). Mice immunized with a transfected vaccinia virus produced polyclonal antibody that was also capable of inhibiting sexual stage development (47), and a yeast-expressed recombinant Pfs25 elicited transmission-blocking polyclonal antibodies in both mice and monkeys (4).

Cytoadherence antigens. Several molecules mediating infected erythrocyte adherence to vascular endothelial cells have been identified but remain less well characterized than parasite molecules from other parts of the life cycle (45). One is sequestrin, a 270-kDa protein expressed on the surface of infected erythrocytes and the ligand for CD36, a recognition protein expressed by vascular endothelium and its experimental surrogate, C32 melanoma cells. A monoclonal antibody to CD36 was used to immunize rabbits and produce an idiotypic antibody. This idiotypic antibody mimicked the CD36 binding domain for attachment to infected erythrocytes and binds to infected erythrocytes, thereby preventing cytoadherence in vitro (66). An immunogen capable of inducing antibody equivalent to this idiotypic antibody may be useful in inducing an anticerebral malaria immunity, and a monoclonal antibody equivalent of the idiotypic antibody itself could be used as a passive protection agent. Other data indicate, however, that the in vivo situation may be more complicated than that predicted by the CD36-based in vitro model (38). The sequestrin gene, however, remains to be cloned.

Anti-illness antigens. There is much suspicion that cytokines such as tumor necrosis factor, the interleukins, and gamma interferon mediate the onset of illness in infected persons (30, 71, 88). Two glycoproteins (60 and 77 kDa) released upon schizont rupture have been isolated and shown to induce the release of tumor necrosis factor from human monocytes (94). Tumor necrosis factor is a potent pyrogen, and ruptured schizonts induce the release of large amounts of it from cocultured monocytes (54), suggesting that neutralization of these two glycoproteins may be useful in illness reduction.

MALARIA VACCINE DELIVERY SYSTEMS

Delivery systems are required to assure the optimal interaction among vaccine epitopes, antigen-presenting cells, and effector cells (B and T lymphocytes). A common component of a delivery system is an adjuvant that is a nonspecific immunopotentiator. The only adjuvant currently in general use is aluminum hydroxide (alum). Early *P. falciparum* sporozoite vaccine trials (3, 35) suggested that higher levels of antibodies

to sporozoites were required, and as a result, new delivery systems designed to improve antibody response have been developed. An adjuvant consisting of mycobacterial cell wall skeleton, monophosphoryl lipid A, and squalane (Detox) was used with a *P. falciparum* sporozoite vaccine to immunize humans; it induced significantly higher antibody titers than did the same immunogen delivered with alum (40, 76).

Liposomes, another alternative to alum, are lipid spheres that self-assemble in aqueous solution. In a recent study, liposomes that contained a *P. falciparum* CS protein epitope and monophosphoryl lipid A were constructed. This preparation induced 5 to 15 times higher antibody levels than did the same immunogen adsorbed to alum. Sera from the subjects vaccinated with the highest doses inhibited by 92% the invasion of cultured hepatoma cells by *P. falciparum* sporozoites (28).

A synthetic muramyl dipeptide combined with monophosphoryl lipid A was used as the adjuvant to immunize rabbits to MSP-1. This preparation induced titers equivalent to those induced with Freund's complete adjuvant, a very potent preparation not available for use in humans due to significant toxicity (46).

Another approach to the induction of immune responses is to use recombinant live vectors. These vectors may prove to be the most effective method of inducing CTL responses and in delivering the recombinant parasite antigens they express directly to antigen-presenting cells. A recombinant vaccinia virus containing the CS protein gene from *P. falciparum* was used to immunize mice which developed a CTL response that specifically killed target cells expressing the CS protein. This study provided the first demonstration of a CTL epitope on a malaria parasite (53). A herpesvirus, pseudorabies, carrying the CS protein gene from *P. yoelii* has been used successfully to induce both high antibody titers and a genetically restricted, antigen-specific, $CD8^+$ T-cell-dependent CTL response to target cells expressing *P. yoelii* CS protein (87).

Salmonella typhimurium engineered to express the CS protein of *P. yoelii* was used to immunize mice, and a CS-specific CTL response developed (26). The mice were not, however, protected from subsequent sporozoite challenge. The CS protein of the rodent malaria parasite, *P. berghei*, has been inserted in an attenuated *S. typhimurium* strain and then used to immunize mice orally. The mice developed a genetically restricted, antigen-specific, $CD8^+$ T-cell-dependent immune response that protected some of the mice against malaria. This protection was antibody independent (1, 86).

The construction of multiple-antigen peptide systems (MAPs) is a very different approach to vaccine development. A MAP is a synthetic oligomeric lysine core with a number of arms branching from it. The arms include B- and T-cell epitopes. Certain configurations of this construct containing a sequence from the CS protein of *P. berghei* proved highly immunogenic even after only one immunization. The antisera generated against the MAP were highly reactive with recombinant CS protein in an immunoradiometric assay and bound to *P. berghei* sporozoites when diluted 1:100,000. MAPs containing both B- and T-cell epitopes induced levels of protection from sporozoite challenge ranging from 50 to 80% (93). Recent studies indicate that MAPs are recognized by antigen-specific T lymphocytes induced by immunization with immunogen containing the same epitopes (31).

MULTIPLE-COMPONENT MALARIA VACCINES

The cloning and sequencing of the malaria antigens just described and other less well characterized ones make the production of a multivalent malaria vaccine a real possibility. An advance has already been made in that direction with SPf66, a synthetic polymer described as consisting of three peptides from the *P. falciparum* merozoite and one peptide from the repeat region of the *P. falciparum* CS protein. Initial studies indicated that this vaccine delayed or suppressed the onset of parasitemia when immunized human subjects were challenged with blood stage parasites (67). A recent trial in 1,548 human volunteers indicates that SPf66 is safe, immunogenic, and protective against *P. falciparum* infection. The vaccine is reported to provide a protective efficacy of 39% (34% against first infections) in a study population containing volunteers of all ages (96).

The goal of a fully protective multivalent vaccine is to make the vaccinated human an incompetent host. The vaccine will be designed to provide some level of immunity to several stages of the parasite life cycle. In theory, then, antibodies to the CS protein and SSP2 neutralize the majority of sporozoites while they are still in the bloodstream. The few that survive invade liver cells, but because the parasitized liver cells express *Plasmodium* antigens such as LSA-1, CS protein, and SSP2, $CD8^+$ and $CD4^+$ CTL and perhaps antibody-dependent immune mechanisms destroy the great majority of these infected liver cells. Every liver schizont that survives can produce as many as 30,000 merozoites, so it is important to reduce the parasite burden in liver as much as possible. Surviving merozoites released into the blood will be neutralized by antibodies to antigens such as MSA-1, MSA-2, and EBA-175. A few merozoites may survive to invade erythrocytes but antibody to antigens like RESA and SERA will neutralize those. The vaccine will also induce neutralizing antibody to sexual stage antigens such as Pfs25 so that any vaccinated persons developing an uncontrolled blood stage infection will be unable to infect mosquitoes.

It is unlikely that the first generation of malaria vaccines will provide comprehensive protection under all circumstances. There may be different vaccines for different situations. One vaccine may work best in persons chronically exposed to high levels of transmission, perhaps due to natural boosting. Another vaccine might be most useful in inducing short-term protection for travelers. It is very possible that vaccines will prevent the development of serious disease without actually preventing infection. The discovery and characterization of the antigens described above, the development of improved delivery systems, and the increasingly refined understanding of the immunology of malaria all give good reason to be optimistic about the future of malaria vaccine development.

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