Perspective

Progress Toward Malaria Preerythrocytic Vaccines

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THE WORLD HEALTH ORGANIZATION ESTIMATES THAT there are 270 million new cases of malaria every year and that 2.1 billion people live in malarious areas (*1*). Malaria is transmitted to humans by anopheles mosquitoes that inoculate malaria sporozoites, most commonly either *Plasmodium falciparum* or *P. vivax*, during feeding. The parasite has a complex life cycle in the human host and presents a number of potential targets for vaccine-induced immune attack.

Development of effective synthetic or recombinant peptide vaccines against malaria has been slower than some had anticipated. The reasons for the delay are not intrinsic to malaria but reflect the challenge in developing modern subunit vaccines: assemble defined synthetic or recombinant peptides with adjuvants and delivery systems in such a way as to consistently elicit protective immune responses. A complication peculiar to parasites is that protective immune responses to these organisms are usually stage-specific; the parasite can escape from a protective immune response when it transforms from one stage of its life cycle to the next. Therefore, a universally effective malaria vaccine will probably have to include antigens from different stages of the parasite life cycle.

Several antigens from the erythrocytic stages of the parasite have been used in limited trials in monkeys, and one candidate vaccine that has been tested in humans was shown to be partially effective (*2*). Our primary efforts focus on the production of vaccines that induce protective immune responses against the preerythrocytic stages of the parasite: the sporozoites free in the bloodstream before they reach the liver and the exoerythrocytic form (EEF) of the parasite found in the hepatocytes. Protection against sporozoite challenge was obtained more than 15 years ago by immunization of rodents, monkeys, and humans with radiation-attenuated sporozoites (*3*). Although protection was stage- and species-specific, it was not strain-specific. Vaccination with one parasite isolate elicited protection against isolates from different areas of the world. These human studies have recently been repeated (*4*). Six of seven volunteers were protected, and their sera and leukocytes are being used to identify the targets and mechanisms of protective immunity.

In malarious areas, exposure to sporozoites for 20 or more years leads not to protection against infection, but to a partial resistance that generally prevents serious illness and death. The reason for the higher degree of effectiveness of the sporozoite vaccines may be that individuals living in endemic areas are exposed to relatively few and intermittent infective bites. In regions of intense transmission, it is rare for an individual to receive more than 100 infective bites per year, whereas protection by vaccination with irradiated sporozoites requires multiple exposures to hundreds of infected mosquitoes.

Because it is impossible to produce enough sporozoites to immunize large populations, researchers have focused on identifying the relevant sporozoite antigens. Studies in mice resulted in the identification of the major component of the sporozoite surface coat, the circumsporozoite (CS) protein, and showed that monoclonal antibodies directed against its immunodominant B epitope resulted in protection in vivo. This B epitope is located in the central area of the CS protein and consists of tandem repeats of species-specific amino acid sequences. In *P. falciparum* this epitope, Asn-Ala-Asn-Pro (NANP), has been detected in all isolates and thus represents an ideal target for vaccine development.

In the mid-1980s when these observations were made, many researchers thought it would be simple to produce effective vaccines based on the repeats. Initial trials with synthetic or recombinant peptides administered with aluminum hydroxide resulted in induction of only modest levels of antibodies in most recipients. However, volunteers with the highest levels of antibodies to sporozoites were protected against *P. falciparum*, and, in others, onset of parasitemia was delayed, indicating that a large proportion of sporozoites had been destroyed.

Studies in animals of passive transfer of monoclonal antibodies to the repeats of the CS protein leave little doubt that antibodies (and Fab fragments) can provide protection against sporozoite-induced malaria. The challenge is for researchers to engineer vaccines that elicit levels of antibodies in humans with the appropriate specificity and sufficient affinity to destroy all of the sporozoites before they are sequestered in hepatocytes.

Three components of a subunit vaccine that is designed to produce antibodies to a short peptide epitope are the immunogen or B cell epitope, the carrier protein that activates T cells to "help" the appropriate B cells, and the vaccine delivery system, which provides optimal interactions of the B and T cell epitopes in the vaccine with the host's immune system. The problem of consistently producing high levels of antibodies to the CS repeats, $(NANP)_n$, has been solved by changes in carrier proteins and in delivery systems. When the $(NANP)_n$–B cell epitope was conjugated to *Pseudomonas* toxin A (the carrier protein) and administered with the adjuvant aluminum hydroxide (the delivery system), antibody levels were increased fivefold over those of previous vaccines (*5*). In another construction, $(NANP)_n$ was fused with 81 amino acids from the nonstructural protein of influenza A and mixed with an adjuvant containing monophosphoryl lipid A (MPL), the cell wall skeleton of mycobacteria, and squalane (*6*) or was incorporated with MPL into liposomes (7). When either preparation was administered to volunteers, the geometric mean concentrations of antibodies to CS were 25 to 33 μg/ml, as compared to 2.3 μg/ml when the same vaccine was administered with aluminum hydroxide.

However, the level of antibodies to the CS repeats has not always correlated with protection (*8*), suggesting that specificity may be as important as quantity of antibody. This view is supported by the observation that a recombinant vaccine including only nine amino acids of the central repeat region of the *P. vivax* CS protein (*9*) did not induce antibodies in monkeys or humans to a four–amino acid protective epitope within the nonamer (*10*). Construction of immunogens that focus the antibody response may be necessary to consistently achieve protection.

Studies in mice suggest other approaches for increasing antibody levels. One strategy involves the use of synthetic vaccines that consist of multiple antigen peptides (MAPS). A MAPS vaccine including B and T epitopes of *P. berghei* CS protein elicited high titers of antibodies and protected 80% of mice from sporozoite challenge (*11*). Another approach is to couple the CS protein peptides to

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carriers that humans are widely sensitized against. Many children in malarious areas are exposed to tuberculosis, immunized against tuberculosis with *Bacille* Calmette–Guerin (BCG), and immunized against tetanus toxoid. The protein-purified derivative of mycobacteria (PPD), BCG (*12*), and a defined epitope from tetanus toxoid (*13*) have provided excellent T cell help for NANP in mice.

After the last immunization with synthetic and recombinant CS vaccines, antibody levels have generally decreased by at least 50% within 6 months. This may be satisfactory for nonimmune travelers to endemic areas, but residents require long-term protection. It is necessary to maintain antibodies at high levels for years, preferably with a single dose of vaccine. The solution may involve novel adjuvants and vaccine formulations that lead to timed and prolonged release of antigen that would provide their own booster doses.

Another approach relies on natural exposure to sporozoites to boost antibody responses. For this to occur, the CS vaccine would have to contain T helper epitopes present in the native CS protein. The observations of restricted murine T cell recognition of CS protein peptides have led researchers to speculate that it might not be possible to engineer a CS vaccine that could sensitize T cells of most humans. This hypothesis was supported by limited field studies showing that lymphocytes from a majority of individuals did not recognize a series of CS-derived peptides (*3, 14*). However, a peptide from the COOH-terminal region of the CS protein was recognized by lymphocytes from most individuals, suggesting that it might function as a universal T epitope. Also, NANPNVDPNANP (V, Val; D, Asp), a nonvariant part of the repeat domain, was recognized by $CD4^+$ T cell clones from a volunteer vaccinated with irradiated sporozoites (*15*). These and other T cell epitopes could be included in vaccines, but whether they will provide T cell help is not known.

Only antibodies to the central repeats of the CS proteins have been shown to be protective in vivo. However, because antibodies to other sporozoite and liver stage antigens (*16*) inhibit sporozoite invasion of hepatocytes, there may be additional antigens that could be included in vaccines.

Antibodies are not the only protective effector mechanism induced by sporozoite vaccines. Immunity elicited by irradiated sporozoites can be eliminated in some strains of mice by treatment with antibodies to $CD8^+$ T cells, and adoptive transfer of immune T cells is protective. The EEFs have been identified as targets of this cell-mediated immunity (*17*). The liver stages of the parasite are attractive targets for vaccine development because they offer additional antigens for attack, and, in contrast to sporozoites, which remain for minutes in circulation, human malarias develop within hepatocytes for a minimum of 5 days. The effector mechanisms directed against EEF are almost certainly either cytotoxic T cells (CTLs) that destroy the infected hepatocytes or cytokines (such as interferon γ) that inhibit EEF development.

There is substantial evidence in rodent models that T cells recognize CS (*18–21*) and non-CS epitopes (*22, 23*) and that they mediate protection, and that the protection can be additive (*23*). However, the mechanisms of sensitization of effector T cells and destruction of liver stages in vivo are unknown. CTLs recognize malaria antigens on infected hepatocytes in vitro (*17, 18*), but it has not been established that they directly contact infected hepatocytes in vivo. CTLs may also recognize parasite antigens on macrophages or other antigen-presenting cells and release lymphokines that inhibit liver stage development.

Work is under way to construct human vaccines that induce CTLs. Methods include the transformation of live vectors such as *Salmonella* (*19*), BCG, and vaccinia with the relevant malaria genes and the use of liposome-associated immunogens, ISCOM particles (antigen complexed to saponin) (*24*) and peptides that represent the epitope (coupled to a lipid tail) (*25*). Work on these vaccines was initially based only on experiments in mice because humans have never been shown to produce CTLs to any malaria protein. However, three of four volunteers immunized with irradiated *P. falciparum* sporozoites have now produced CTLs to a single defined epitope on the CS protein (*26*).

This human CTL site and a murine T helper epitope on the CS protein are polymorphic among strains of *P. falciparum*, suggesting to some investigators that variants have been selected by CTLs and that vaccine-induced CTLs will select for resistant parasite strains. If polymorphic CTL epitopes on the CS protein are the major contributors to protective immunity and if variation in the epitopes alters recognition by CTLs, then protective immunity to sporozoites will be strain-specific. It is therefore encouraging that volunteers vaccinated with one strain of irradiated *P. falciparum* sporozoites have been consistently protected against challenge with other strains and that a single amino acid change in the *P. falciparum* CTL epitope did not alter human CTL recognition (*26*).

An effective CS vaccine could lead to antibody selection of repeat variants. These have not been detected in *P. falciparum*, but unrelated variant repeats are present in *P. vivax* (*27*), and extensive variation in repeats occurs in monkey malarias. If *P. falciparum* repeats vary without affecting parasite viability, then the formulation of vaccines will be complicated. However, single nucleotide substitutions have been detected in the codons for NANP in many isolates of parasite, but, without exception, they are all synonymous. This may signify that variants are selected against and that NANP repeats have a survival value for *P. falciparum*.

Obstacles remain, but great advances have been made in the 6 years since the characterization of the gene encoding the *P. falciparum* CS protein. The pace of development of sporozoite and blood stage vaccines and the rapid application of basic new immunologic principles to vaccine formulation are reasons for optimism, even though a highly effective, long-acting, multivalent vaccine against malaria will probably not be available in the next few years.

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