Malaria Vaccine Development
Status Report

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Introduction

Malaria continues to claim an estimated 2 to 3 million lives annually and to account for untold morbidity in the approximately 300 to 500 million people infected annually[1].

Four species of protozoan parasites cause malaria in humans: *Plasmodium falciparum, P. vivax, P. malariae*, and *P. ovale. P. falciparum* is responsible for the majority of deaths and most of the severe forms of disease, including cerebral malaria. At-risk groups include those in whom immunity has not yet developed (travelers, young children in endemic areas, etc.) and those in whom immunity has diminished (pregnant women, and people from endemic areas who have ceased to be routinely exposed to infection). Malaria is often cited as a substantial impediment to economic and social development in endemic regions.

Malaria is considered a re-emerging disease, due largely to the spread of drug-resistant parasite strains, decay of health-care infrastructure and difficulties in implementing and maintaining vector control programs in many developing countries.

Malaria is reported frequently in U.S. travelers and military or other personnel deployed in endemic areas. While nowhere near the levels reported in the U.S. through the 1940's, malaria transmission still occurs sporadically in this country due to the persistence of mosquitoes capable of transmitting the parasite. Each year there are over 1,000 cases of imported malaria reported in the U.S.

As a result of the spread of drug-resistant parasites and insecticide-resistant mosquitoes, in many respects there are now fewer tools to control malaria than existed even 20 years ago. Because of malaria's growing global burden, its control is essential. Historically, vaccines have been one of the most cost-effective and easily administered means of controlling infectious diseases, yet no licensed vaccines exist for malaria. Accumulating basic and clinical research suggest that effective vaccines for malaria can be developed and could significantly reduce morbidity and mortality, and potentially reduce the spread of infection.

History of Vaccine Development Efforts

Malaria parasites have complex life cycles and, thus, distinct developmental stages, each of which has multiple antigens that could serve as targets of an immune response. A *pre-erythrocytic* vaccine would protect against the infectious form injected by a mosquito (sporozoite) and/or inhibit parasite development in the liver. In a previously unexposed individual if a few parasites were to escape the immune defenses induced by a *pre-erythrocytic* vaccine, they could eventually multiply and result in full-blown disease. An *erythrocytic* or *blood stage* vaccine would inhibit parasite multiplication in the red cells, thus preventing (or diminishing) severe disease during the blood infection. A *sexual stage* vaccine does not protect the person being vaccinated, but instead interrupts the cycle of transmission by inhibiting the further development of parasites once they-along with antibodies produced in response to the vaccine-are ingested by the mosquito. Transmission-blocking vaccines could play a role as part of a multi-faceted strategy directed to elimination of parasites from low-transmission areas or as a means of protecting a vaccine or drug directed at *pre-erythrocytic* or *erythrocytic* stages against the spread of resistant parasites. An optimal vaccine would have the ability to elicit protective immunity that blocks infection as well as prevents pathology and interrupts transmission of parasites, and would most likely be a combination vaccine comprised of subunits from different parasite stages.

Five observations predict the eventual success of vaccine development for malaria:

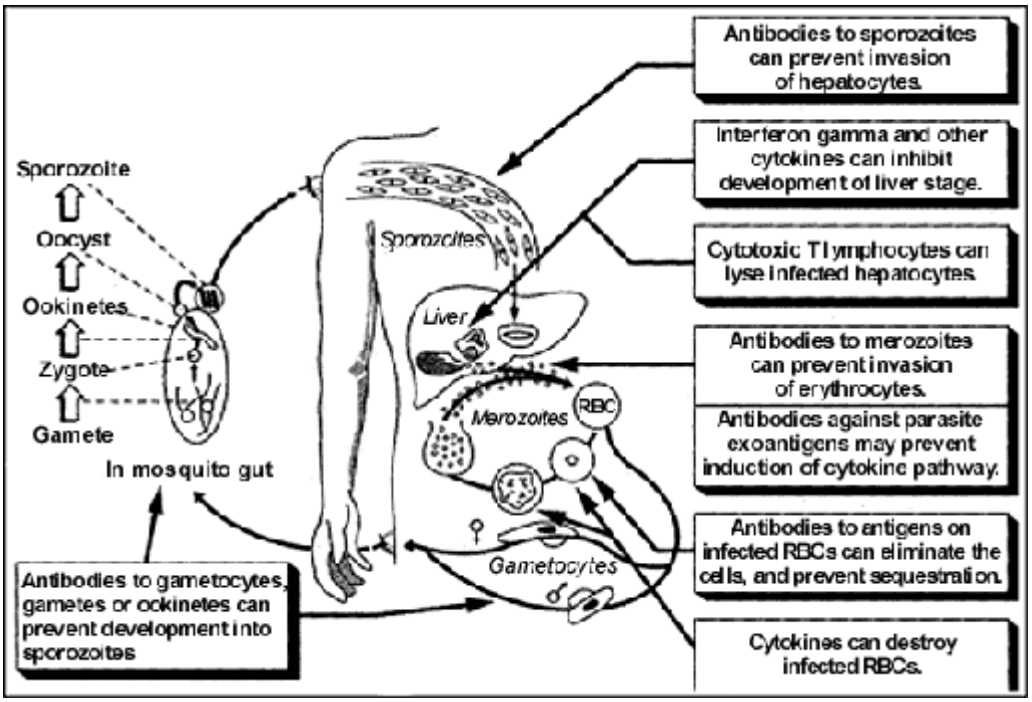
- Human populations residing in malaria endemic areas naturally acquire protective immunity against disease, although the patterns of immunity vary with malaria transmission patterns.
- Several studies showed that immunoglobulin purified from the blood of immune adults from endemic regions can passively transfer protection against *P. falciparum*.
- Clinical studies carried out since the 1970's demonstrated that experimental vaccination with attenuated sporozoites can effectively immunize patients against a subsequent malaria infection.
- Animal models of malaria clearly substantiate the potential for induction of protective immunity with defined vaccines.
- Two recent clinical trials of defined vaccines in endemic regions have reported significant, though limited, efficacy.

More than 30 distinct antigens identified in various life cycle stages of the malaria parasite have been proposed, at some level, as potential vaccine candidates based on observations such as: surface expression of the antigen on one or more life cycle stages; in vitro inhibitory (e.g. invasion-blocking) effects of specific antibodies; or, in vivo experiments showing protective effects of either direct immunization or passive transfer of antibody in animal models[2]. Several early vaccine candidates, many based on the circumsporozoite (CS) protein, the dominant surface antigen of the sporozoite stage, progressed into Phase I/II clinical trials but were halted by problems of low immunogenicity and efficacy or, in some cases, by reactogenicity. Overall, those candidates that have proceeded to trials have generally had some form of corporate co-sponsorship. Only one candidate vaccine, SPf66, based on antigens from both merozoite and sporozoite stages, has undergone extensive field trials. Efficacy was reported in several early clinical trials in South America, and one in Africa, but results from subsequent trials in Africa and Southeast Asia were not as promising[3,4]. Recent clinical studies of a vaccine composed of CS antigen and hepatitis B surface antigen (RTS,S) demonstrated that adjuvant plays a critical role in successful immunization; these studies employed the same antigenic construct with 3 different adjuvant formulations, only one of which (SBAS2, an oil-in-water emulsion also containing 3-deacylated monophosphoryl lipid A and QS21) induced significant protective immunity[5].

These results underscore the need not only to identify the right antigenic components for a vaccine, but also to find presentation and delivery methods that induce qualitatively and quantitatively appropriate immune responses.

Experimental observations indicate that protective immunity may involve multiple different immune responses, both humoral and cellular (Fig. 1)[2,6]. The focus has been largely on induction of antibodies in the case of blood-stage and transmission-blocking vaccines. *Pre-erythrocytic* vaccine developers initially focused on induction of antibodies, then on CD8+ cytotoxic T lymphocytes (CTL), and now additionally on T cell derived cytokines. Interferon gamma (IFNg) and other cytokines appear to play a role in the elimination of liver stage parasites, possibly through induction of mediators such as nitric oxide that kill parasites within hepatocytes (Fig. 1).

Figure 1 — Possible Mechanisms of Host Defense Against Malaria



To date, no pattern of immune response fully predictive of protection has been identified or validated. Naturally occurring immunity wanes rapidly in the absence of ongoing parasite exposure, and protection has been similarly short-lived in those few subunit vaccine trials that have demonstrated measurable efficacy. Such a vaccine might be useful for travelers. Unless new technologies can be found to improve the longevity of vaccine-induced resistance, however, it is likely that a vaccine to be used in endemic areas will need to take advantage of natural boosting provided by ongoing parasite exposure in order to provide long-lived protection.

In natural infection, unregulated immune responses may contribute to the pathogenesis of disease. For example, an association of cerebral malaria with high plasma levels of pro-inflammatory cytokines has been reported[8]. Thus, the potential for enhanced immunopathogenesis must also be taken into account in vaccine development efforts.

State of the Science

To date, most of the effort on vaccine development has focused on *P. falciparum* for several reasons: 1) high mortality from infection; 2) capability for experimental challenge infection; and, 3) relative ease of in vitro studies and availability of animal models for in vivo studies.

While *P. vivax* has a wider geographic distribution than *P. falciparum*, including in emerging economies such as Southeast Asia, India and Brazil, work on vaccine development has been impeded by several technical obstacles, such as the difficulty of culturing the parasite in vitro.

The thinking underlying the development of different types of malaria vaccines is summarized in **Table 1.**

Because it is impossible to be all-encompassing in the review of current research on malaria vaccines within the limitations of this document, the summary below aims only to illustrate some of the newest approaches to vaccine development.

Recombinant vaccines

The Walter Reed Army Institute of Research (WRAIR) and SmithKline Beecham Biologicals (SBBio), through a partnership extending uninterruptedly over the past 17 years, are developing a multi-antigen, multi-stage malaria vaccine based upon recombinant protein antigens. This collaboration has led to development of the CS-based RTS,S vaccine, which in combination with SBAS2 adjuvant repeatedly induced protection of volunteers in a Phase IIa trial. Subsequent re-challenge of volunteers revealed that protection waned substantially by 6 months after the last immunization. The first field trial of RTS,S/SBAS2, conducted by the Medical Research Council in The Gambia, reported 65% efficacy in adult males in a regions of intense transmission where both homologous and heterologous *P. falciparum* strains are prevalent. Efficacy persisted for 2 months and diminished afterward. The results from The Gambia further validate the standardized sporozoite challenge model employed in Phase IIa trials as a useful predictor of the efficacy of sporozoite-based vaccines in the field. In partnership with SBBio, USAID, the Naval Medical Research Center (NMRC) and others, WRAIR is conducting preclinical, clinical, and field trials to: 1) optimize RTS,S/SBAS2 vaccine regimens; 2) evaluate RTS,S with improved adjuvants; 3) develop the blood-stage antigen MSP-1 as a potential component of a multi-stage, multi-antigen vaccine; and 4) explore prime/boost strategies. Other WRAIR initiatives include development of: the *pre-erythrocytic* Liver Stage Antigen-1 (LSA-1); blood-stage antigens Merozoite Surface Protein (FVO strain and 3D7 mutant) and Apical Membrane Antigen-1 (AMA-1); and, attenuated Venezuelan Equine Encephalitis Virus as a platform for antigen delivery.

In a collaborative program between investigators at Oxford University and SBBio, detailed characterization of the cellular immune response to the RTS,S/SBAS2 vaccine is underway. Planned clinical trials include prime-boost studies of RTS,S boosted with a recombinant modified vaccinia virus Ankara encoding the CS protein.

The NIAID Malaria Vaccine Development Unit (MVDU) is focusing on recombinant proteins derived from blood stages and sexual stages of parasite development.

The MVDU has facilities for protein expression in a variety of recombinant systems as well as subsequent process development. Once produced and purified, blood-stage antigens are being tested in Aotus monkeys to identify the most promising candidates. Other aspects of the developmental pathway include determination of optimal formulation in rhesus immunization studies, search for immunologic assays that may correlate with protective immunity, exploration

of synergistic responses to different parasite antigens, and the execution of human clinical trials. Blood-stage vaccines currently under development in the NIAID MVDU include the C-terminus of MSP1, and recent protection studies in the Aotus model system have shown promising results with a 42 kd MSP-1 protein produced in baculovirus in collaboration with Novavax. A similar approach is also being tested by investigators at the University of Hawaii. A second candidate - PfEMP1 - is expressed on the surface of infected red cells and is thus available to the immune system. A region of this variant parasite antigen that mediates binding of the infected cell to CD36 on vascular endothelial cells has shown promising results in an Aotus trial. The MVDU is also conducting research on other blood-stage candidates, including MSP3, MSP4, MSP5, and AMA1, in most cases as a collaborative effort with other investigators. Transmission-blocking vaccines under development at the MVDU include Pfs25 and Pvs25, sexual stage (ookinete) antigens expressed by *P. falciparum* and *P. vivax*, respectively. Clinical grade Pfs25 has been produced and a plan is underway for evaluating these antigens for safety and immunogenicity in clinical trials later this year. Clinical grade Pvs25 is also being prepared for Phase I testing.

Investigators at New York University are investigating the use of CS-based multiple antigenic peptides (MAP) for induction of anti-sporozoite immunity. A synthetic MAP vaccine containing minimal T and B cell epitopes from the repeat region of the *P. falciparum* CS protein with alum and QS21 elicited high levels of parasite-specific antibodies in a recent clinical trial, but immunogenicity was HLA-restricted. Newer methods for MAP synthesis and inclusion of universal epitopes are currently being explored.

The CDC malaria vaccine program is focused on development of multivalent, multistage vaccine formulations that contain a series of antigenic domains from all of the developmental stages of the parasite. The multivalent, multistage malaria vaccine development strategy, which is aimed at inducing "multiple layers" of long-lasting, effective immunity, takes into consideration the immunogenicity and genetic diversity of antigenic fragments contained in stage-specific proteins. Two *P. falciparum* candidate vaccines are under investigation. One, an ~41 kd protein called FALVAC-1, contains 21 B-and T-cell epitopes from a variety of pre-erythrocytic, erythrocytic and sexual stages: CS, LSA1, MSP1, SSP2, MSP2, AMA1, RAP1, EBA-175, and Pfg27. FALVAC-1 has been expressed in a baculovirus expression system in collaboration with National Institute of Immunology, New Delhi, India, and Protein Sciences Corporation, Connecticut. Mouse, rabbit, and monkey immunization studies of FALVAC-1 with various adjuvants demonstrated induction of immune responses that recognizes different stages of the parasite. These observations provide proof of the principal that recombinant antigens containing antigenic fragments from different stage-specific antigens can induce responses against different stages of parasites. A second candidate, FALVAC-2, containing the 19 kd fragment of MSP1, the third epidermal growth factor domain of Pfs25, Region II of EBA-175, as well as 30 B-cell epitopes and 25 T cell epitopes from a total of 13 stage-specific antigens, is under development. Similar approaches are underway for the development of multivalent, multistage *P. vivax* vaccines. CDC has entered into a Collaborative Research and Development Agreement (CRADA) with the Bharat Biotech. International Limited (BBIL), Hyderabad, India, for production of GMP-grade candidate vaccine antigens. The goal for the next 5 years is to test multivalent, multistage *P. falciparum* and *P. vivax* recombinant vaccines in non-immune persons and individuals living in malaria endemic areas.

The Australian malaria vaccine program is developing prototype asexual stage vaccines based on the 190L fragment of MSP1, MSP2, the Ring Associated Surface Antigen RESA, AMA1, and the Rhoptry Associated Proteins RAP1 and RAP2.

Five human vaccine trials have been conducted with combinations of recombinant MSP1, MSP2 and RESA in the Montanide ISA 720 adjuvant (SEPPIC), with the most recent being a Phase IIb trial in children in a highly endemic area of Papua New Guinea. Further extensive trials are

planned to extend these promising results to younger children and further optimize this formulation. In test animals, including monkeys, AMA1 and RAP2 have given very encouraging protection. The first Phase I human trial has been conducted with AMA1, and further Phase I trials with AMA1 and RAP2 are planned for later this year.

DNA vaccine and prime-boost approaches

The Naval Medical Research Center (NMRC), in collaboration with Vical, Inc., USAID, Aventis Pasteur, Entremed, Inc., and multiple investigators around the world, has initiated a DNA-based malaria vaccine development effort called the "Multi-Stage DNA Vaccine Operation" or "MuStDO".

The goal during the next 3 to 4 years is to assess a 5 gene pre-erythrocytic stage vaccine (MuStDO 5) and a 15 gene pre-erythrocytic plus erythrocytic stage vaccine (MuStDO 15) as DNA vaccines alone, and as the priming component of a prime-boost regimen with recombinant protein in adjuvant and recombinant viruses as the boosters. These studies are ultimately aimed at assessing the capability of the vaccines to prevent blood stage infection entirely in naïve individuals visiting highly endemic areas of Africa, and to prevent death in infants and young children in Africa. Thus far, initial Phase I studies in the U.S. using a single gene *P. falciparum* CS DNA vaccine have established safety as well as immunogenicity for CD8+ CTL as well as CD8+ and CD4+ IFNg-producing cells. Plans are underway in collaboration with WRAIR and SBBio to examine the effects of boosting these individuals with RTS,S/SBAS2. In May 2000, a Phase I/IIa study will be initiated in which the 5 gene vaccine will be given with and without a plasmid expressing the cytokine GM-CSF to enhance immunogenicity. Future plans include studies of minigene DNA, recombinant protein, and recombinant virus vaccines based on epitopes from stage specific proteins discovered through the *P. falciparum* genome sequencing project.

Researchers at Oxford are assessing the safety, immunogenicity and efficacy of a DNA prime-MVA (modified vaccinia virus Ankara) boost regime in healthy volunteers. The insert, which is the same in the DNA and the MVA components, is a polyepitope string fused to the entire TRAP antigen (Thrombospondin-Related Anonymous Protein, also known as Sporozoite Surface Protein 2, SSP2, which is expressed primarily by sporozoites and liver stage parasites). The delivery of DNA by intramuscular route and by a needleless delivery device into the skin is being compared. MVA is delivered intradermally. Initial studies of both DNA and MVA vaccines established adequate safety and immunogenicity. The first prime-boost studies of volunteers were initiated in December, 1999, and are ongoing, with challenge studies anticipated later this year. Plans are underway to test both DNA and MVA vaccines in The Gambia in late 2000.

Transgenic vaccines

Genzyme Transgenics Corp. and NIAID have established a collaboration for preclinical development of an MSP-1-based vaccine in transgenic animals.

The 42 kd fragment of MSP1 has been produced in milk of transgenic mice, and a purification process is now under development. If the purified product can be shown to protect Aotus monkeys, it will provide supporting data supporting the development of other transgenic animals such as goats.

Genomic and proteomic approaches

In 1996, a collaborative international effort was undertaken to sequence the complete genome of *P. falciparum*.

To date, two of the parasite's 14 chromosomes have been completely sequenced and the sequences of many of the remaining chromosomes are nearing completion. Although there have been significant technical hurdles in sequencing this A-T rich organism, it is now estimated that essentially all of the parasites approximately 6000 genes are available in existing databases. Thus, the malaria community and vaccine developers have access to virtually all of the genes encoding the antigens and proteins expressed by this organism. Although not validated, computer algorithms are being conceived to identify genes whose expressed products are potential candidate vaccine antigens by virtue of their predicted cell surface localization, stage specific expression, or structural features that may interact with components of the human host immune system to initiate a protective response. Accompanying the development of such computational methods are high throughput methods (DNA chips and microarrays, and proteomics) of differential gene and protein expression to identify and characterize antigens that may be appropriately exposed to the immune system. A challenge for malaria investigators is the limiting amounts of material that are available for certain stages of the parasite's life cycle, especially the liver stages.

Investigators are developing strategies to identify genes whose products are essential to parasite survival and/or contribute to disease manifestations.

Interfering with the function of these gene products with targeted immune responses is another approach to malaria vaccine development. New tools of molecular genetics, e.g. efficient and global gene knockouts, are needed to make this approach truly feasible and economically viable.

In addition to the targeted and rationale approaches mentioned above, investigators are developing high throughput "shotgun" methods for candidate vaccine identification.

For example, studies are underway to immunize mice with expression libraries prepared in genetic immunization vectors to help identify candidate vaccines in an unbiased, systematic manner using the *P. yoelii* rodent model of malaria.

The human genome project is providing immunologists with access to all of the immune response genes. It is anticipated that future developments will enable investigators to determine the expression of all of these genes on chips and microarrays from large numbers of individuals. Analysis of gene expression from these individuals should lead to identifying immune correlates of protection and disease. Moreover, such methods will prove useful in studies of vaccine efficacy and safety.

The Power of Collaboration

It is widely agreed that successful development of malaria vaccines will require the collaborative efforts of government, academia, and industry, and that each sector has unique capabilities to contribute. A 1996 IOM workshop on malaria vaccines[9] reported that "the public sector must take the lead, given the costs of vaccine research and development and present beliefs that expected returns on investment will cover only a portion of the research and development outlay. The pharmaceutical and biotechnology industries must play a major role in resolving technical issues relating to appropriate expression and purification of antigens, vaccine formulation, and manufacturing technology, but new industrial development efforts will come only in conjunction with a successful, coordinated public sector effort that first proves the feasibility and value of a given technical approach". It would be ideal for the public and private sectors to establish a consortium to identify critical technical problems and work together on their solution.

Available public sector resources

A survey conducted by the Wellcome Trust[10] found that the total identifiable global expenditure on malaria research in 1993 was just $84 million, only a portion of which was directed toward vaccine development.

Since that time, however, the situation has improved markedly. Research on malaria vaccine development is being supported through programs of the U.S. government (NIAID, DoD, USAID, CDC), Wellcome Trust, Australian government, World Health Organization, European Commission, and others, at laboratories and field sites throughout the world. Several of these organizations have either expanded ongoing efforts in malaria vaccine development or established new programs. In addition, a Malaria Vaccine Initiative was recently launched by the Bill and Melinda Gates Foundation.

Public sector support for research to identify product leads and help with clinical evaluation has been identified as a key incentive for increasing pharmaceutical research and product development for diseases of low-income countries[11].

Much of the recently expanded malaria effort within the public sector has been specifically aimed at putting in place the various aspects of a vaccine development pathway that will provide for testing of "proof of principle" through limited clinical trials in endemic regions.

In 1997, the NIAID undertook a research plan for malaria vaccine development. The NIAID plan established an integrated program between the extramural Division of Microbiology and Infectious Diseases (DMID) and the Division of Intramural Research, which takes advantages of the strengths of the two groups to create a malaria vaccine development pathway that extends from discovery research through clinical trials in endemic areas. A Malaria Vaccine Development Unit (MVDU) has been created within NIAID's intramural laboratories, which focuses on process development for recombinant blood stage and transmission-blocking vaccine antigens and development of antigen-specific assays. Blood-stage antigens for development within the MVDU will either be selected from those under investigation in the intramural malaria program or collaborative interaction with investigators in the international extramural malaria program. NIAID extramural supports translational research on any potential vaccine candidate through its extensive program of grants to not-for-profit as well as for-profit institutions. Through the Small Business Innovative Research (SBIR) and SBIR-at-NIAID programs, NIAID funds malaria vaccine research and development at several small biotechnology companies. These projects support a spectrum of activities, including validation and optimization of recombinant protein expression systems, optimization of recombinant protein and nucleic acid immunogens, as well as validation, optimization, preclinical and early phase clinical evaluation of platform technologies. Access to primate testing is available through intramural resources and collaboration with CDC. NIAID is also putting in place a major new contract designed to provide access to additional resources for process development, cGMP production and regulatory support for IND development. Domestic clinical trials may be performed at the NIH Clinical Center or through DMID's Vaccine and Treatment Evaluation Unit contracts. NIAID is expanding clinical trial capacity through collaborations in Mali and Ghana, and through international clinical research awards to other countries where malaria is endemic. DMID has also recently awarded a contract for international clinical trial support, which offers assistance with trial design and monitoring.

The Military Infectious Diseases Research Program supports malaria vaccine development efforts at two major sites in the United States, the Naval Medical Research Center (NMRC) and the Walter Reed Army Institute of Research (WRAIR). In addition, Department of Defense (DoD) laboratories in Kenya, Egypt, Indonesia, Thailand and Peru work on malaria vaccine development. These facilities conduct work in six areas: 1) genomics, bioinformatics and antigen discovery; 2) basic immunology research; 3) preclinical research and development; 4) assay

development; 5) product development; and, 6) clinical trials. NMRC, in collaboration with USAID and NIAID, has established additional field capability in Ghana. In addition to the capability of doing human experimental challenge studies in the U.S., and field studies in all of the overseas laboratories, the DoD program has capacity for nonhuman primate studies of candidate malaria vaccines. Studies on *P. falciparum* and *P. vivax* in Aotus monkeys are conducted at facilities in Peru, Panama and at WRAIR. Rhesus monkey immunogenicity studies are conducted in Thailand and at NMRC. Monkey malaria *(P. knowlesi)* immunization and challenge studies in Rhesus monkeys are also conducted at NMRC and collaborating laboratories. The DoD also has established a manufacturing facility at WRAIR capable of producing clinical grade recombinant vaccine antigens, to which other agencies may also obtain access.

CDC has supported for over two decades a program and facilities for conducting immunogenicity and efficacy studies on malaria vaccines in non-human primate model systems. In addition, CDC maintains a field station in collaboration with the Kenya Medical Research Institute (KEMRI). CDC/KEMRI is conducting a multi-disciplinary study that combines epidemiologic and laboratory investigations of clinical manifestations of malarial illness, parasite infection, host immune responses, in vitro correlates of protection, and host, parasite and mosquito vector characteristics. Through these studies CDC has developed a well-characterized field site for malaria vaccine testing in western Kenya. Discussions are underway with the Indian Council of Medical Research for field-based vaccine-related studies in India that may lead to additional field testing capability.

During more than 30 years, the United States Agency for International Development (USAID) has supported efforts towards malaria vaccine development, first focusing on discovery in academic institutions and, later, on more downstream development, largely in collaboration with partners in the public sector. The USAID focus is on vaccines to protect children and pregnant women in endemic areas from severe disease and death, with less emphasis on the prevention of parasitemia per se. Currently the two major elements of the USAID Malaria Vaccine Development Program (MVDP) are (1) the MSP-1 effort at WRAIR and (2) the DNA vaccine effort at NMRC (MuStDO). The USAID provided most of the impetus and support for the initiation and continuation of the MSP-1 effort and will also support the evaluation of combination MSP-1/RTS,S formulations. The MuStDO was initiated through joint NMRC/USAID support. Field trials within the current planning horizon are goals of both of these programs. The USAID MVDP has for many years been a core supporter and user of the pilot production facility at WRAIR as well as the nonhuman primate testing facility at CDC. USAID has provided considerable support to collaborative efforts with NIAID (Phase I testing of an earlier MSP-1 formulation and of a CSP multiple antigen peptide with New York University, initial funding of the MVDU, and support of nonhuman primate testing of MSP-1 and PfEMP-1 at CDC). The MVDP has also supported the Australian program through funding of RAP2 studies at the Queensland Institute of Medical Research and CDC, early evaluation of MSP-4 at Monash University, and the earlier development of a trial site in Papua New Guinea.

The Australian malaria vaccine program is a collaboration between the Papua New Guinea Institute for Medical Research, the Swiss Tropical Institute and the Australian Government funded Co-operative Research Centre for Vaccine Technology (CRC-VT). The main partners in the CRC-VT malaria program are The Queensland Institute of Medical Research and The Walter and Eliza Hall Institute of Medical Research. Contract production of antigens for clinical trials is being undertaken by Australian biotechnology companies. The aim is to produce a vaccine directed against the asexual blood stages of malaria parasites.

The UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) plays an important role in malaria vaccine research and development at the global level, providing a broad inventory of resources to the research community ranging from training and

capacity building for scientists in malaria-endemic countries (including GLP and GCP) to development of a series of guidelines for clinical testing of malaria vaccine candidates in humans. In addition to its portfolio of discovery research, TDR collaborates with investigators in generating product development proposals for advanced candidates, promotes partnerships and projects in malaria-endemic countries including technology transfer activities, and provides independent monitors for clinical trials (e.g. RTS,S in The Gambia and Kenya, AMA1 in Papua New Guinea, Pfs25 in Hong Kong).

The European Commission (EC) has two research programs dealing with malaria vaccines. One is the International Collaboration, termed INCO-DEV. This research program has been funding collaborative research with developing countries' institutions for close to 20 years. Malaria vaccines are a priority. INCO-DEV is co-financier of the European Malaria Vaccine Initiative (EMVI). Another major EC player is the Life Sciences Program, Key Action Two - Control of Infectious Diseases. This program's vaccine component is currently financing a consortium dealing with asexual stage vaccines, and a consortium dealing with transmission blocking vaccines. The third player is EMVI, which involves the Commission as well as European Union Member States. EMVI's mandate is fairly narrow, including support of GMP production and phase I, possibly phase II, clinical trials and collaboration with the African Malaria Vaccine Testing Network (AMVTN) in establishing clinical trials in Africa of malaria vaccines.

Obstacles to vaccine development

There are obviously a number of scientific questions that must be addressed in the course of further development efforts on malaria vaccines. These have been discussed at length elsewhere[2,7], and include issues such as how to induce appropriate (protective, long-lasting, nonpathogenic) immune responses, how to structure combination vaccines, how to deal with parasite antigen diversity and antigenic variation, as well as how to deal with human genetic restriction of immune response and/or genetic predilection toward detrimental responses.

There are also a number of hurdles related to translational research and evaluation of candidate vaccines. These include issues regarding the appropriateness and accessibility of animal models. Other technical hurdles relate to the need to identify assays for ongoing validation of candidate antigens through process development and scale-up production, as well as assays predictive of protection for assessment of immunogenicity and efficacy in clinical trials. In addition, much careful thought must be given to clinical trial design. This is especially true for blood stage vaccines, where the feasibility of experimental challenge infection is extremely controversial and the optimal measurement of efficacy is reduced morbidity/mortality, as well as for sexual stage vaccines, where the ultimate measurement of efficacy is interruption of malaria transmission[7].

It is anticipated that the scientific obstacles cited above can be overcome through research supported by expanded public sector programs. Moreover, new or expanded public sector programs have targeted one of the primary logistic obstacles cited in the 1993 IOM report[7] - the need for cGMP production facilities for pilot lots of malaria antigens. The need remains, however, for help from the pharmaceutical and biotechnology industries in resolving technical issues relating to appropriate expression and purification of antigens, vaccine formulation, and manufacturing technology. There can be no question that the dearth of committed industrial partners has limited progress in malaria vaccine development. The vaccine candidate that is currently farthest advanced in clinical trials, RTS,S, has clearly benefited from the expertise provided by SBBio at all stages of this vaccine's research and development history. Likewise, other candidates have benefited greatly from partnerships with industry that provide access to proprietary technologies and reagents. These types of public-private sector collaborations must be fostered, and any perceived legal impediments overcome.

In the 1996 IOM report on "Vaccines against Malaria",[9], much emphasis was placed on the need for increased coordination of U.S. governmental activities on malaria vaccine research and development in order to strengthen interactions with industry. Indeed, it is true that expanded efforts implemented since 1996 have largely pursued parallel tracks within the different agencies (both in the U.S. and other countries), and to date the number of potential vaccine candidates has clearly supported this approach. This parallelism may be further exacerbated with the predicted discovery of many more new malaria antigens through genomics research. Nonetheless, with limited resources for downstream development, priorities must eventually be identified. This would be greatly facilitated by mechanisms allowing for development of combination vaccines, employing antigens and/or adjuvants from different sources, as well as head-to-head comparisons of various candidates in preclinical and clinical trials. Identification of mechanisms to allow such sharing of proprietary reagents and information would contribute enormously to the rational development of malaria vaccines.

Conclusion

The need for enhanced public-private sector collaboration to support development of new public health tools for "orphan" diseases such as malaria has been emphasized repeatedly in a number of venues over recent years. The U.S. government has shown interest in the development of new mechanisms for pursuing this goal. Important recent developments include: the President's Millenium Vaccine Initiative and White House meeting on new partnerships to develop and deliver vaccines to developing countries; the FY 2000 Congressional mandate for development of a challenge grant program at NIH to promote joint ventures with biotechnology, pharmaceutical and medical device industries; and, proposals to amend the Internal Revenue Code to provide tax credits for research related to vaccine development for malaria and other "widespread" diseases. Furthermore, government agencies have put in place programs to co-sponsor the discovery, development and testing of new malaria vaccine candidates. The U.S. government, other international organizations and philanthropic groups are working on new ways to assure a market for these products. This is an extremely opportune time to explore methods to eliminate impediments to collaboration and put these much-needed partnerships in place. As recently stated by U.S. President Clinton, "We can save millions of lives together, and we ought to do it."

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References:

1. Weekly Epidemiological Record of the World Health Organization, 1996, 71(3):17.
2. Hoffman SL et ASM Press, 1996, *Malaria Vaccine Development: A Multi-immune Response Approach.* Washington, DC
3. Miller L H and Hoffman S L, 1998, *Nature Medicine* 5:520
4. Facer CA and Tanner M, 1997, *Advances in Parasitology* 39:1

5. Stoute JA et al., 1997, *New England Journal of Medicine* 336:86
6. Good MF and Doolan DL, 1999, *Current Opinion in Immunology* 11:412
7. Malaria: Obstacles and Opportunities, 1991, *National Academy Press* Washington, DC
8. Riley EM, 1999, *Immunology Today* 20:228
9. Vaccines Against Malaria: Hope in a Gathering Storm, 1996, *National Academy Press* Washington, DC.
10. Malaria Research: An Audit of International Activity, 1996, *PRISM* Wellcome Trust, London, UK
11. Orphans and Incentives: Developing Technologies to Address Emerging Infections, 1997, *National Academy Press* Washington, DC

Table 1 - Characteristics of Potential Malaria Vaccines

Vaccine Type	Goal	Principal Target Population	Advantages	Caveats	Possible Immune Pathways	Research Challenges	
						Problem	Solutions
Pre-erythrocytic	Prevent or reduce disease	• Nonimmune travelers and residents from areas of low transmission • Children and pregnant women in endemic areas, with or without a bloodstage vaccine	• Easy to test for efficacy in human volunteers • Repeated exposure should provide boosting • Prevents disease by blocking the parasite before it infects RBCs	• In a nonimmune individual, if one parasite escapes from the liver to infect RBCs a lethal infection can develop • A large population trial will be required to test impact on severe disease and mortality in Africa	1. Antibodies to block sporozoite invasion of liver cells 2. T cell responses against infected liver cells (IFNg and CTL)	• Must maintain high antibody titer • Limited immuno-genicity, epitope variation, genetic restriction of immune responses	• Combine induction of antibodies and T cell responses • Use adjuvants and delivery systems that maintain strong immune response • Use multiple immunogens
Blood-Stage	Reduce severe disease	Children and pregnant women in endemic areas	• Repeated infection provides boosting • Model in New World monkeys for testing *P. falciparum* and *P. vivax* vaccine candidates	• Antigenic diversity and antigenic variation • A large population trial will be required to test impact on severe disease and mortality	1. Antibodies against merozoite surface antigens to block invasion of red blood cells 2. Antibodies against malaria proteins expressed	• High antibody titer likely to be required • Immune response may select for mutant parasites • Antigenic variation may limit effectiveness	• Combine multiple synergistic immunogens • Use adjuvants and delivery systems that maintain antibody levels • Combine blood-stage vaccine with transmission-blocking

					on surface of infected RBCs 3. Cell-mediated immunity		vaccine • Immunize against functional domains that are less variant
Transmission-Blocking	• Reduce parasite transmission • Limit spread of parasites resistant to other vaccines	• Endemic areas with low transmission as a single vaccine • All endemic areas as a combined vaccine with blood-stage and/or pre-erythrocytic	In vitro assay exists for assessing biological activity of transmission-blocking antibodies	• Does not provide protection from disease • Some candidate immunogens are not seen by humans in the course of infection, in which case natural boosting will not occur • Measurement of impact will require vaccination of entire communities	Antibodies to gametes and ookinetes	Must maintain high antibody titer in absence of boosting (for some candidates)	Use adjuvants and delivery systems that maintain high antibody levels