PERSPECTIVES: PARASITOLOGY

Malaria—from Infants to Genomics to Vaccines

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A recent Keystone Symposium considered the many challenges posed by malaria and progress in developing a vaccine against the *Plasmodium* protozoan parasite that causes this disease (*1*). Principal hurdles to vaccine development include the complexity of the *Plasmodium* life cycle, and determining which proteins from which life cycle stages a vaccine should target (see the figure). Identifying appropriate *Plasmodium* antigens will be critical for constructing a subunit vaccine comprising individual parasite proteins rather than the whole parasite.

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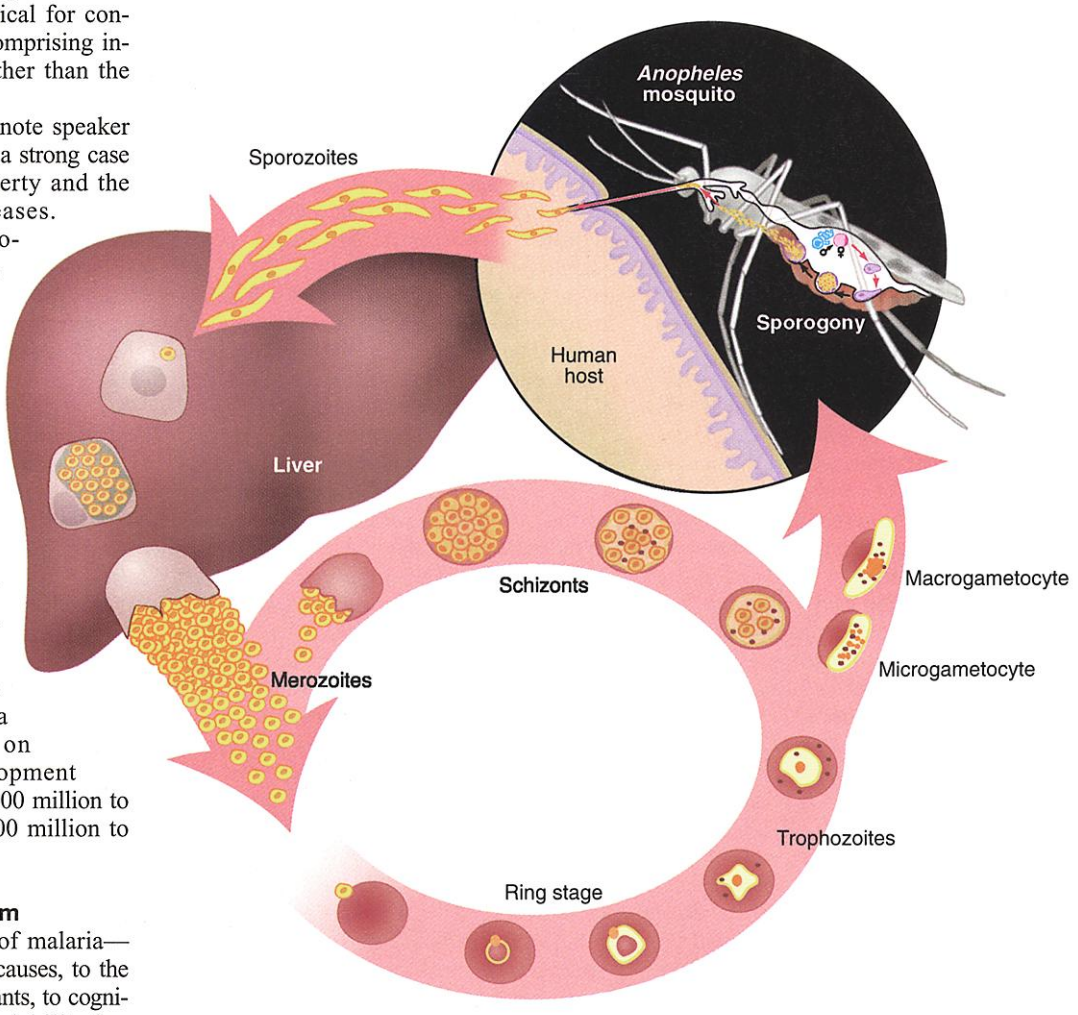
Opening the meeting, keynote speaker Jeffrey Sachs (Harvard) made a strong case for the linkage between poverty and the prevalence of tropical diseases. Sachs presented a macroeconomic analysis of malaria, concluding that malaria and other tropical diseases are major influences on the health and economic development of Africa. The macroeconomics report of the World Health Organization (WHO) released this year (*2*) estimates that malaria alone reduces the economic growth of Africa by more than 1% per year, adding up to hundreds of billions of dollars of lost income in the long run. Sachs challenged the audience to participate in preparing a plan to increase spending on malaria research and development from the current amount of $100 million to $150 million annually, to $500 million to $1000 million annually.

Defining the Malaria Problem

The epidemiological impact of malaria—from the number of deaths it causes, to the number of low-birthweight infants, to cognitive impairments and other debilitating symptoms—is huge. Kevin Marsh (KEMRI-Wellcome Trust Collaborative Research Program, Kenya) presented data showing that although childhood mortality from all causes is decreasing in Africa, malaria mortality is actually increasing. Reasons for this include the spread of chloroquine-resistant *Plasmodium falciparum*, and the severe, often fatal syndromes—anemia, cerebral malaria, and respiratory distress—associated with disease caused by this parasite. The patterns of clinical disease and the mortality rates vary significantly with location and transmission intensity, an important point to consider when planning trials of candidate vaccines. For example, could a vaccine that does not protect against mild disease in fact protect against severe disease?

In any discussion of malaria vaccines, the feasibility of vaccination in the field must be considered. Long-term studies in which human volunteers were immunized with irradiated *P. falciparum* sporozoites demonstrated the threshold required for greater than 90% protection against subsequent challenge with the parasite (Stephen Hoffman, Celera Genomics). Although the number of human volunteers was small (n = 14), protection was sustained for at least 10 months and was effective against challenge with other strains of *P. falciparum*.

Andrew Read (Univ. of Edinburgh) pointed out that malaria parasites are very



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A malicious life. The life cycle of *Plasmodium*, the malaria parasite. Sporozoites injected by the mosquito into the human host invade liver cells, where each sporozoite forms thousands of merozoites. Released merozoites enter the bloodstream and invade erythrocytes, developing into trophozoites, which then undergo schizogony to produce merozoite progeny that then repeat the cycle of erythrocyte invasion. Some merozoites develop into the male and female sexual stages (gametocytes) that are taken up by the mosquito in a bloodmeal. Fertilization, ookinete formation, and sporozoite production take place in the tissues of the insect vector, completing the life cycle.

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polymorphic. He raised the possibility that the use of vaccines against blood-stage antigens that do not elicit complete immunity may inadvertently result in increased prevalence of parasites of greater virulence. More virulent parasites circulating in the vaccinated population would put unvaccinated individuals at greater risk. He argued that selection for increased virulence would not occur with an irradiated sporozoite vaccine or a transmission-blocking vaccine (directed against parasite stages in the mosquito). An increase in virulence would probably take decades to occur and could even happen in the presence of a decrease in overall malaria mortality. In addition, it has been difficult to establish the degree of variability in virulence among strains of *P. falciparum*. Both Andrew Read and Andrew Clark (Penn State) emphasized that the parasite population must be monitored both before and after vaccination to follow possible changes in virulence with time and to allow compensatory adjustments to be made to the vaccine.

Genes, Genomics, and Malaria

The interplay between host and parasite genetics was discussed by Louis Miller (NIH). The ancient scourge of malaria has strongly selected for polymorphisms (variations) in certain human genes, as evidenced by the prevalence of sickle cell anemia, the thalassemias, glucose 6-phosphate dehydrogenase, and the Duffy chemokine receptor. The impact of malaria on polymorphisms in hemoglobin C, glycophorin B, and complement receptor type I has recently become apparent. Miller emphasized the need to identify unique allelic variants in populations living in malarially endemic regions in order to facilitate pinpointing of parasite proteins that could be targets of vaccine-induced immunity.

Speakers also considered ways to mine human and *P. falciparum* genome sequences to elucidate interactions between the parasite and its human host. Ross Coppel (Monash Univ., Australia) cited an approach to constructing a subunit vaccine by rational selection of a group of structurally related parasite proteins such that a crucial plasmodial functional pathway would be completely blocked. Coppel showed that a multi-subunit vaccine comprising several related merozoite surface proteins containing epidermal growth factor–like domains elicited protection against parasite challenge in a mouse model of malaria. The PlasmoDB *P. falciparum* genome database should facilitate recognition of areas of antigenic variability in parasite proteins, which would identify these proteins as potential vaccine targets that are recognized by the host immune system. Coppel pointed out, as did other speakers, that the *P. falciparum* genome will yield hundreds of new target proteins but that we need better vaccine technology (for example, better delivery systems and adjuvants) to be able to exploit this information.

Protective Host Immune Responses

Eleanor Riley (London School of Hygiene and Tropical Medicine) summarized the immune responses in children living in malarious areas. She reported on the links between the innate and adaptive immune systems and their interaction in determining disease susceptibility. Notably, malaria-induced anemia is often found in younger children, but as the children age and develop effective immune responses, cerebral malaria becomes more common. This raises the question of whether host immune responses to *Plasmodium* undergo immunologic regulation as the host matures, thus altering the pathogenic course of malaria.

Protective immune responses to sporozoite and liver stages of infection were discussed by Denise Doolan (Naval Medical Research Center). Immunizing rodents with irradiated sporozoites revealed that activation of antigen-specific $CD8^+$ T cells producing interferon-γ (IFN-γ) is the crucial immune event. In addition, an effective irradiated sporozoite (pre-erythrocytic stage) vaccine must elicit the $CD4^+$ T cell responses required for $CD8^+$ T cell activation. Consistent with this, prime-boost vaccine strategies (using a DNA prime and a virus boost) also require both $CD8^+$ and $CD4^+$ T cell activation to obtain protection.

Allan Saul (NIH) discussed transmission-blocking antibodies elicited by sexual stage–specific antigens. Interestingly, many of these target antigens are only expressed in the mosquito vector and are not subject to immune selection by the vertebrate host. Antibodies to these antigens block fertilization of the sexual-stage gametes and subsequent parasite development in the mosquito. An ex vivo assay—feeding mosquitoes with parasites and antibodies through a membrane—provides an easy way to measure transmission-blocking immunity.

Antigenic Targets Eliciting Protection

Daniel Carucci (Naval Medical Research Center) described a high-throughput proteomics approach for identifying stage-specific malaria proteins (particularly of sporozoites and pre-erythrocytic stages) that are potential vaccine targets. Using liquid microcapillary electrophoresis coupled with tandem mass spectrometry, his group has identified more than 950 proteins from all stages of the *P. falciparum* life cycle (sporozoites, asexual and sexual blood stages, merozoites, and gametocytes). These proteins include integral membrane proteins not generally identified by 2D gels, proteins predicted to be differentially expressed (for example, metabolic enzymes in the trophozoite), and sexual stage–specific proteins expressed by gametocytes. The hope is that some of these proteins will prove to be new protective antigens. Alessandro Sette (Epimmune Inc., San Diego) described efforts to identify epitopes in a number of pre-erythrocytic proteins that are recognized by both $CD8^+$ and $CD4^+$ T cells, and to optimize these multiple epitope vaccines.

In contrast to the T cell responses elicited by sporozoite and liver-stage parasites, B cell production of antibodies is the primary means of immune protection against blood stages of infection. Antibodies against merozoite surface proteins inhibit merozoite invasion of erythrocytes and their development into trophozoites (see the figure). Tony Holder (National Institute for Medical Research, Mill Hill, UK) plans to develop a vaccine using a modified recombinant major merozoite surface protein (MSP1), whose target epitopes have been defined by structural analysis.

The large PfEMP1 proteins encoded by the parasite and also expressed on the surface of the infected host erythrocyte were the vaccine targets selected by Patrick Duffy (Seattle Biomedical Research Institute). These proteins, also called var or variable antigens, have been implicated in the binding of infected erythrocytes to host endothelial cells and to the placenta. Although there has been progress in identifying the specific var domains responsible for binding, the large number of PfEMP1 genes in the plasmodial genome and their antigenic variability among different parasite isolates presents a major barrier to their use in vaccines.

Preclinical Research and Development

Activation of antigen-specific T and B cells was discussed by Rafi Ahmed (Emory Univ.) and Garnett Kelsoe (Duke Univ). Ahmed described the generation of $CD8^+$ T cell memory, which is initiated by the early mitotoic expansion of these cells after antigen stimulation. His data support a model in which $CD8^+$ memory T cells slowly proliferate in response to interleukin 15 in the absence of continued antigenic stimulation. Kelsoe summarized B cell activation: initial uptake of antigen by dendritic cells, their interaction with antigen-specific T cells, and the T cell–B cell interactions that take place within the follicles of secondary lymph nodes. Follicular dendritic cells are critical for affinity maturation of the antibody response and generation of memory B cells.

In a practical counterpoint, several speakers reported their efforts to activate

antigen-specific T cells by immunizing human volunteers with: DNA vaccines (Stephen Hoffman), multi-antigenic peptides (Elizabeth Nardin, New York Univ.), or recombinant hepatitis B particles (Urszula Krzych, Walter Reed Army Institute of Research). DNA immunization elicits antigen-specific cytotoxic T lymphocytes and IFN-γ–producing $CD8^+$ T cells. This prime can be boosted by parasite challenge, but has not resulted in primary antibody production. Adrian Hill (John Radcliffe Hospital, Oxford) reported better $CD8^+$ and also $CD4^+$ T cell responses with a prime-boost approach using a DNA vaccine prime followed by a recombinant virus boost, particularly with higher doses of both the DNA and the MVA strain of vaccinia. Only small amounts of antibody were produced, but Hill reported that IFN-γ levels in the ELISPOT assay were the best in vitro correlate of a delayed rise in parasitemia after parasite challenge in human volunteers.

Louis Schofield (WEHI, Melbourne, Australia) provided an update on the development of an antitoxin vaccine. His rationale is to prevent or ameliorate malaria by vaccinating against parasite products that may exacerbate symptoms of the disease. Glycosylphosphatidylinositol (GPI) released by the parasite may be a functional endotoxin driving inflammation and interfering with host metabolism, but the actual role of this molecule in disease progression has not been resolved. Immunizing mice with a chemically synthesized *P. falciparum* GPI provided substantial antidisease protection against challenge with the murine malaria parasite *P. berghei*. Thus, GPI may contribute to pathogenesis, suggesting the feasibility of a synthetic antitoxin vaccine.

Carole Long (NIH) has identified laboratory correlates of protective immune responses (both B and T cell based), directed primarily against blood-stage parasites. Measurable properties of antibodies include their titer, avidity, specificity, and biological activity (for example, their ability to inhibit parasite invasion and growth in culture). Preclinical studies in animal models need to identify surrogate markers of protection that are specific, quantitative, and, if possible, functional. Such markers will facilitate more rapid and less costly testing of candidate vaccines.

In any consideration of malaria vaccine trials, the challenge continues to be defining clinical endpoints and patient selection criteria. Pedro Alonso (Univ. of Barcelona) led a panel of experts working in malarially endemic areas in a discussion of case definitions and relevant clinical endpoints for vaccine trials in children and adults. The panel emphasized the importance of parasite genotyping for monitoring the impact of vaccination on parasite population dynamics.

Clinical Trials of Malaria Vaccines

A review of malaria vaccines concentrated on those being tested for efficacy in phase II clinical trials. The most advanced vaccine (RTS,S)—directed against sporozoites and liver-stage parasites—has been developed by the U.S. Army and GlaxoSmithKline. This vaccine includes a portion of the circumsporozoite protein of *P. falciparum* expressed in the hepatitis B surface antigen and formulated with the proprietary adjuvant ASO2. Joe Cohen (GlaxoSmithKline) reported that about 1200 doses of the RTS,S vaccine have been administered to human volunteers in the United States and to semi-immune volunteers in a field study in the Gambia. American vaccinees showed partial (40 to 50%), short-lived protection when challenged with viable sporozoites. The vaccine is to be tested in further phase II trials and its efficacy improved. T and B cells from vaccinated volunteers are being tested to find in vitro correlates of protection (Ursula Krzych). Circumsporozoite protein-mediated IFN-γ production elicited from baseline and RTS,S-immune lymphocytes indicated that this inflammatory cytokine is elevated in individuals protected by the RTS,S vaccine and that both $CD4^+$ and $CD8^+$ T cells are involved in this response. Significantly, protracted protection, albeit observed in only one of five volunteers, was associated with sustained IFN-γ production.

The use of *P. falciparum* sporozoite and liver-stage antigens in multivalent DNA-based vaccines was described by Tom Richie (Naval Medical Research Center). His group tested a single-gene DNA vaccine in three trials, and a five-gene DNA vaccine (with and without DNA encoding the cytokine human granulocyte-macrophage colony-stimulating factor) in one trial. Inclusion of cytokine DNA conferred no obvious advantage, and the single-gene vaccine did not confer protection. However, administration of the *P. falciparum* five-gene DNA vaccine did prime volunteers for boosting of their antibody and T cell responses with a single exposure to sporozoites inoculated by infected mosquitoes. This critical finding suggests the value of the five-gene DNA vaccine in the field.

Hill and colleagues in Oxford and the Gambia are attempting to induce T cells specific for sporozoite and liver-stage antigens with various heterologous prime-boost strategies. Hill presented data summarizing 15 different clinical trials both in the United Kingdom and in the Gambia. In contrast to DNA alone or the viral vector alone, significant protection against heterologous strain challenge was observed after prime-boost immunization. Priming was achieved with a DNA vaccine or a fowlpox vaccine encoding the sporozoite antigen, thrombospondin-related anonymous protein (TRAP), as well as a string of epitopes from other parasite antigens; the boost consisted of MVA vaccinia virus carrying the DNA of these same antigens.

In a third approach to developing vaccines against sporozoite and liver-stage antigens, Ruth Nussenzweig (New York University) and Ashley Birkett (Apovia Inc.) summarized the preclinical development of Apovia's CorVax particles. These particles comprise a recombinant hepatitis B core antigen expressing both T and B cell epitopes from the malaria circumsporozoite protein. Self-assembled particles produced in *Escherichia coli* are very stable and highly immunogenic in animal models.

Vaccines for erythrocytic and sexual parasite stages have not been as extensively tested in humans. Gray Heppner (Walter Reed) summarized their blood-stage vaccination program. MSP1 formulated with the adjuvant ASO2 has been tested in phase I safety trials in the United States and in a sporozoite challenge study; future field trials in Kenya are anticipated. Robert Sauerwein (Nijmegen Univ. Medical Center) summarized the European Union's plans for human trials of both sporozoite and blood-stage vaccine candidates containing the GLURP, AMA1, LSA3, and MSP1 parasite proteins. The NIH's Malaria Vaccine Development Unit has produced recombinant transmission-blocking (Pvs25, Pfs25) and blood-stage (MSP1, AMA1) vaccine candidates (under Good Manufacturing Practice conditions) in readiness for U.S. phase I safety trials.

There were spirited discussions about what could be done to speed up the process of malaria vaccine development. Predictions of when an efficacious vaccine would be available ranged from 7 to 23 years. There was a general consensus that an international consortium should be established to provide an integrated forum for individuals and groups involved in malaria vaccine development to exchange and develop ideas; articulate and communicate strategies and requirements; and organize advocacy efforts. With the *P. falciparum* genome yielding new vaccine candidates, the fine-tuning of existing vaccine strategies, and encouraging results from early field trials, the hope that a malaria vaccine will eventually be developed is not misplaced.

References

1. Malaria's Challenge: From Infants to Genomics to Vaccines; Keystone Symposium, 3 to 8 March 2002, Keystone, Colorado.
2. *Report of the Commission on Macroeconomics and Health of the World Health Organization* (WHO, Geneva, 2002).