The Challenge of Malaria

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Plasmodium falciparum, like the human immunodeficiency virus (HIV), causes a major disease in the tropical world that will become worse in the years ahead, with no guaranteed solution at hand. This threat is highlighted most dramatically by a recent Institute of Medicine (IOM) report on malaria (*1*). In this Perspective, I have developed what I see as the research priorities to develop new tools for reducing mortality and for control and eradication of malaria.

Malaria is one of the few diseases for which we can estimate past mortality rates on the basis of today's gene frequencies. These estimates depend on the assumption that the mutation in the β chain of hemoglobin A that generates hemoglobin S, common in Africa, and in the red cell membrane protein band 3, common in Southeast Asia (*2*), were selected because such mutations, when heterozygous, confer resistance to severe malaria. Given this assumption, the mortality from malaria can be predicted from the frequency of these genes with the use of the Hardy-Weinberg equation (see figure). The mortality in recent years has been less because of the widespread use of the antimalarial drug chloroquine.

In Africa south of the Sahara, where methods that are conventionally used to control the mosquito vector have not proved feasible, the spread of chloroquine resistance is rapidly eliminating this primary tool to reduce morbidity and mortality. The impact was documented at a hospital in Zaire where a sudden increase in admissions and mortality from malaria was precipitated by the appearance of chloroquine-resistant *falciparum* malaria in the region (*3*). Resistance is also developing to the affordable, alternative drug combination: pyrimethamine-sulfadoxine.

Research to lessen the impact of malaria immediately is focused on evaluating the effectiveness of insecticide-impregnated bed nets, drugs that are far along in development such as ginghaosu derivatives (*4*), and better ways to apply the less-than-perfect, currently available tools. The United States should continue to contribute to this research aimed at mitigating the effects of malaria in the short term, but I believe that our most valuable and unique contribution would be to find better tools to apply against *P. falciparum* and its vectors.

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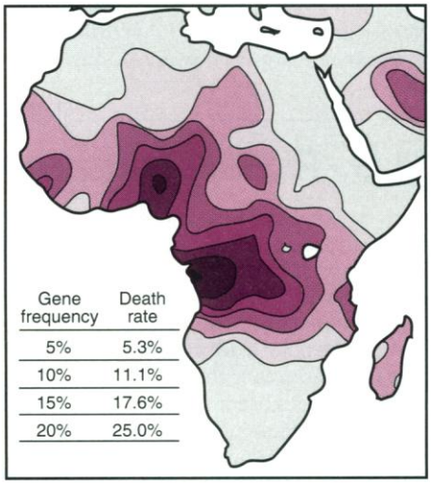
What longer term research approach is likely to have the earliest and most dramatic impact on disease and deaths? It is my opinion that vaccines are in this category (*5*)—specifically, an effective vaccine against the asexual erythrocytic parasite. First, this is the only stage in the life cycle that causes disease. Even partial vaccine-induced immunity, which controls an evolving infection by decreasing parasite density in the blood, will lessen mortality. Second, immunity eventually does develop in older children and adults after repeated infections. Third, vaccines have been successful in every animal model of malaria, including *P. falciparum* in New World monkeys. For example, purified antigens from parasites effectively induce protection against otherwise lethal malarias (*1*, *5*). I do not wish to minimize the difficulties development of a successful vaccine must overcome, which include parasite evasion mechanisms, the reluctance of industry to become involved in malaria vaccine development, and the inherent difficulties in testing vaccines against asexual blood stages for efficacy in humans. If we could involve industry in the development process even though large profits cannot be expected, then I trust that the application of modern biology could solve the scientific problems. In the past, science has had a great impact on health through making available clean water supplies, pasteurization of milk, chemotherapy, insecticides, and viral and bacterial vaccines. I would like to believe that an equivalent contribution today would be the development of a malaria vaccine.

Of lesser priority, but still of great importance, would be the development of vaccines that would interrupt transmission at the sporozoite or sexual stages. Such vaccines may, in combination with other methods of control, eradicate malaria from regions of low to moderate transmission such as Sri Lanka. For travel to malaria-endemic areas where chloroquine is no longer effective in protecting against malaria, sporozoite vaccines offer great promise.

Attack against the mosquito vector has always offered the only hope for eradication of the parasite. It is only through eradication that we would no longer have the continual concern about emergence of drug- and vaccine-resistant parasites. To appreciate the potential of this approach, it is only necessary to remember the dramatic effect the insecticide DDT had on reducing or eliminating malaria from many parts of the world. It is also important to note that malaria is a serious problem in Africa because of the high rates of transmission—a direct result of the *Anopheles gambiae* complex of mosquitoes, the most efficient vectors in the world (*6*). It is not surprising, then, that when *An. gambiae* was introduced into other parts of the world such as Brazil and Egypt, epidemics occurred where malaria was previously easy to control (*7*). The problem was eliminated when *An. gambiae* itself was eradicated. The eradication of *An. gambiae* is out of the question in Africa, at least with the technology that we can envision.

There is a new program at the World Health Organization, with support by foundations such as the MacArthur Foundation and the Wellcome Trust, to alter the capacity of mosquitoes to transmit malaria. The program focuses on factors in the mosquito that kill the parasite or block the parasite's development (*8*) and on methods to introduce genes for these factors into vector populations. Two observations make me optimistic about the feasibility of such an undertaking. It has been assumed that the parasite is able to mutate to escape any barrier in the mosquito that we may introduce, as if the parasite's adaptability is unlimited. This may not be the case. Mammalian malarias are transmitted only by *Anopheles* mosquitoes. Although *Culicine* mosquitoes regularly bite malaria-carrying humans, none is involved in the transmis-



Hemoglobin S gene in Africa. The most intense maroon areas have a frequency of 14% or more, with decreasing frequencies radiating from there. (**Inset**) Risk of death from malaria in hemoglobin AA children calculated from the equilibrium frequency of the hemoglobin S gene. Map adapted with permission © from W. F. Bodmer and L. L. Cavalli-Sforza, *Genetics, Evolution, and Man* (Freeman, San Francisco, 1976).

sion of the infection. There must be some block to the development of the parasite in *Culicine* mosquitoes that the human parasite is unable to overcome.

Introduction of genes encoding refractoriness into field populations of mosquitoes remains a major obstacle to implementation of such control strategies. A transposable element, the P element, first appeared in *Drosophila melanogaster* around 1950 and spread in a non-Mendelian fashion to flies of this species around the world (*9*). We might use an analogous transposable element of mosquitoes in a cassette with genes for refractoriness to malaria to introduce these genes into vector populations.

The third leg of basic research aimed at developing new tools is the identification of biochemical pathways unique to the malaria parasite and subsequent development of poisons specific to the parasite. One such area is hemoglobin digestion. The parasite, after digestion of hemoglobin, reorganizes heme into a nontoxic compound, hemozoin pigment. The polymeric structure of hemozoin pigment has recently been identified, and the polymerizing enzyme activity is blocked by chloroquine and other related antimalarial compounds (*10*). Furthermore, the locus for the chloroquine resistance gene (*11*), which encodes a drug-efflux mechanism (*12*) unrelated to the polymerase, has been identified. Understanding the structure and function of the polymerase and efflux mechanism should open the way for drug design to reverse chloroquine resistance.

Malaria, unlike AIDS, is not a major health problem for citizens of the United States; it is a problem of the unseen sick and dying in the villages of the tropical world. Our goal must be to develop tools and ways of delivering these tools to limit disease and to prevent malaria at a cost that is affordable and sustainable in these populations with limited resources. Success will demand continual support of basic scientists and involvement of industry. Can we ignore this challenge?

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Mosquito Molecular Genetics: The Hands That Feed Bite Back

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Mosquitoes are the most important vector of human parasitic diseases. Every year they transmit over 250 million new cases of malaria, filariasis, and viral disease. It is therefore surprising that only recently has mosquito molecular genetics been pursued with the same rigor and zeal characteristic of other aspects of parasitic disease control and treatment. In the last few years, research efforts funded by the John D. and Catherine T. MacArthur Foundation and the National Institutes of Health here in the United States, and by the Wellcome Trust in Great Britain, have catalyzed a renaissance of interest in the vectors of parasitic diseases. New knowledge of the molecular bases of vector-parasite interactions and population structure in mosquitoes should ultimately lead to novel ways to control disease transmission.

A workshop (*1*) sponsored by the MacArthur Foundation brought together vector molecular biologists with scientists who have made significant progress in other, well-studied organisms, most notably *Drosophila melanogaster*. Together, these two groups assessed the state of mosquito molecular biology and molecular genetics.

Many of the investigators are working toward developing strains of mosquitoes that are refractory or resistant to parasites. These strains will be released to control the transmission of disease by replacing the existing populations. Better diagnostic methods will help these efforts by defining those species or strains that should be the targets of genetic control.

In Africa there are six described members of the *Anopheles gambiae* complex, three of which overlap in their geographical range. Different members of a complex can coexist in a single locale, but only one may be actually transmitting the parasites. Members of the *An. gambiae* species complex have been differentiated by their polytene chromosome banding patterns (*2*), and the derived maps are as detailed as those available for *Drosophila*. However, these maps have yet to lead to techniques that would allow quick and accurate identification of large numbers of animals in the field. At the workshop, various molecular approaches for species identification were presented. Ribosomal (F. H. Collins, Centers for Disease Control–Atlanta) and mitochondrial (A. Cockburn, U.S. Department of Agriculture–Gainesville) DNA variation potentially can provide sensitive measures of species differentiation. The ribosomal RNA genes of mosquitoes have the typical structural features of the eukaryotic rDNA cistrons. Included in the intergenic spacer regions (IGS) are sequences of DNA that vary among populations (*3*). Regions of the IGS were scanned for two base-pair sequence polymorphisms among the members of the species complexes. Polymerase chain reaction (PCR) primers containing two 3′-terminal nucleotides that overlap the polymorphisms can distinguish among members of the complex. Only those animals with an exact match to the primers amplify a product. Mitochondrial DNA variability is also being evaluated in the *An. quadrimaculatus* species complex of the Americas, but probes have yet to be developed. PCR techniques with random amplified polymorphic DNA (RAPD) sequences (C. Louis, Institute of Molecular Biology and Biotechnology–Crete) are being investigated, but it remains to be seen whether they are generally applicable.

Another necessary step in developing strains of mosquitoes that are resistant to parasites is the identification of the gene or genes required for disease transmission. For example, certain genetic variants of *An. gambiae* and *Aedes aegypti*, the yellow fever mosquito (see figure), have reduced capacity to transmit the pathogens responsible for parasitic diseases, including malaria and filariasis. In order to identify the genes responsible for this reduced transmission, certain technological achievements must be made. The three central workshop topics—genome mapping, transposable elements, and transformation strategies—point to the challenges facing molecular biologists working with mosquitoes, notably, a lack of ability to transform mosquitoes at useful frequencies and an inability to isolate genes

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