Malaria as a Reemerging Disease

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INTRODUCTION

Malaria is a clear example of a reemerging disease. The annual number of cases (incidence) fell sharply 30–35 years ago as the result of a malaria eradication campaign (1, 2). At that time, malaria was eliminated from Europe, most Asian regions of the USSR, the United States, and most of the Caribbean (3). However, it was not eradicated in Southeast Asia, South America, or Africa, and has reemerged in Sri Lanka, Madagascar, and elsewhere (3). Thus, the incidence of malaria today is greater than 40 years ago and is increasing because of antimalarial drug resistance (4, 5), insecticide resistance (6), and the effects of civil strife—dislocation of susceptible refugee populations from nonendemic areas to areas with malaria transmission (7, 8). The incidence of malaria complications (morbidity) and deaths (mortality) is likewise increasing because of drug resistance (9). These changes also reflect the failure thus far of alternative control strategies, such as vaccine development. The net result is that the increasing morbidity and mortality of malaria affect not only the health of the developing world, but also (prevent) its economic development. This review begins by examining the two major reasons that the malaria eradication campaign was unsuccessful, antimalarial drug resistance and insecticide resistance. It then examines alternative malaria control strategies such as the development of antimalarial agents effective against drug-resistant parasites, of bednets and curtains impregnated with pyrethroid insecticides, and of malaria vaccines, concluding with a discussion of the balance that will likely be necessary between basic and applied research for effective malaria control.

MALARIA ERADICATION

During the 1950s, the World Health Organization and other international organizations made a commitment to eradicate malaria (1, 2). However, that commitment was based on two incorrect assumptions: 1) human malarial infection could be eliminated by treatment with antimalarials such as chloroquine and 2) transmission could be eliminated with residual insecticides such as dichlorodiphenyltrichloroethane (DDT). First, the prevalence of chloroquine resistance (which was increased by massive chloroquine use during the malaria eradication campaign) made it impossible to clear Plasmodium falciparum parasitemias in many regions of Southeast Asia and South America with chloroquine (the safest and least expensive antimalarial). Second, both insecticide resistance and exophilic transmission undermined the efficacy of residual insecticides. In addition, massive problems with logistics, planning, resource allocation, and a lack of operational research contributed greatly to the failure of malaria eradication (3). Finally, although there are a number of ways in which malaria is not an ideal candidate for eradication (table 1) (10–12), a logical analysis of these factors was impossible at that time because the smallpox eradication campaign did not begin until a decade later, and did not succeed until 1977 (13, 14). Because of the major logistical problems involved, the malaria eradication campaign formulated by the World Health Organization in the 1950s focused primarily on Southeast Asia and South America (1) rather than sub-Saharan Africa, where the intensity of transmission and the morbidity and mortality of malaria were greatest.

ANTIMALARIAL DRUG RESISTANCE

The factors which permitted the emergence of drug resistance, and have increased its prevalence, are inadequately understood. In part, this is because controlled experiments are difficult to perform with human populations under the conditions of natural malaria transmission. Nevertheless, two factors important for the emergence of resistance to other antimi-
choloroquine-resistant \( P.\ falciparum \) is in vivo: 1) extensive use of (selection with) treatment based on single drugs and 2) suboptimal treatment using drug doses lower than those recommended. Both factors were present during the malaria eradication campaign and are likely to have been important for the emergence of chloroquine resistance. Chloroquine was the only antimalarial used by most national malaria programs and was given by mass distribution to entire populations. In fact, chloroquine was actually added to table salt in Southeast Asia, South America, and sub-Saharan Africa (17-19).

In addition, individual patients often received suboptimal treatment because a number of malaria programs used lower drug doses in order to economize and because patients often shared their pills with other family members, especially children. Thus, even if the emergence of chloroquine resistance in \( P.\ falciparum \) is an extremely unusual genetic event (occurring only once or twice during the last 50 years), the power of selection exerted by mass chloroquine distribution and contiguous spread may have been sufficient to account for the subsequent spread of chloroquine-resistant \( P.\ falciparum \) throughout South America, Southeast Asia, and Africa (20-22).

Similarly, mass chemophylaxis with pyrimethamine and its introduction into table salt were associated with the relatively rapid emergence and contiguous spread of pyrimethamine resistance in Tanzania (23-25). Taken together with the effects of civil strife (7, 8), contiguous spread of resistant parasites based on drug pressure from chemophylaxis may account for much of the spread of drug resistance in malaria.

**CHLOROQUINE RESISTANCE**

Because chloroquine is safe even during pregnancy (26, 27), effective for the treatment of severe malaria (28), and economical (29), the development and spread of chloroquine resistance is one of the most important factors in the current, worldwide resurgence (reemergence) of malaria. The emergence and spread of chloroquine resistance has had a devastating impact on the morbidity and mortality of \( P.\ falciparum \) malaria (9) and has exacerbated the difficulties of treating severe malaria due to \( P.\ falciparum \) infection. In fact, many \( P.\ falciparum \) infections in Southeast Asia are now resistant to chloroquine, mefloquine, halofantrine, and pyrimethamine-sulfadoxine, and partially resistant to quinine and quinidine (30, 31). Despite the recent emergence of chloroquine-resistant *Plasmodium vivax* (32) in Thailand, Myanmar, Brazil, Indonesia, Colombia, and other countries (33-36), chloroquine resistance is a less frequent and less severe problem with *P. vivax* than with \( P.\ falciparum \). However, chloroquine remains effective for the treatment of *Plasmodium ovale* and *Plasmodium malariae* infections, most *P. vivax* infections, and for *P. falciparum* infections from selected areas without resistance such as Haiti (37).

**Chloroquine-resistant \( P. falciparum \)**

From the time of its synthesis and early use in the late 1940s (38-41) until the early 1960s, there was no evidence of chloroquine resistance. However, in the early 1960s, chloroquine resistance was reported from both South America (Colombia and Venezuela) (42) and Southeast Asia (Thailand) (43). Subsequently, chloroquine-resistant *P. falciparum* spread from Colombia and Venezuela to contiguous regions of the Amazon Basin in South America (20, 22), and from Thailand to contiguous regions of Southeast Asia (21, 22). Chloroquine-resistant *P. falciparum* first appeared in Africa almost two decades later, in 1978 (44), although the route (or means) by which it reached Africa is unclear. One hypothesis is that chloroquine-resistant *P. falciparum* was imported by laborers from Southeast Asia who worked on projects such as the railway from Mozambique (Beira) to Zaire (Kinsasa). If this hypothesis is correct, molecular markers based on the chloroquine resistance gene or its flanking sequences may distinguish chloroquine-resistant parasites from Southeast Asia or Africa versus South America, based on techniques such as Southern blots (restriction digests followed by hybridization). Unfortunately, because the genetic basis of chloroquine resistance is unknown (45, 46), no geographically based genetic markers are now available.

Studies by a number of investigators, including ourselves, suggest that the basis of chloroquine resistance in *P. falciparum* is the rapid efflux (excretion) of chloroquine by resistant parasites (figure 1) (47, 48), which are thus resistant because they fail to accumulate biologically significant intracellular concentra-

**TABLE 1. Criteria that favor disease eradication**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Smallpox</th>
<th>Malaria</th>
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<tbody>
<tr>
<td>Distinctive (diagnostic) clinical presentation</td>
<td>Yes (rash)</td>
<td>No (fever, malaise)</td>
</tr>
<tr>
<td>Subclinical human illness</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Seasonality, periods of low transmission</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effective vaccine after infection</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Life-long immunity after infection</td>
<td>Yes</td>
<td>No (frequent reinfestation)</td>
</tr>
<tr>
<td>Non-human vector</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-human reservoir</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

* Modified from Stuart-Harris (11).
tions of chloroquine. Verapamil, which inhibits chloroquine efflux, thus enhances both chloroquine accumulation (49) and the activity of chloroquine (47) against otherwise-resistant parasites. Studies based on the ability of verapamil to enhance chloroquine accumulation with P. falciparum isolates from Southeast Asia, South America, and Africa suggest that there may be only one mechanism of chloroquine resistance worldwide because verapamil has increased the chloroquine accumulation of all chloroquine-resistant P. falciparum isolates tested thus far (50).

**Chloroquine-resistant P. vivax**

In contrast, chloroquine-resistant P. vivax has appeared relatively recently. Chloroquine-resistant P. vivax is most common in Southeast Asia (32–34) but has also been reported from South America (35, 36). The data available suggest that these infections typically respond to treatment with oral mefloquine or halofantrine (32, 51) and that they may respond to treatment with amodiaquine (52). However, because the frequency of chloroquine-resistant P. vivax is relatively low (even in the areas where it was first reported), chloroquine remains the first-line treatment for persons with P. vivax infection, even in areas where chloroquine-resistant P. vivax has been identified, unless the patient developed his/her P. vivax infection while on chloroquine chemoprophylaxis.

Because there are as yet no studies of the mechanism responsible for this resistance, it is not clear whether the mechanism of chloroquine resistance in P. vivax is similar to that in P. falciparum (47–50). Because it is now possible to grow chloroquine-resistant P. vivax parasites in vivo in monkey models (53) and to perform limited short-term in vitro susceptibility testing (54), it should now be possible to address this question by testing whether verapamil increases chloroquine accumulation in chloroquine-resistant P. vivax as it does in chloroquine-resistant P. falciparum (49). Unfortunately, because of the potentially confounding effects of the host immune response, and because P. vivax parasites do not grow in the usual in vitro culture system (55), it has been more difficult to follow the spread of chloroquine-resistant P. vivax than chloroquine-resistant P. falciparum. No molecular markers are currently available for chloroquine resistance in P. vivax.

**Pyrimethamine Resistance**

Pyrimethamine (Daraprim®; Burroughs Wellcome, Research Triangle Park, North Carolina) alone is typically used for chemoprophylaxis. In areas such as West Africa, it is usually taken as two (25 mg) pills on Sunday (i.e., “Sunday, Sunday” medicine). Because pyrimethamine is rarely used alone for treatment (it is usually used in combination with sulfadoxine (Fansidar®, Hoffmann-LaRoche, Nutley, New Jersey)), there is virtually no clinical experience with which to compare in vitro and in vivo pyrimethamine-resistant phenotypes. Thus, the data available on resistance to pyrimethamine are based on the identification of specific point mutations in the dihydrofolate reductase (DHFR) enzyme, which is the molecular target of pyrimethamine, and on the effects of those point mutations on in vitro susceptibility testing results with pyrimethamine (50 percent inhibitory concentration—the (nanomolar) concentration of drug required to inhibit parasite (P. falciparum) growth by 50 percent in vitro (IC_{50}). The clinical relevance of these observations has been assumed but has not been proven.

**Pyrimethamine-resistant P. falciparum**

P. falciparum parasites with pyrimethamine-resistant genotypes (point mutations associated with elevated IC_{50}’s to pyrimethamine alone) have been identified in South America, Southeast Asia, and Africa (56–58). Although the point mutations in DHFR associated with pyrimethamine resistance in vitro are separated from each other in the linear nucleotide/amino acid sequence, they converge at the pyrimethamine binding site (active site) when the DHFR molecule is viewed in three dimensions based on its x-ray crystallographic structure (figure 2) (59, 60). As
FIGURE 2. Three-dimensional representation of the dihydrofolate reductase (DHFR) binding site. Amino acids thought to be important in resistance to DHFR antagonists converge at the dihydrofolate binding site when DHFR is viewed in three dimensions. These amino acids include: Asn-108 in place of Ser-108 in the C α-helix for pyrimethamine resistance; Val-16 in place of Ala-16 in the A β-strand for cycloguanil resistance; and Ile-51 and Arg-59 in place of Asn-51 and Cys-59 for resistance to both pyrimethamine and cycloguanil. Reproduced with the permission of the National Academy of Sciences of the United States (60).

demonstrated by the studies of Wellems and his colleagues, specific point mutations such as the change from Ser to Asn at position 108 increase the IC50’s observed with pyrimethamine by ≥2,000-fold over those observed with the wild-type enzyme (60, 61). In contrast to Leishmania and other protozoa, in which amplification of the DHFR gene is the principal mechanism of resistance to antifolates (62), there is no evidence for amplification of DHFR among P. falciparum isolates resistant in vitro to pyrimethamine, cycloguanil (the active metabolite of proguanil), or other dihydrofolate reductase inhibitors.

Studies performed by several groups have now established that polymerase chain reaction (PCR)-based assays can be used to detect P. falciparum parasites with the pyrimethamine-resistant genotype, e.g., Asn 108 rather than Ser 108 in DHFR (57, 58). Based on selective mismatching of primers at their 3’ end, there is now a PCR assay which identifies the specific mutations responsible for in vitro resistance to pyrimethamine and cycloguanil in P. falciparum (figure 3). Recent work by Plowe et al. (63) demonstrates that this technique can be used on-site to monitor the relative prevalence of parasites with the pyrimethamine- or cycloguanil-resistant genotypes in endemic countries. This assay is extremely useful for monitoring the prevalence of these mutations because it does not require in vitro parasite growth with the attendant risk of losing parasite clones which grow well in vivo, but not in vitro (64, 65).

FIGURE 3. Polymerase chain reaction (PCR) for resistance to dihydrofolate reductase (DHFR) antagonists. With 1 of 3 reverse (3’) primers for the wild type (drug-sensitive) pyrimethamine-resistant and cycloguanil-resistant enzymes, respectively, and a single forward (5’) (forward) primer, the 337 bp amplification product is obtained only when the reverse primer used matches the nucleotide sequence in the DHFR. As indicated by the asterisk (*), the critical match (mismatch) which determines the specificity of this reaction is at the 3’ end of the reverse primer at amino acid 108 in DHFR (nucleotide 323 in the DHFR coding sequence) (59, 60, 63).

Pyrimethamine-resistance in P. vivax

The limited data available suggest that P. vivax is resistant to treatment with pyrimethamine alone in vivo, and may also be resistant to sulfadoxine plus pyrimethamine (Fansidar®) (66, 67). However, because P. vivax can be grown in vitro only with difficulty, there have been no reports about the in vitro susceptibility (IC50’s) of P. vivax to pyrimethamine or sulfadoxine. Based on the experience with P. falciparum, one would expect P. vivax resistant to pyrimethamine in vitro to have one or more of the point mutations associated with in vitro resistance to pyrimethamine in P. falciparum (57, 58, 63).

RESISTANCE TO COMBINATIONS OF DHFR ANTAGONISTS AND SULFONAMIDES (FANSIDAR®)

Combinations of DHFR antagonists and sulfonamides, such as Fansidar®, are increasingly important because they are almost as economical as chloroquine (29) and because they are often effective against chloroquine-resistant P. falciparum in vivo (68, 69). For these reasons, one country in sub-Saharan Africa (Malawi) with a high prevalence of chloroquine-resistant P. falciparum (68, 70, 71) switched to Fansidar® from chloroquine as its first-line antimalarial in 1993. Although the preliminary data suggested that the switch from chloroquine to Fansidar® decreased the incidence of complications such as severe malarial anemia (W. Nkhoma, Ministry of Health, Lilongwe, Malawi, personal communication, 1995), a more recent report suggests that the frequency of RI and RII level resistance to Fansidar® in vivo is increasing (72), and, thus, that this strategy may have improved the clinical outcome for a limited period of time (2–3 years).
In contrast, in vivo Fansidar® resistance is well-established in both South America and Southeast Asia. In particular, the experience in Southeast Asia suggests that widespread use of Fansidar® for the treatment of chloroquine-resistant P. falciparum is likely to be followed by widespread resistance (73–75). At the present time, Fansidar® treatment failures are so common in Southeast Asia that Fansidar® is no longer a reliable choice for the treatment of P. falciparum infection.

Despite the information available on point mutations associated with pyrimethamine resistance, the mechanisms (mutations) responsible forFansidar® resistance are not defined. Isolates from areas where in vivo Fansidar® resistance is common often have one or more of the DHFR point mutations associated with pyrimethamine resistance in vitro (59–61). However, two major difficulties remain before one can expect the same level of molecular understanding about sulfonamide and Fansidar® resistance that has been achieved with pyrimethamine resistance. The first major difficulty is that point mutations in the dihydropteroate synthase (DHPS) target site of the sulfonamides (76, 77) must be clearly related to sulfonamide resistance in vitro, as point mutations in the DHFR have been related to pyrimethamine resistance in vitro (59–61). This step is essential in order to clarify the relation between specific point mutations in DHPS and Fansidar® failure in vivo. The second major difficulty is that there are no generally accepted conditions for testing Fansidar® and other similar combinations of DHPS/DHFR inhibitors in vitro (65, 78). Because p-amino-benzoic acid and folate concentrations may have a profound effect on such testing (78), a reliable in vitro system to test these combinations will be necessary in order to correlate antiparasitic activity in vivo with specific point mutations. Finally, because combinations, such as Fansidar® contain two drugs, it is possible that combinations of point mutations (in the DHFR and DHPS), each of which has minimal or undetectable effects alone, may have profound (synergistic) effects together.

Fansidar®-resistant P. falciparum has been reported frequently from Southeast Asia and South America, but rarely from sub-Saharan Africa. Although a number of point mutations have been identified in the DHPS of P. falciparum (76, 77), the relation between these mutations and Fansidar® resistance is incompletely defined for the reasons described above. Although this question is complex, it is of immense importance for countries such as Malawi which have switched to Fansidar® as their first-line antimalarial. Because the prevalence of Fansidar® resistance remains low in most of sub-Saharan Africa (68, 70), studies to define the molecular basis of Fansidar® resistance should begin in areas with established foci of Fansidar®-resistant P. falciparum such as Malawi (72), or in parts of Southeast Asia or South America where Fansidar® resistance is established but treatment is thought to be effective (73–75).

Fansidar® failures with P. vivax have been reported from Southeast Asia (66, 67) and may also occur elsewhere. However, the causes of those failures are unclear because P. vivax can be grown in vitro only with difficulty, and conventional in vitro susceptibility testing is rarely performed. Thus, as yet there have been no studies relating point mutations in the P. vivax DHFR or DHPS to Fansidar® failure in vivo or Fansidar® resistance in vitro (as measured by susceptibility testing).

MEFLOQUINE RESISTANCE

Mefloquine was originally developed because it was active in vitro and in vivo against chloroquine-resistant P. falciparum. For this reason, early studies in Southeast Asia demonstrated that mefloquine was superior to chloroquine for the treatment of serious P. falciparum infections (79). However, after more than 15 years of extensive use, mefloquine resistance has become a significant problem in Southeast Asia, particularly in refugee-populated areas on the eastern border of Thailand with Myanmar (Burma) (80–83). In fact, in those areas, mefloquine is no longer effective for either the chemoprophylaxis or treatment of P. falciparum infection. In contrast, mefloquine remains effective for chemoprophylaxis in most of sub-Saharan Africa (84, 85), even in regions where chloroquine is ineffective such as East Africa (44, 86).

The mechanism responsible for mefloquine resistance is unknown, although it is associated with amplification of the pfmdrl parasite homolog of the gene responsible for multidrug resistance in mammalian cancer cells (87–89). Penfluridol enhances the activity of mefloquine against otherwise mefloquine-resistant parasites (90), similar to the effect of verapamil on the activity of chloroquine against otherwise chloroquine-resistant parasites. However, in contrast to chloroquine resistance (47–50), there is no evidence for mefloquine efflux from mefloquine-resistant parasites or for penfluridol enhancement of mefloquine accumulation by otherwise mefloquine-resistant parasites.

HALOFANTRINE RESISTANCE

Halofantrine is a phenanthrene methanol structurally similar to chloroquine which is active in vitro and in vivo against many chloroquine-resistant P. falciparum (30, 91). During the past 10 years, it has been
used extensively in areas of Southeast Asia, such as Thailand, where there has been substantial resistance to both chloroquine and mefloquine (92). However, recent studies suggest that the efficacy of halofantrine may be compromised in precisely those situations (areas with mefloquine resistance) (93), and, thus, that the utility of halofantrine may be much more limited than expected initially. Molecular studies suggest that resistance to halofantrine is also associated with amplification of the pfmdrl gene (93).

Despite the increasing prevalence of resistance to halofantrine in Southeast Asia, there is no evidence for halofantrine or mefloquine resistance in sub-Saharan Africa (84, 85). Recent experience suggests that mefloquine, halofantrine, and Fansidar® are generally effective even in regions of sub-Saharan Africa with extensive chloroquine resistance.

MULTIDRUG RESISTANCE AND PARTIAL RESISTANCE TO QUININE

In areas such as Thailand, there are multiply resistant strains of *P. falciparum* which are resistant to chloroquine in vivo and in vitro, to mefloquine in vivo and in vitro, and to halofantrine in vivo and partially resistant to quinine and quinidine in vivo and in vitro (94, 95). Because many of these parasites are also resistant in vivo to treatment with pyrimethamine plus sulfadoxine (Fansidar®) (95), they pose a critical problem in drug resistance and malaria control that has not yet been addressed.

ALTERNATIVE STRATEGIES FOR TREATMENT OR PREVENTION OF DRUG-RESISTANT *P. FALCIPARUM* INFECTION

Alternative chemotherapeutic strategies

Because of the problems posed by multiply resistant *P. falciparum*, there is a need for alternative strategies that circumvent drug resistance or control malaria by other means. Potentially effective alternative chemotherapeutic strategies include: 1) development of antimalarial derivatives effective against multiply resistant *P. falciparum* (including aminoquinolines, trioxanes (artemisinin derivatives), and others), 2) antimalarial combinations (recognizing the potential for problems due to differences in absorption and metabolism among drugs in the combinations), 3) alternative strategies for rural versus urban areas (when surveillance suggests differences in the prevalence of resistance between rural and urban areas), and 4) reinstitution of drugs such as chloroquine several years after they have been replaced as the first-line antimalarial by other agents. Artemisinin derivatives are attractive alternatives because they act rapidly in vivo (96) and are active against otherwise drug-resistant *P. falciparum* parasites (97). Unresolved questions about their use include neurotoxicity (98) and potential fetotoxicity. Recent controlled studies suggest that the artesinin derivative artemether is as effective as quinine for the treatment of cerebral malaria in children (99) and severe malaria in adults (100), and that the incidence of neurologic sequelae among children treated with artemether is no greater than with quinine (99).

Vector control could be achieved by reducing the entire vector population (which is impractical in regions as large as sub-Saharan Africa) or by reducing human-vector contact (use of residual insecticides such as DDT, bednets, or curtains impregnated with pyrethroid insecticides (see below)).

Vaccine development

Alternatively, one could produce immune responses in the human host that interfere with transmission through the mosquito (transmission-blocking immunity) or reduce the rates of infection or disease. Although transmission-blocking activity can be produced by antibodies against antigens on the gametocyte/gamete surface, it is not yet clear which (humoral or cell-mediated) immune responses will protect against infection or disease in human subjects.

ANTIMALARIALS ACTIVE AGAINST MULTIPLY RESISTANT *P. FALCIPARUM*

During the last year, studies of the structure-activity relations responsible for aminoquinoline resistance have demonstrated that the diaminoalkane side chain is critically important for antimalarial resistance. By altering the length of the diaminoalkane side chain in chloroquine and other aminoquinoline antimalarials, it has been possible to synthesize a new class of antimalarials which are as active as chloroquine against chloroquine-susceptible *P. falciparum* in vitro. Of particular interest are analogs with ethyl, propyl, isopropyl, decyl, or dodecyl side chains which are as active against chloroquine-, mefloquine-, and multiply resistant *P. falciparum* as chloroquine is against chloroquine-susceptible *P. falciparum* (101). These results demonstrate that the diaminoalkane side chain is a critical determinant of antimalarial resistance, presumably because analogs that are active against otherwise resistant parasites are not (recognized or) excreted by the efflux mechanism responsible for chloroquine resistance (47–50).

INSECTICIDE RESISTANCE

The spraying of insecticides such as DDT under the eaves of houses was a central strategy in the malaria control
eradication program (1, 6) and was responsible for many of its initial successes (3, 102, 103). However, the subsequent development of resistance to DDT was one of the most important reasons that malaria eradication failed, and it continues to be an important factor in the worldwide resurgence (reemergence) of malaria. Resistance to insecticides such as DDT is now widespread and compromises vector control in all the regions of the world where malaria is endemic. In addition, the environmental and ecologic effects of DDT have led to pressure to abandon its use for those reasons (104).

Although limited information is available about the mechanisms responsible for the action of DDT and for DDT resistance, these are important questions because of potential cross-resistance to other insecticides, such as the pyrethroids (105–107), which have been used to impregnate bednets and curtains for malaria control. The information available suggests that both DDT and pyrethroids act, in part, by repelling (rather than killing) mosquitoes (108). Thus, fewer mosquitoes enter houses which have been sprayed with DDT as a residual insecticide and houses that contain permethrin-impregnated bednets or curtains. Although the factors responsible for the killing activity of DDT and the pyrethroids are incompletely defined, they may involve voltage-gated sodium channels (109). Thus, it is possible that DDT- and pyrethin-resistant anopheline vectors may have altered sodium channels which make them resistant to those insecticides (110). Although the mechanisms actually responsible for DDT resistance are unknown, factors which have been implicated include enzymes that degrade DDT (dehydrochlorinase activity) linked to a glutathione-S-transferase (111, 112).

Alternatives to DDT for vector control and for reducing the amount of vector-human contact include bednets or curtains impregnated with pyrethroid insecticides such as permethrin or deltamethrin. Studies in a number of malaria-endemic countries have shown that this strategy can reduce the number of anopheline mosquitoes and anopheline bites by ≥95 percent (113) and the prevalence of infection by ≥40–45 percent (114, 115). However, its impact on the incidence of severe disease is controversial; one study suggested that insecticide-impregnated bednets had no impact on severe disease despite marked reductions in the entomologic inoculation rate (116). Conversely, more recent reports suggest that bednets reduce malaria morbidity and mortality, but may be less effective in areas with more intense transmission (117–119). Because the initial reports of reduced morbidity and mortality were from areas with low intensity transmission such as The Gambia (120, 121), additional studies will be necessary in areas with more intense transmission to determine whether insecticide-impregnated bednets and curtains are similarly effective under those conditions. Additional factors likely to limit the efficacy of insecticide-impregnated bednets or curtains include biting by anopheline mosquitoes outside houses or in the daylight hours and resistance to the pyrethroid insecticides (122, 123). Resistance to pyrethroid insecticides is emerging in part because of their extensive agricultural use.

Thus, the use of insecticide (pyrethroid)-impregnated bednets or curtains is an intriguing alternative, which should reduce the entomologic inoculation rate and the incidence of new infections. However, it is not yet clear whether this strategy will reduce severe disease and death in areas with the most intense transmission. Additional factors likely to compromise the efficacy of pyrethroid-impregnated bednets and curtains include pyrethroid resistance and exophilic vectors.

VACCINE DEVELOPMENT

With the major problems that now threaten strategies based on antimalarials and vector control, vaccine development is an alternative that must be considered carefully. The several rationales for developing a malaria vaccine include prevention of infection, prevention of disease, and reduction of transmission. In addition, the goals of a malaria vaccine vary in different populations. The goal of a malaria vaccine in endemic areas, such as sub-Saharan Africa, is to prevent severe disease and death among children who are exposed repetitively throughout their lives and ultimately develop protection from severe disease, but not infection (the semi-immune state). The goal of a malaria vaccine for expatriates is to prevent infection among nonimmune travelers exposed for short periods of time (usually 2–3 weeks). In contrast, the goal of a transmission-blocking vaccine is to reduce the intensity of transmission by immunizing the infected resident population. The persons immunized should benefit directly from antidisease and antiinfection vaccines, but not from transmission-blocking vaccines, which benefit the population indirectly by reducing the entomologic inoculation rate, and should be effective only in persons with circulating parasites (who would otherwise transmit the infection from their bloodstream to the anopheline mosquito vector). Boosting is also a potentially critical factor, and will be essential for vaccines given to children and adults in endemic areas (who should be boosted repetitively if the relevant antigens are exposed during natural infection). Conversely, protection against infection among expatriates

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must be achieved without boosting in order to prevent illness from short-term exposures. Because immune responses which protect against the mosquito (sporozoite) stage of the parasite are not effective against either the asexual bloodstream (merozoite) or sexual bloodstream and mosquito (gamocyte/gamete) stages of the parasite, most investigators expect that an effective malaria vaccine will need at least three different antigens (sporozoite, merozoite, and gametocyte) to protect against infection (sporozoites) and disease (merozoites and other blood stage antigens) and to reduce transmission (gametocytes or gametes). Although several candidate vaccine antigens have been tested in humans, none have been shown to be both safe and effective, i.e., to reliably prevent infection or disease in humans.

**SPOROZOITE VACCINES (PREVENTION OF INFECTION)**

Since the seminal discovery of Nussenzweig and her colleagues almost 30 years ago (124), it has been clear that immunization with irradiated sporozoites provides protection against sporozoite-induced malaria, and that immunization with recombinant sporozoite antigen (the four-amino acid peptide—asparagine (N), alanine (A), proline (P) (NANP)—repeat found in circumsporozoite protein (CSP)) elicits antibodies that react with (and neutralize) sporozoites in vitro. However, because antibodies directed against the NANP repeat of CSP do not protect against sporozoite-induced *P. falciparum* infection in vivo (125), and because persons who are regularly reinfected in endemic areas also have anti-NANP antibodies (126), other factors (such as cell-mediated immunity) must be important in the protection elicited by x-irradiated sporozoites (127, 128). Although the reasons for the lack of protection observed after immunization with recombinant NANP remain unclear, they are thought to include the role of cell-mediated immunity in protection against sporozoite-induced infection, the location of the determinants responsible for cell-mediated responses in a hypervariable region of the CSP molecule, and immune restriction (129). During a series of trials with a number of different formulations, the fraction of persons protected against subsequent challenge with *P. falciparum* sporozoites by mosquito inoculation has rarely exceeded 30 percent (130, 131). Thus, despite their initial promise, the outlook for sporozoite-based vaccines is uncertain, even with the addition of sequences designed to enhance cell-mediated responses to the NANP repeat.

**SYNTHETIC PEPTIDE VACCINES**

Apart from sporozoite vaccines, the only vaccine construct which has been subjected to extensive human testing is the SPf66 synthetic peptide vaccine developed by Manuel Patarroyo and his colleagues (132). This vaccine candidate was developed by covalently linking the parasite peptides which elicited the strongest humoral and cellular responses in *Aotus* monkeys. SPf66 contains an 11 peptide sequence from the amino terminal region of merozoite surface protein-1 (MSP-1), several copies of the NANP repeat in CSP, and two additional peptides thought to be from parasite proteins, although parasite proteins with those sequences have not yet been identified. A number of studies suggest that immunization with SPf66 is safe (133). However, it is not clear whether SPf66 is effective, i.e., whether immunization with SPf66 reduces the frequency of infection or severe malaria. Studies performed thus far suggest that the protective efficacy of immunization with SPf66 is between 0 percent and 30 percent (134, 135). In summary, although immunization with SPf66 is safe, its efficacy is no greater than 20–30 percent, and it may be ineffective.

**MEROZOITE VACCINES (PREVENTION OF DISEASE)**

Studies of merozoite (asexual) antigens such as MSP-1 suggest that antibodies to those antigens may limit parasitemia in vivo (136), and might thus prevent complications associated with high parasitemias such as cerebral malaria. Erythrocyte binding antigen 175 (EBA-175) is of interest because it may be necessary for the *P. falciparum* merozoite to enter a red blood cell (137). Apical membrane antigen-1 (AMA-1) has been localized to the rhoptries and may also be involved in parasite entry into the red cell (138). Although MSP-1, EBA-175, AMA-1, and several other asexual antigens are vaccine candidates (136–138), none have been shown to be effective in humans for the prevention of infection or disease.

**GAMETOCYTE VACCINES (PREVENTION OF TRANSMISSION)**

Studies using serum with high titers of antibodies to gametocyte/gamete antigens (*P. falciparum* sexual stage antigens 25 and 230 (Pfs25, Pfs230)) indicate that antibodies against these antigens can block transmission in the mosquito (139, 140). Because antibodies to Pfs25 are rarely present among exposed persons in endemic areas (141), immunization of humans with recombinant Pfs25 will be necessary to determine whether such antibodies are capable of blocking transmission under natural conditions. Experiments in an-
imals indicate that antibodies to Pfs25 block transmission in membrane-feeding experiments (139), and thus suggest that such antibodies to Pfs25 may block transmission in the field. In contrast, recent data suggest that antibodies to Pfs230 occur naturally (142), and thus that they may be responsible for some natural transmission-blocking activity.

CONCLUSIONS: PUBLIC HEALTH (DISEASE CONTROL) STRATEGIES VERSUS BASIC RESEARCH

In planning disease control strategies, there are often conflicts between those who believe that the means for control are at hand and simply need to be applied and those who believe that the critical issue is a lack of fundamental scientific knowledge (143). Malaria is clearly an example of this conflict. For instance, there are investigators who believe that application of bednets and curtains will eradicate malaria or reduce its consequences more effectively than any other measures likely to be available in the short-term or intermediate future. Therefore, they have argued that all available resources should be invested in bednets and curtains, emphasizing locally sustainable maintenance and reimpregnation of bednets and curtains. Conversely, other investigators believe that malaria control strategies based on bednets and curtains are inadequate and that better control will require major improvements in antimalarials. Thus, they have argued for a greater investment in antimalarial drug development. An effective vaccine would clearly be an economical public health strategy (if it could given only one to three times within existing (e.g., EPI) programs, were immunogenic in young children, and were enhanced by boosting during natural infection). However, at present, there are no known antigens or vaccine candidates which have produced ≥80 percent protection from infection or disease or reduced transmission by ≥80 percent in human trials. Thus, most investigators believe that malaria eradication is not a realistic possibility in the near future (10–15 years) and that additional basic science breakthroughs, such as better drugs, better vector control, or partially effective vaccines, will be necessary to achieve malaria control.

In conclusion, I believe that the eradication of malaria will require at least two resources which are not available at the present time: 1) an effective vaccine (which prevents infection, reduces transmission, is enhanced by natural boosting, and can be given at an early age) and 2) safe, nontoxic antimalarials effective against drug-resistant parasites (to cure persons who develop symptomatic infection and thus prevent morbidity and mortality, and reduce transmission). Although vector control can reduce the intensity of transmission, the natural anopheline reservoir in areas such as sub-Saharan Africa is great enough that sustained reduction of that reservoir may be an unrealistic goal in the next 10–15 years. Likewise, although ancillary measures such as improved housing associated with economic development are important, they are likely to be necessary (but not sufficient) for eradication.

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