tives of the traditional Chinese herbal treatment Qinghaosu. Donors have been reluctant to support the introduction of artemisinin into Africa, both because of its high unit cost relative to chloroquine and other first-line drugs—chloroquine costs ~10 cents for a curative regimen whereas artemisinin costs $1—and out of fear that artemisinin too will rapidly generate resistance. To counteract this risk, artemisinin-based compounds should be introduced in combination with other antimalarial drugs. Ironically, the delay in sponsoring such an approach is leading to the indiscriminate spread of artemisinin-based monotherapies through informal drug supply networks in Africa.

Encouragingly, in regions of Africa containing intensive economic activity (mines, oil fields, rubber plantations, urban zones, tourist sites), corporate malaria control efforts bolstered by public support are making a big difference (*12*). Successful corporate efforts generally rely on an intensive mix of environmental vector-control measures, individual protection of workers through household residual spraying, and case management. Recent initiatives by the world economic forum and other business groups plan to link these corporate efforts to broader international malaria control programs, particularly through formal public-private partnerships (*13*).

Longer term, more sweeping solutions will come from new drug discovery and especially vaccine-development efforts based on recent genomic advances. No major pharmaceutical company reports a concerted malaria research effort. The Gates Foundation has valiantly aimed to spearhead new research by supporting the drug-development MMV and the MVI. MMV has the declared goal of developing one new antimalarial drug every 5 years at a cost of $150 million, or $30 million per year, plus significant "in-kind" industry support. These numbers are below most estimates of drug-development costs, and are very unlikely to cover the high expenses of drug trials. A reasonable estimate of total worldwide public and private annual spending on malaria drug and vaccine research is less than $100 million, or less than one-seventh of 1% of the $70 billion or more of annual worldwide biomedical R&D, for a disease that accounts for about 3% of the worldwide disease burden as measured by disability-adjusted life years (*14*). R&D donor needs for drugs and vaccines are around $1 billion per year on a sustained basis, compared with current annual spending of less than $150 million.

The RBM consortium, headquartered at WHO, should serve as the nerve center of a renewed global effort to fight malaria. This consortium should immediately prepare a comprehensive strategy that includes an operational multiyear plan of action together with a full assessment of donor funding needs. The proposed budget should clearly delineate the separate needs for current prevention and treatment programs, largely funded through the GFATM and the World Bank; the rapid development, clinical testing, and procurement of artemisinin-based and other drug combinations; and the outlays for R&D for new drug discovery and vaccine development, including effective systems for high-cost clinical trials. Annual outlays by donors must reach several billion dollars per year for a generation or so to get malaria under control in endemic areas of Africa and Southeast Asia. But this will be a very small price to pay for millions of lives saved per year and for hundreds of millions of people to be given the chance to escape from the vicious cycle of poverty and disease.

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VIEWPOINT

Plasmodium Chloroquine Resistance and the Search for a Replacement Antimalarial Drug

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Genetic and biochemical research is providing new information on the mechanism of chloroquine resistance. Drug discovery initiatives are finding new leads that have favorable pharmaceutical properties and efficacy against chloroquine-resistant malaria.

The discovery of chloroquine and its subsequent worldwide use against malaria in the 20th century produced one of the greatest public health advances ever achieved by a drug against an infectious disease. Chloroquine's efficacy, affordability, easy administration, and low toxicity led to marked reductions in morbidity and mortality across the Americas, Africa, Asia, and Oceania. Chloroquine remained effective for decades. Despite its distribution in massive quantities (including distribution in the salt supplies of some countries), many years passed before chloroquine resistance (CQR) began to spread. *Plasmodium falciparum*, the most malignant of the four human malaria parasite species, showed foci of CQR in Southeast Asia and South America in the late 1950s, Papua New Guinea in the 1960s, and East Africa in the late 1970s. The steady and unremitting spread of CQR from these foci could only be met by a few alternative drugs, all of which were more expensive, encountered resistance problems of their own, or were less safe and more difficult to use than chloroquine itself. Morbidity and mortality from *P. falciparum* malaria consequently resurged, especially among children in Africa (*1*). Malaria caused by *Plasmodium vivax*, second only to *P. falciparum* malaria in its impact on health and economic development, remained responsive to chloroquine everywhere until a little over a decade ago, when

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chloroquine-resistant *P. vivax* began to spread in Southeast Asia and probably South America (*2*). Chloroquine-resistant *Plasmodium malariae* was also reported recently in Indonesia (*3*). Of the four human malaria parasite species, only *P. ovale* remains without reports of CQR.

The acute need for a replacement drug having the advantages and efficacy that once characterized chloroquine is a driving priority for malaria research. Success depends on the selection of appropriate drug targets, identification of good drug candidates that hit these targets with specific and prompt action, and stable and continued susceptibility of these targets when they are under therapeutic pressure. Fortunately, these efforts are being supported by new research partnerships and are beginning to benefit from modern tools of biomedical and pharmaceutical discovery. Genomics and proteomics studies have been receiving a particularly strong boost from results of the *P. falciparum* genome sequencing project, reported this month from three major sequencing centers (*4*). The sequences of seven of the parasite's 14 chromosomes are now closed and the remaining chromosomes are in advanced states of assembly, representing a formidable accomplishment on an AT-rich genome that has been notoriously difficult to clone and sequence. A new platform for discovery is set by the abundant information now available on *P. falciparum* genome organization and gene content.

Chloroquine acts by binding to heme molecules released from the hemoglobin that is digested by malaria parasites as they grow within their host red blood cells. This binding interferes with the process by which heme is normally incorporated into inert crystals and detoxified, thereby effectively poisoning the parasites (*5*). Because heme is not a parasite-encoded molecule that can mutate under drug pressure, malaria parasites have had to solve the difficult problem of chloroquine's toxicity by evolving a mechanism either to prevent drug-heme interactions or to control the damage from these complexes. Chloroquine-resistant *P. falciparum* parasites achieve this by reducing chloroquine accumulation in their acid digestive vacuoles where the drug does its damage. Linkage analysis of a genetic cross, positional cloning studies, and DNA transfection experiments have associated the CQR phenotype of *P. falciparum* with complex point mutations in the gene encoding PfCRT, a putative transporter or channel that is probably involved in chloroquine flux and proton equilibrium across the digestive vacuole membrane (*6*). Recent allelic exchange studies conclusively show that this CQR phenotype can be conferred on chloroquine-sensitive *P. falciparum* by replacing the endogenous *pfcrt* gene with mutant alleles from chloroquine-resistant parasites of South American or Asian origin (*7*).

Although the results of chloroquine treatment are largely determined by parasite drug susceptibility itself, host factors including immunity can have important effects on clinical outcome. Children who experience repeated malaria episodes in endemic regions eventually acquire levels of nonsterilizing immunity (premunition) that enable some to clear infections of chloroquine-resistant parasites after chloroquine administration (*8, 9*). The ability of particular individuals to achieve such clearance may be associated with human genetic variations that, individually or in combination, affect malaria pathogenesis and host responses to infection. Population studies based on high-throughput technologies and dense maps of single-nucleotide polymorphisms (SNPs) from the human genome offer an approach to identifying such variations (*10*). Understanding these variations and the mechanisms that operate at the drug response–immunity interface may help us to address some interesting questions: Can knowledge of the host factors that influence chloroquine treatment outcome be useful to vaccine development efforts? Will erythrocytic-stage vaccines improve chemotherapeutic responses to antimalarial drugs?

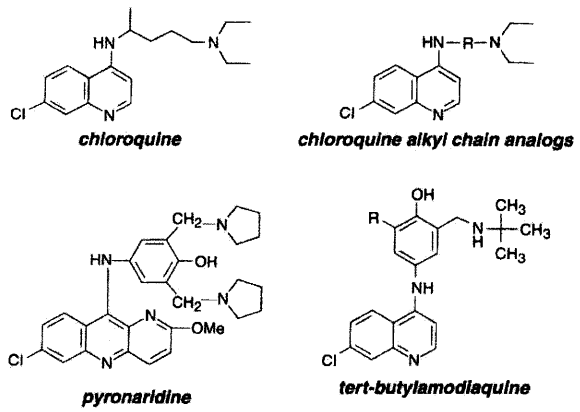

Fig. 1. Structure of chloroquine and some related synthetic compounds that are effective against chloroquine-resistant as well as chloroquine-sensitive *P. falciparum*. Drug binding depends on the stacking interactions between the 7-chloro-substituted quinoline ring and products of heme released from the digestion of host cell hemoglobin (*23*). Modifications of the side chain of chloroquine are thought to affect structural interactions involved in the drug resistance mechanism. Whether compounds effective against chloroquine-resistant *P. falciparum* are also active against chloroquine-resistant forms of *P. vivax* and *P. malariae* has not been established.

Evidence now indicates that amino acid substitutions in PfCRT (including a key $Lys^{76} \rightarrow Thr$ change) determine the CQR phenotype of *P. falciparum* in all malarious regions where it occurs. Strong support for this conclusion comes from recent studies that used SNPs and microsatellite variations in the *P. falciparum* genome to investigate parasite population structures, to follow selection in response to drug pressure, and to test chromosome segments for selective sweeps associated with drug resistance. Chromosome-wide surveys have identified abundant SNPs in coding regions subject to selection pressure as well as synonymous SNPs under little or no selection pressure (*11, 12*); analysis of the synonymous SNPs has traced today's *P. falciparum* populations to a small homogeneous ancestry of parasites that probably existed 100,000 to 180,000 years ago (*12*). The evidence that universally associates *pfcrt* mutations with sweeps under chloroquine selective pressure derives from related genome-wide surveys with *P. falciparum* microsatellites. These surveys indicate that *pfcrt* mutations gave rise to CQR at least four independent times (*13*). Mutations in the *P. vivax* homolog of *pfcrt*, however, are not associated with CQR (*2*); this finding suggests a genetic basis for CQR in *P. vivax* that is different from that in *P. falciparum*. Other loci for CQR in *P. vivax* and *P. malariae* may reflect the deep evolutionary divisions of these species from *P. falciparum* and may help to explain the different delays in their acquisition of CQR. Because *P. vivax* and *P. malariae* are very difficult to manipulate experimentally, genome data and linkage disequilibrium studies of these species with polymorphic markers may provide essential information on genetic loci selected by drug pressure. The potential value of knowing the CQR determinants in *P. vivax* and *P. malariae* as well as in *P. falciparum* is strong motivation to push forward and complete the genome sequencing and comparative analysis of these species.

The *P. falciparum* genome database will also be valuable in searches for possible modulators of the CQR phenotype and for other genes involved in more complex mechanisms of resistance to drugs such as quinine. In carefully controlled laboratory assays, variations are often detected in the chloroquine concentrations that different chloroquine-resistant *P. falciparum* lines can survive under tissue culture conditions. The influence that these variations may have on in vivo (clinical) resistance depends on their magnitude and whether they reflect true modulations of the CQR phenotype, as opposed to artifacts from in vitro laboratory measures of parasite response (*9*). The *P. falciparum* Pgh-1 P-glycoprotein–like molecule encoded by *pfmdr1* is an example of a determinant that has been associated with modulations of the CQR

phenotype (*14*), although it did not increase the risk of chloroquine treatment failures in association with *pfcrt* mutations (*8*). Mutations in the *pfmdr1* gene may therefore represent fitness adaptations to physiological changes from the *pfcrt* mutations, analogous to the compensatory alterations that occur in other pathogens after the acquisition of core resistance determinants (*15*). Effects from possible modulators of CQR phenotypes may vary with the genetic background of *P. falciparum* in different geographical regions. If so, methods that dissect quantitative trait loci in genetic crosses can be applied to map chromosome segments and search the database for genes involved in these effects. Determinants of more complex forms of drug resistance (e.g., those responsible for decreases in quinine efficacy in South America and Southeast Asia) may also be identifiable through these approaches.

Searches for new drugs to meet the need once filled by chloroquine require targets that are parasite specific and can be hit with prompt and lethal effect. Drugs identified by these searches, however, may encounter forms of resistance from one or two target mutations and soon lose their reliability for treating cases of *P. falciparum* malaria. Examples of such target mutations include parasite dihydrofolate reductase mutations that counter the action of pyrimethamine and cytochrome b mutations that counteract atovaquone; both alterations quickly produce resistance under drug pressure. Pyrimethamine and atovaquone have consequently been formulated with different target inhibitors such as sulfadoxine (Fansidar combination) and proguanil (Malarone combination), respectively. Combinations of inhibitors that hit the same active site but exert opposing selective forces on particular target mutations may also offer an effective strategy to slow the spread of resistant strains (*16*). New targets in metabolic pathways mined from the *P. falciparum* genome sequence, although subject to these considerations, provide some exciting prospects for drug searches. Among these are targets in the type II fatty acid and the mevalonate-independent (1-deoxy-D-xylulose-5-phosphate or DOXP) isoprenoid pathways of the parasite apicoplast, a chloroplast-like organelle (*17, 18*). Already, fosmidomycin—an inhibitor of DOXP reductoisomerase evaluated previously for its antibacterial activity—has been found to have significant antimalarial activity and is currently being tested in a synergistic combination with clindamycin (*19*).

Among old and new targets for antimalarials, the host heme molecule attacked by chloroquine remains one of the most attractive for drug development. Antimalarials that act on heme or take advantage of its potential for oxy radical activation can produce prompt clearances of parasites from the bloodstream. Important examples in addition to chloroquine include the quinine alkaloids and endoperoxide-containing artemisinin derivatives used in the treatment of severe malaria. The mechanism of CQR in *P. falciparum* probably involves specific structural interactions between chloroquine and amino acid substitutions in PfCRT that were slow to evolve because of their complexity. This could be good news for drugs that attack heme detoxification but are not recognized by the CQR mechanism, as *P. falciparum* may again find it difficult to develop mutations required for resistance. Indeed, a number of 4-aminoquinoline analogs of chloroquine and other inhibitors of heme polymerization have been identified that are effective against both chloroquine-resistant and chloroquine-sensitive *P. falciparum* parasites (Fig. 1) (*20–22*). The favorable pharmaceutical properties of certain of these compounds have established them as promising leads for drug discovery, and some are being pursued in private-public partnering arrangements sponsored by organizations such as the Malaria Medicines Venture (www.mmv.org). Although the standards established by chloroquine are high and the process required to bring new drugs forward is expensive, the development of an affordable chloroquine replacement effective against CQR malaria will be an achievement that more than justifies the necessary effort.

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VIEWPOINT

The *Plasmodium falciparum* Genome—a Blueprint for Erythrocyte Invasion

Alan F. Cowman and Brendan S. Crabb

Erythrocyte invasion by *Plasmodium falciparum* involves multiple ligand-receptor interactions and numerous apparent redundancies. The genome sequence of this parasite reveals new gene families encoding proteins that appear to mediate erythrocyte invasion.

The invasion of human red blood cells by the extracellular merozoite form of *Plasmodium falciparum* is a process central to the pathogenesis of this devastating pathogen. Human erythrocytes demonstrate remarkable diversity with regard to the surface molecules they express. Critical to the success of *P. falciparum* is the flexibility this parasite shows when attaching to and invading host red blood cells. Consequently, *P. falciparum* is able to invade erythrocytes that are antigenically different (as a result of age or allelic diversity) through a number of alternate pathways that involve distinct receptors. After attachment to its target host cell, the merozoite reorients itself such that its pointed (or apical) end becomes positioned at the site of entry (Fig. 1). Thus, the proteins of the merozoite surface, together with those of the organelles associated with the apical end, are considered vital to erythrocyte invasion. It is clear from the recently completed *P. falciparum* genome sequence that many of the molecules thought to be involved in invasion are members of larger gene families (*1, 2*). Although much remains to be learned, the specific involvement of these molecules in determining independent invasion pathways, as well as their importance in the induction of antiparasite immunity, is becoming clearer. The availability of the *P. falciparum* genome has already provided a wealth of information that has greatly accelerated our understanding of the complex process of invasion.