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To face the challenges of malaria

It is indeed a privilege to edit a special section on Malaria in *Current Science*, commemorating 75 years of publication. The malarial parasite (*Plasmodium*) itself was identified by Leveran almost 125 years ago and Ronald Ross established the incriminating role of the mosquito in spreading the disease more than 100 years ago.

Malaria continues to afflict 300–500 million people all over the world and take its toll of 1–2 million deaths every year. The *Plasmodium* species is of ancient origin and both *P. vivax* and *P. falciparum*, the two major species accounting for more than 95% of global malaria, are considered to be parasites of the hominid lineage before the origin of the modern man. The availability of the genome sequence of *P. falciparum* and, more recently, a comparative analysis of the genomes of *P. falciparum* isolates from different geographic regions have led to an assessment of the genetic diversity of the different populations and on this basis led to an estimate of time interval to the Most Recent Common Ancestor (MRCA) of the reference strains. A dramatic population expansion of *P. falciparum* 10,000 years ago, perhaps from a single ancestor, is an attractive concept, but the genetic diversity across the genome is quite heterogenous. SNP, microsatellite or mitochondrial genome analysis give different estimates and some estimate the time interval to MRCA to over 100,000 years. Studies available with *P. vivax* indicate that it is much older than *P. falciparum*, perhaps originating from a non-human primate related to Asian monkeys. Though *P. falciparum* and *P. vivax* express differential disease patterns, molecular analysis of mitochondrial and nuclear genomes reveal an almost similar level of diversity. Molecular analysis also indicates that *P. falciparum* possibly originated in Africa and *P. vivax* in Asia.

Apurup Das and colleagues (**page 1516**) highlight all of the above aspects and point out that majority of the hotspot regions of diversity in *P. falciparum* are around diverse cell surface molecules responsible for cytoadherence, antigen-coding genes and genes contributing to drug resistance, suggesting that genetic diversity is influenced by environmental pressure for selection and survival. Coldspots of low diversity are around genes coding for metabolic functions, suggesting the stabilization of housekeeping genes. Thus, genomic diversity studies provide target identification for drug and vaccine development. Parasite population diversity can also explain differences in drug efficacy and resistance development in a given geographical location.

More than three decades of effort has not led to a vaccine to protect against malaria. Is it a mirage? The best protection strategy is to inject irradiated sporozoites and no candidate vaccine has reached its efficacy. In fact there is a move to mass produce such irradiated sporozoites, but the logistics are not clear. Two articles on vaccine strategies against malaria highlight the issues involved. V. S. Chauhan (**page 1525**) analyses the complex reasons contributing to the elusiveness of a successful malaria vaccine. These include the complex life cycle of the parasite necessitating the appropriate identification and use of a cocktail of antigens, extensive polymorphism in the key malaria-antigen candidates, lack of correlates to protective immunity and unavailability of a suitable animal model for testing vaccine efficacy. However, there is now hope than ever before for the availability of at least a partially effective vaccine in the foreseeable future, emanating from some of the vaccine trials underway. One such example, is the encouraging response to the pre-erythrocytic, RTS, S/ASO_2 vaccine where DNA sequences coding for part of the central repeats (NANP) and B and T cell epitopes of the C-terminal part of the circumsporozoite protein (CS) were fused to hepatitis B surface antigen DNA. The resulting multimeric particle expressed in yeast was adjuvanted with ASO_2, an adjuvant formulation containing an oil water emulsion of deacylated monophosphoryl lipid A and QS21. This vaccine has been found to be safe and in extended trials in children from Gambia and Mozambique, the partial protection observed in the first six months, namely reduced incidence of severe malaria by 57.7% and 29.9% reduction in the risk of clinical malaria was found to last for 18 months. This is really a significant breakthrough.

An important element in malaria control is to prevent the parasite spread and a parasite transmission-blocking vaccine is an ideal tool. Nirbhay Kumar (**page 1535**) highlights the challenges. Proteins synthesized in the gametocytes including pre- (Pf 230 and Pf48/45) and post-fertilization (Pf25 and Pf28) stages are the key target antigens. However, expression of these properly folded recombinant proteins has remained a challenge in view of the multiple cysteine-rich sequences. Animal studies have revealed that DNA-based vaccines may be able to circumvent the problem, that will also provide an opportunity to prepare a cocktail vaccine of pre- and post-fertilization antigens, even including antigens from other stages of parasite development.

In the absence of a vaccine to protect against malaria, therapeutic option lies heavily on antimalarial drugs. Unfortunately, the parasite, *P. falciparum* in particular, has developed resistance to the frontline drug chloroquine as well as the second line antifolate drugs, leaving artemisinin derivatives as more or less the only efficacious drug in the basket. This is a serious situation, especially in Africa, in view of the higher cost of artemisinin and the potential for resistance development on extensive

use or misuse of the drug to treat malaria. While attempts are underway to develop artemisinin-based combinations to prolong the life of this precious antimalarial, several groups are looking into the identification of new drug targets and development of new pharmacophores. Padmanaban and colleagues (**page 1545**) have reviewed the attempts to identify various drug targets in the metabolic pathways of the sub-cellular organelles in the parasite and to develop new candidate drug molecules. Promising leads have been obtained with the metabolic pathways in the apicoplast. An interesting development in the author's laboratory is the identification of curcumin from turmeric as a potential antimalarial in combination with artemisinin derivatives. Certain other natural compounds have also been reported to have antimalarial activity. While several lead compounds are available, many would fail in the subsequent steps necessary to cross before a candidate molecule can become a drug. The potential to develop resistance, efficacy and safety in vulnerable populations such as pregnant women and children and finally affordability of the drugs would be the crucial factors.

While resistance development to antimalarials, especially to chloroquine and related drugs, is a major issue to tackle, the mechanisms involved are still debated and this knowledge would be useful in designing new molecules. David Warhurst (**page 1556**) elaborates on the debate. It is held that accumulation of protonated CQ $2H^+$ into vacuolar water at pH 4.8 is necessary for its antimalarial activity. However, the haemozoin crystallization process as such is closely associated with neutral lipid nanospheres in the aqueous medium of the vacuole. Therefore, hydrophobicity of the drugs in interfering with the haematin detoxication process may be an important parameter to be considered and that the interaction of chloroquine with the growing β-haematin crystal, preventing its growth may be an incidental process. Similar mechanisms would be applicable to other modified 4-aminoquinolines. The question of increased drug efflux brought about by appropriate amino acid replacements in PfCRT versus decreased biding of CQ to haematin under acid conditions, contributing to drug resistance is still debated. While PfCRT has assumed an important role in the efflux process, an interaction with PGH-1 appears to be a factor to be considered in chloroquine resistance and perhaps even in the case of resistance to arylaminoalcohols such as quinine and quinidine.

It is obvious that measurement of the actual pH inside the parasite, the digestive vacuole in particular, is a key to explain several processes such as drug influx and efflux as well as the haemozoin crystallization process. Lanzer and Rohrback (**page 1561**) highlight the potential of live cell imaging in the efforts to identify novel targets for rational intervention. Live cell fluorescence microscopy can be used to study several processes, including pH homeostasis, Ca^{2+} signalling, protein trafficking and cell motility. They highlight the pitfalls in intracellular pH measurements in the parasite. Problems that arise because of the small size of the parasite with numerous compartments influencing spatial resolution, photosensitivity of the parasite components, inherent problems in the use of dyes such as acridine orange to measure pH-dependent fluorescence, have all been brought out. An appropriate candidate to use appears to be pHluorin, a ratiometric, pH-sensitive green fluorescent protein. The essential conclusion is that the digestive vacuole pH may not be different between CQ-sensitive and CQ-resistant strains and a pH gradient cannot explain the phenomenon of resistance. Similar problems are encountered with studying the Ca^{2+} dynamics in the parasite, which plays an important role in invasion, maturation, synchronization, exflagellation and gamete formation. Different techniques with indicator Ca^{2+}-sensitive fluorescence dyes have given confusing results. The authors suggest that the use of aequorin, pericam or other ratiometric Ca^{2+}-sensitive GFP derivatives expressed in the parasite cytosol can enable accurate determination of at least free cytosolic Ca^{2+}. Live cell imaging is a powerful tool to obtain faithful images of subcellular features leading to an understanding of structure and function.

Finally, neither vaccines nor drugs can eliminate malaria, so long as the mosquito vector is not eliminated or at least controlled. India did achieve a dramatic decrease in malaria cases in the 1960s due to vector control using DDT, but since then there has been a resurgence due to inadequate follow up and development of insecticide resistance. A. P. Dash and colleagues (**page 1571**) emphasize the necessity to have knowledge of the vector species and their bionomics. The situation is complicated, since most of the *Anopheles* vector causing malaria in India are species complexes and members differ considerably in biological characteristics that determine vector potential, host preference, resting behaviour and response to insecticides. Identification of sibling species becomes necessary. The authors review vector control strategies using insecticide-based approaches in conjunction with non-insecticidal control methods. The use of appropriate insecticide-impregnated bed-nets and bioenvironmental approaches using larvivorous fishes have proved effective, although genetic manipulation of the vector is a debatable strategy.

The articles cover a broad spectrum on strategies to face the challenge of malaria and I hope malarialogists as well as the general reader would find them useful and interesting.

G. Padmanaban
e-mail: geepee@biochem.iisc.ernet.in