Clinical review

Science, medicine, and the future

Malaria

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Sixteen years ago, when I was a medical student interested in malaria, my professor of medicine asked me what the chances were of developing a malaria vaccine. So far, a successful vaccine is still being sought, but recent advances in the molecular biology of malaria suggest that one may become available soon, along with better diagnostic, preventive, and treatment strategies.

Malaria is a huge and growing problem, especially in Africa: it kills one to two million children each year, causes disease in a further 400 million individuals, and accounts for 25-50% of all hospital admissions. Moreover, mortality associated with cerebral malaria (caused by infection with *Plasmodium falciparum*) has not improved in the past 30 years.[1] *P falciparum* is now virtually untreatable with chloroquine in most parts of the world, and many strains of *P vivax* are also resistant. Although quinine is often the antimalarial drug of choice for chloroquine resistant strains, it is poorly tolerated and compliance is therefore low. Newer antimalarials such as mefloquine are both expensive and not always effective. Some parasites are now resistant to most antimalarials. This drug resistance makes choice of chemoprophylaxis for travellers difficult as well.

In the past 15 years research has expanded considerably from preventive strategies and development of vaccines to include better understanding of the pathophysiology of disease, both clinically and at the cellular and molecular level.[2] A recent review of malaria research by the Wellcome Trust highlights some of these achievements and points to important directions for future study.[3] In this article I review some of these advances in laboratory studies of malaria and discuss how they may affect future control measures and clinical management.

Possible future developments

- A malaria vaccine containing a mixture of polypeptides, parasite DNA, or attenuated parasites
- New antimalarial drugs developed from "rational" approaches and combination chemotherapies
- Alternative routes by which to administer antimalarial drugs in rural settings
- Rapid identification of drug resistance in parasites
- Adjunctive therapies to improve survival of patients with severe malaria
- Interference with transmission of malaria by genetic manipulation of mosquito vectors

Molecular biochemistry

How parasites meet their developmental needs when they are tucked away within a red cell has always been intriguing (fig 1). Parasites hijack existing red cell proteins as well as introducing completely new biochemical pathways in the red cell to meet their voracious demands for substrates (such as glucose and some amino acids) needed for multiplication. In the last 24 hours of their 48 hour life cycle, parasites increase in size and divide to produce up to 36 daughter parasites. To do this, parasites make many hundreds of new proteins, only a few of which have been identified.

The falciparum genome is 10 times more complex than that of a bacterium, but about 100 times simpler than that of its human host. A collaborative project to sequence the falciparum genome is now under way and should be completed within the next three to four years. The results will provide information allowing the function of new parasite proteins to be elucidated. This information can then be used to screen inhibitors that specifically target identified parasite proteins. This will provide the basis for rational drug design.

Other technological advances using oligonucleotides immobilised on "chips" may also simplify the rapid identification of important parasite genes such as those responsible for drug resistance or parasite virulence. Early assessment of these parasite characteristics in a patient's blood sample could identify those with falciparum malaria at greatest risk of developing severe disease. Currently, assessment of disease severity is based on clinical indicators of host response to infection, such as the presence of hypoglycaemia or lactic acidosis, and parasite determinants of severe infection have not yet been adequately identified.

The recent ability to inactivate genes in parasites has now explained the function of some genes (such as for the circumsporozoite protein) the sequences of which have been known for years. Knockout studies will continue to expand knowledge of newer sequences as they are acquired.

Disease mechanisms

P falciparum grows and multiplies logarithmically in red cells after passing through the host's liver. This asexual stage of development can, within a few 48 hour cycles of replication, give rise to high parasite burdens within the patient (over half of red cells infected). Soon after the discovery of the malaria parasite, it was observed that infected red cells stuck to capillaries (fig 1). This propensity of infected cells containing mature parasites to sequester along capillary walls prevents their clearance by the reticuloendothelial system. One of the clinical consequences of this cytoadherence is that the peripheral parasitaemia (assessed on a blood film) does not necessarily reflect the total parasite burden in the host. The total burden of parasites is much larger than the population that is circulating, and it is this sequestered mass of parasitised erythrocytes which causes microvascular obstruction in the capillary beds of organs such as the brain, giving rise to the severe syndromes such as cerebral malaria.

In the host there are several ligands that are expressed on capillaries which provide receptors for the attachment of parasitised red cells. These receptors are either constitutively expressed (such as CD36) or potentially upregulated in response to mediators released in the course of infection. For example, intercellular adhesion molecule-1 is upregulated in response to increased circulating levels of tumour necrosis factor triggered by parasites. In the past two years the parasite encoded molecules that are transported to the red cell surface and attach to these receptors have also been identified.[4] These var gene products are (as their name implies) highly variable both antigenically and in their primary sequence, and are transported to the surface of the red cell to allow cytoadherence to capillaries and to allow the parasite to replicate. There may be up to 150 different var genes in a parasite, occupying about 5% of the whole genome. The var gene products also cause infected cells to stick to uninfected red cells, forming clusters or "rosettes," which may also be important in pathogenesis of severe malaria (fig 1). The design of strategies to reverse or prevent the primary event of infected cell sequestration, which leads to severe disease, is now possible.

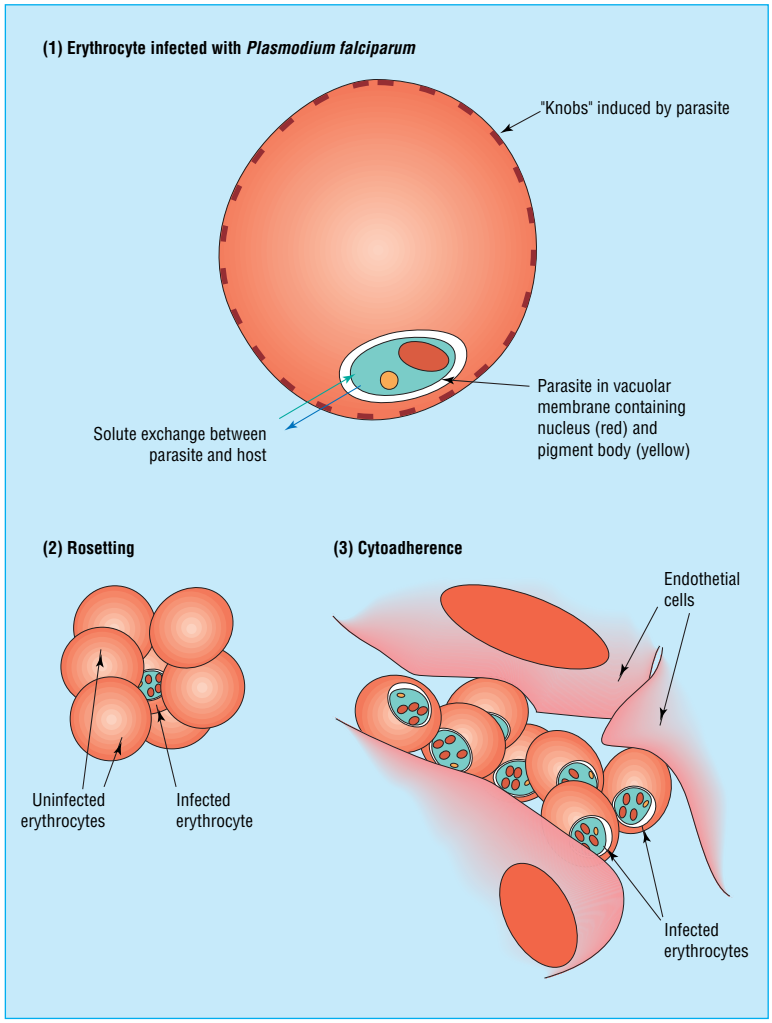

Fig 1 Important processes in host-parasite interaction which may be amenable to future interventions. (1) The infected erythrocyte undergoes complex alterations which enable the parasite to regulate solute exchange with the host. Parasites also express proteins on the surface of the red cell that are important in pathophysiology. Some of these proteins aggregate in accretions termed "knobs," including products of the var gene family, which mediate the processes of rosetting and cytoadherence. (2) Rosetting is the adherence of uninfected red cells to an infected red cell, forming aggregates that may impede microcirculatory flow. (3) Cytoadherence is the adhesion of infected red cells containing relatively mature parasites to endothelial cells lining capillaries and postcapillary venules. Host ligands mediating cytoadherence are expressed on endothelial cells and are vascular adhesion molecules belonging to the integrin and other families. Cytoadherence results in microvascular obstruction and impaired delivery of oxygen and possibly other metabolic precursors to vital organs

Treatment strategies

There are two ways in which mortality from malaria can be reduced: by discovering better antimalarial drugs or by attacking fundamental points in the disease process. Existing antimalarials are effective in clearing parasites in patients with severe malaria, but, because antimalarial treatment takes time to become fully effective, patients die from complications of infection. I believe that, in the long term, the way to reduce mortality in patients with malaria will be to give, alongside the primary antimalarial drug, early adjunctive therapies based on the recognition of complications associated with severe disease. Thus, ways to treat lactic acidosis in malaria, to reduce the deformability of red cells (and increase microvascular flow), to treat convulsions, and to manage anaemia are being, or are likely to be, examined as adjuncts in managing severe disease (see box). Anti-sequestration agents will also join this list when treatment strategies based on recent molecular advances have been validated in laboratory models of cytoadherence. To extend this list of adjuncts further requires sophisticated studies of pathophysiological mechanisms which focus on clinical aspects of the malaria and link these to advances in the laboratory.

New antimalarial drugs are still needed urgently, however, because of the rapid spread of multidrug

Potential adjunctive therapies in treating falciparum malaria

- Exchange transfusion
- Oxpentifylline
- Desferrioxamine
- Dichloroacetate
- Antisequestration agents
- Anticonvulsants

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Fig 2 Typical rural setting of a malaria endemic area on the Thai-Burmese border

resistance. The pharmaceutical industry has, unfortunately, not prioritised research into antimalarials because malaria afflicts people who can ill afford expensive new drugs. One promising recent class of antimalarial is the artemisinin compounds developed by Chinese scientists. Derivatives are now widely available in many parts of South East Asia and Africa, but are not yet licensed for use in Europe and America. Artemisinin derivatives are rapidly parasiticidal, even against parasites resistant to most other classes of antimalarial, and may soon become an established alternative to quinine, currently the first choice for treating severe malaria. Treatments that combine more than one antimalarial drug stand a better chance of being effective against multidrug resistant strains, as well as in slowing the development of further resistance.

Managing malaria in rural areas presents a considerable challenge (fig 2). Patients are often already unconscious when they arrive at small treatment centres, where facilities for resuscitation and parenteral treatment are limited. In this setting a simple approach to treatment, such as the use of antimalarial suppositories, may improve the outcome for severely ill patients. Artesunate (one of the artemisinin derivatives) in suppository formulation is currently being studied in children and adults who are too ill to take oral antimalarial treatment.

Vaccines

The principal problem in developing an antimalarial vaccine has been identifying one or more parasite peptides that generate effective immunity when administered with non-toxic adjuvants.[5] There are, as yet, no clearly identified human markers of immunological protection in people living in areas of high malaria transmission. Antigenic variation of parasites in the human host multiplies these difficulties further. A first generation antimalarial vaccine (Spf66) which underwent rigorous clinical trials did not live up to its accompanying advertising,[6] but there are now many more candidates under development. The difficulty with this choice of candidates is knowing which one (or combination) to select for further development. Field studies which examine immune responses in endemic areas may help to clarify criteria for selection.

In addition to these conventional polypeptide vaccines, advances in DNA vaccines are also being applied to malaria. DNA works as a vaccine after injection by entering into cells and expressing the encoded protein in host tissue in a manner which elicits powerful immunogenic responses. It is easier to synthesise, manipulate, and store DNA than peptides, making DNA vaccines an attractive option for future vaccine studies once their safety is established. While awaiting approval from regulatory bodies for human use, such vaccines are useful in identifying potential immunogens in animal models.

Preventive strategies

Malaria can maintain itself only through its insect vectors. An important focus of research is therefore to intervene in ways which reduce the transmission of malaria by the insect host. Insecticide impregnated bed nets have already produced substantial reductions in disease and mortality in some settings,[7] although this may incur the longer term risk of reducing development of natural immunity in populations. Their overall usefulness is being assessed in larger field trials.

In the future it may be possible to genetically engineer mosquitoes to make them resistant to the malarial parasite, or unable to transmit it, and which can compete with and perhaps replace existing populations of mosquitoes. This approach would enhance the effectiveness of antimalarial vaccines and bed nets in reducing disease in a community.

Conclusions

Advances in molecular technology have accelerated understanding of malaria as a parasite and a disease. While the potential for molecular advances in guiding new treatment strategies is being explored, in the short term we still need improvements in existing antimalarial therapies. These improvements will depend on supportive treatments which ameliorate pathophysiological processes contributing to mortality, as well as newer antimalarials that are effective against drug resistant parasites. In addition, the exciting developments described above suggest that it will eventually be possible to contain the problem of malaria by a combination of insect control measures, better antimalarial drugs, adjunctive therapies, and, not least, that hitherto elusive malaria vaccine.

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