

The 1996 Runme Shaw Memorial Lecture: Malaria-Past, Present and Future

D A Warrell, **DM, DSc, FRCP*

Abstract

Falciparum malaria may have infected Homo sapiens (and perhaps H erectus) in the Asia Pacific region for more than 100 000 years. This estimate is based on the gene frequency of α -thalassaemia, the protection it affords against falciparum malaria and assumptions of untreated mortality from the infection. Up until the end of the 19th century, there was a high mortality from malaria in the coastal parts of Malaya, but the malaria control campaign, begun in 1901 at Klang, was described by Sir Ronald Ross as the first successful antimalarial work in the (then) British Empire. This was extended to Singapore in 1911. When the Far Eastern Association of Tropical Medicine held its Fifth Biennial Congress in Singapore in 1923, malaria was still a major killing disease in parts of Malaya and Sarawak. The mechanism of life-threatening malaria involves cytoadherence of parasitised erythrocytes in microvascular beds, a process enhanced by the products of macrophage activation induced by malarial pyrogen. Improvements in the chemotherapy of life-threatening falciparum malaria with chloroquine and quinine have been countered by the emergence of resistant strains. Artemisinin derivatives may become the treatment of choice during the coming decade. Apart from traditional anti-mosquito methods, control of malaria now involves the use of insecticide-impregnated bed nets, new entomological strategies, including genetic manipulation of mosquitoes and selective chemoprophylaxis. Antigenic diversity and antigenic variation of the malaria parasite have so far defeated attempts to produce an effective vaccine.

Ann Acad Med Singapore 1997; 26:380-7

Key words: Artemisinin, Cytoadherence, Macrophage activation, Malaria control in Malaysia and Singapore, Malaria vaccine, Plasmodium falciparum

Introduction

Today, malaria is the single most important parasite causing morbidity and mortality in the world, and is second only to *Mycobacterium tuberculosis* as the single most important infective agent. The global incidence of malaria is estimated at around 400 million clinical cases per year, of which the large majority occur in sub-Saharan Africa. The annual mortality from malaria is between 1 and 3 million. Virtually all of these deaths are caused by *Plasmodium falciparum* (malignant tertian malaria). The other malaria parasites of humans, *P vivax*, *P ovale* and *P malariae*, can cause unpleasant acute febrile symptoms and repeated attacks, as in the case of relapses or reinfections with *P vivax*, can produce significant morbidity, economic hardships if the victim is an adult, and chronic anaemia. The few deaths from these "benign" malarias result from rupture of enlarged spleens, general debility and intercurrent infections. However, there is historical evidence of high mortality from vivax malaria when it was prevalent in parts of Europe, for example in England in the 17th and 18th centuries.^{1,2} This review is confined to consideration of falciparum malaria.

The Past

For how long has *P falciparum* infected humans? Studies of the morphological and biological characters of human and primate malaria parasites led Garnham³ to hypothesise the emergence of virulent *P falciparum* (Laverania) in mid-Pleistocene times when humans diverged from the higher apes. Recent studies of gene sequences of plasmodia have been interpreted in different ways. Waters et al⁴ analysed small subunit ribosomal RNA (SSU rRNA) of plasmodia infecting several classes of vertebrates and found that *P falciparum* was closest to the avian malarias. They suggested a transfer from birds to humans in relatively recent times. They argued that the transmission dynamics of *P falciparum* infection required large human populations in stable relationship with mosquito vectors, circumstances conceivable only after the origins of agriculture, perhaps as recently as 5000 years ago. However, *P falciparum* infection can be maintained in quite small populations with an apparently low rate of infection such as the pygmies of the Ituri forest of Zaire. Escalante et al⁵ studied conserved regions of the gene coding for the circumsporozoite protein (CSP) of 12 species of human and primate

* Professor of Tropical Medicine and Infectious Diseases

Director, Centre for Tropical Medicine, University of Oxford

Address for Reprints: Prof David A Warrell, Centre for Tropical Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU, United Kingdom.

plasmodia. *P reichenowi*, of which the natural hosts are chimpanzees and lowland gorillas, is morphologically almost identical to *P falciparum*, and was found to be the most similar genetically. These authors conclude that *P falciparum* is an ancient human parasite, associated with humans since the divergence of homonids from the hominoids most closely related to them, perhaps 8.9 million years ago. If this interpretation is correct, we may imagine that ancestral species in Southeast Asia, such as *Homo erectus* ("Peking man"), whose fossil remains have been found in parts of China and Indonesia, may have suffered and died from falciparum malaria.

The gene frequencies of a number of potentially deleterious erythrocyte abnormalities, such as sickle cell disease, thalassaemias and glucose-6-phosphate-dehydrogenase deficiency, reach high levels in countries with endemic falciparum malaria, suggesting a protective effect. For example, along the north coast of Papua New Guinea, in Nepal and in the Andhra Pradesh province of India, the gene frequency of the single α -globin gene deletion may exceed 80%.⁶ The α -thalassaemias result from rearrangements/deletions of the α -globin gene complex on chromosome 16. The time taken for mild α -thalassaemia (α +thal, $-\alpha/-\alpha$) to reach its present gene frequency in parts of Southeast Asia and New Guinea has been estimated at between 50 000 and >100 000 years, by making various assumptions (AVS Hill, personal communication). This implies a "time depth" of malaria of a similar order. However, Flint et al⁶ have argued that malaria selection has operated relatively recently (within the last 5000 years) on human populations. This is the only feasible explanation for the geographical patchwork of different patterns of haemoglobinopathy, the majority of which result from regionally specific mutations arising only once. For example, there is good archaeological evidence that the Vanuatu archipelago has been populated for less than 5000 years, and yet the commonest haplotype $-\alpha^{3.7III}$, which is very unlikely to have been imported, has reached a protective frequency.

Malaria in West Malaysia and Singapore

In the much more recent past, high temperatures, heavy rainfalls, extensive mangrove swamps and luxuriant tropical vegetations contributed, at the end of the last century, to devastating malaria and blackwater fever along the coasts of peninsular Malaysia and Singapore.^{7,8} However, this unpromising environment was to be the site of what Sir Ronald Ross described as the first successful antimalarial work in the (then) British Empire. At the Fifth Biennial Congress of the Far Eastern Association of Tropical Medicine, held in Singapore in 1923, when the foundation stone was laid for the new College of Medicine, some of this pioneering work on malaria control was reviewed. Sir Malcolm Watson,

who was stationed in Klang for 28 years, gave vivid descriptions of the malaria prevention campaign which was started in 1901, a year after the foundation of the Institute for Medical Research.^{7,9} Today, in these healthy surroundings, it may be difficult to appreciate what life must have been like in the early years of this century, when malaria was the most prevalent of all diseases in Singapore and was still increasing as its principal mosquito vector, *Anopheles maculatus*, colonised areas of cleared jungle in the hills. There was an annual peak of malaria cases and deaths from May to July. In July 1911 the death rate was 85.83 per 1000. In that year the antimalarial campaign was started in Singapore. It involved subsoil drainage with concrete channels and pipes and larviciding using crude oil. This resulted in a marked reduction in morbidity and mortality attributable to malaria, shown by comparing average monthly death rates from all causes 1903-1912 with 1913-1922. The death rate in May was reduced from 53.76 to 37.27 per 1000, while the spleen rate fell from about 50% to 0.

In Selangor, malaria transmitted by *A ludlowi* and *A umbrinus* was so intense that the government of the (then) Federated Malay States ordered the closure of Port Swettenham (Pelabuhan Kelang) in 1901. The development of rubber and coconut estates on Pulau Carey in the delta of the Klang and Langat Rivers was also jeopardised. However, the malaria control programme, which was started in the same year, was so effective that the important economic developments near Klang were able to go ahead. Other pioneering control programmes, contributing to the economic growth of Malaysia and Singapore, were at Gemas (Negri Sembilan); in the rubber estates where annual death rates, attributable to malaria rather than dysentery, were sometimes over 200 per 1000; and in Sarawak.

The Present

Falciparum malaria is now confined to tropical countries, in some of which, such as West Malaysia and Singapore, it is wholly or partly controlled. As recently as the 1940s, falciparum malaria caused hundreds of deaths in Italy and some other southern European countries' and there is speculation that global warming might encourage a return of endemic malaria to some of these regions. With the growth of international tourism, medical practitioners in western countries are increasingly aware of falciparum malaria as an imported disease. After the failure of the World Health Organisation's Global Malaria Eradication Campaign (1955-1969) which relied on extensive DDT spraying and chloroquine chemoprophylaxis, the strategy has changed to improving the treatment of affected patients and trying to develop more effective control methods. Three of the major challenges posed by falciparum malaria today are 1) understanding the pathophysiological mechanisms

leading to severe and potentially fatal malaria; 2) development of new antimalarial drugs in the face of increasing drug resistance and 3) improving personal protection against malaria and reducing transmission.

Pathophysiology of Cerebral Malaria

A number of clinical features of *P falciparum* infection have been identified which are associated with a poor prognosis.⁹ Of these severe forms, cerebral malaria is the most common in most populations. This disease has been the focus of clinical and laboratory work based in the University of Oxford for the past 17 years. At the start of the Wellcome-Mahidol University, Oxford Tropical Medicine Research Programme in Thailand in 1979, the daunting case fatality reported for cerebral malaria was almost 50% in some countries.¹⁰ Understanding the pathophysiology of cerebral malaria was crucial if this appallingly high mortality were to be reduced. At that time, the "permeability hypothesis" was the most widely accepted explanation for cerebral malaria. A toxic factor released by the parasites was thought to increase permeability of the blood-brain barrier leading to cerebral oedema, coma and death.¹¹ This idea, supported by some anecdotal clinical observations, had encouraged clinicians to use high doses of corticosteroid, especially dexamethasone, as an ancillary treatment for cerebral malaria. The formal assessment of this popular treatment was carried out in Chantaburi, in eastern Thailand near the Cambodian border,¹² where gem-mining in the jungles attracted large numbers of young men from non-malarious parts of the country, who were vulnerable to severe malaria. The study prompted the development of a practical case definition of cerebral malaria and provided an opportunity to test the permeability hypothesis, since dexamethasone is the most potent known anti-inflammatory agent affecting the blood-brain barrier. In a randomised placebo-controlled trial, 100 adult patients satisfying the new strict definition of cerebral malaria, were given standard intravenous treatment with quinine together with either dexamethasone (total dose 2 mg/kg over 48 hours) or placebo. There was no significant difference in mortality between the two groups (deaths 16/50 dexamethasone; 18/50 placebo; $P = 0.8$) but, in the dexamethasone-treated patients, coma was prolonged ($P < 0.02$) and there was an excess of serious gastrointestinal bleeding and secondary bacterial infections.¹² A subsequent study in New Guinea, in which a much larger dose of dexamethasone was given (11.4 mg/kg over 48 hours) confirmed these observations.¹³ Prolongation of coma by dexamethasone was not consistent with the permeability hypothesis. Additional evidence against this concept was the lack of evidence of raised intracranial pressure (reflected in cerebrospinal fluid opening pressure measured at lumbar puncture)¹⁴ and the absence in the majority of patients of cerebral oedema detectable by computer-

ised tomography (CT) or nuclear magnetic resonance (NMR) scans.^{15,16} Blood-cerebrospinal fluid (CSF) barrier permeability to albumin was not increased during coma as compared to convalescence. However, in African children with cerebral malaria, evidence of cerebral swelling has been found.^{17,18} This is probably due to increased intracranial blood volume. Some children develop clinical signs of cerebral or brain stem herniation.¹⁷⁻¹⁹

Two other hypotheses now provide a more compelling explanation for the pathophysiology of cerebral malaria.

Mechanical hypothesis

Since the end of the 19th century, histopathologists have observed, in the brains of patients dying of cerebral malaria, red blood corpuscles containing mature pigmented parasites apparently blocking the small blood vessels. In Aotus monkeys, experimentally infected with *P falciparum*, parasitised erythrocytes appeared to stick to endothelium of small cardiac blood vessels via electron-dense protuberances (knobs) on their surfaces.^{20,21} An ultrastructural study of needle necropsy samples of brain from patients dying of malaria in Thailand showed similar appearances and provided evidence that selective cytoadherence and sequestration in cerebral venules was a specific feature of cerebral malaria.²² Sequestration was seen only in patients with clinical features of cerebral malaria, not in those dying of non-cerebral malaria and the packing of parasitised erythrocytes was tighter in the brain than in other organs.²² Cerebral blood flow and metabolism were studied in adult patients with cerebral malaria in Thailand.²³ Total cerebral blood flow was not consistently lower in coma than immediately after recovery of consciousness, but the values were low in relation to arterial oxygen content, one of the principal physiological determinants of cerebral blood flow. Cerebral oxygen consumption was low during coma and immediately after recovery of consciousness, but cerebral lactate production decreased significantly with recovery, suggesting that coma in cerebral malaria was associated with a switch to cerebral anaerobic glycolysis.^{23,24}

Erythrocyte surface ligands and endothelial receptors: Recently, there has been a breakthrough in the understanding of an important family of antigens expressed on the surface of *P falciparum* infected erythrocytes. These proteins, known as *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) are large (200-350 kDa) proteins concentrated in the electron-dense knobs under the surface of parasitised erythrocytes. They show both clonal variation, allowing them to evade host immune responses, and adhesive properties involved in the cytoadherence phenomenon. During the search for drug resistance genes in *P falciparum*, a

family of more than 150 highly variable ("var") genes was discovered, constituting about 6% of the whole *P. falciparum* genome. These var genes have been found to encode PfEMP-1. Several of the genes have been sequenced, cloned and partially expressed and binding of PfEMP-1 to several candidate endothelial receptors has been demonstrated.²⁵⁻²⁷ Some of these receptors (Table I), such as CD36 and thrombospondin, seem to be expressed at all times in a wide range of vascular beds and are able to bind *in vitro* to all "wild" isolates of *P. falciparum*. They are regarded as constitutive and their expression is not related qualitatively or quantitatively to disease severity. Other receptors such as ICAM-1 and E-selectin are inducible (show increased expression or are only expressed) in the cerebral blood vessels of patients with cerebral malaria, suggesting that they may be responsible for cytoadherence in this disease.^{28,29} TNF α and other inflammatory mediators are able to induce this expression. A recently discovered endothelial receptor for cytoadherence, the glycosaminoglycan, chondroitin sulphate A,³⁰ is expressed on placental vascular endothelium and may specifically bind a strain of malaria causing maternal infection.³¹ Rosetting, the adherence of non-parasitised to parasitised erythrocytes, is a phenomenon displayed *in vitro* by some parasite strains responsible for severe disease.^{32,33} The rosettes could contribute to microvascular obstruction in cerebral and other vascular beds.

Toxin/cytokine hypothesis

A malarial "toxin" released at schizont rupture, was first suggested by Sir Ronald Ross in 1911. There is now renewed interest in this toxin as a stimulus to macrophages to release TNF α and other cytokines which are responsible for the characteristic fever paroxysms of malaria and for many of the pathological processes of severe disease.³⁴ In African children with cerebral malaria, there is a strong correlation between circulating

levels of TNF α and other cytokines and clinical severity of the infection, case fatality and the incidence of neurological sequelae.^{35,36}

Treatment of malaria and severe sepsis syndrome with anti-TNF Antibodies: In a pilot study in the Gambia, children with cerebral malaria were treated with an anti-TNF monoclonal antibody CB0006. There was a dose-related reduction in fever and a hint of some clinical benefit.³⁷ However, in a large randomised placebo-controlled trial recently completed in the same population, another anti TNF α monoclonal antibody BC7 did not improve case fatality and was associated with a small but significant increase in the incidence of neurological sequelae (Kwiatkowski D, personal communication). Paradoxically, both these monoclonal antibodies appeared to increase the level and persistence of TNF α in the circulation. It is possible that binding to the antibody delayed clearance of the cytokine and did not neutralise its active epitopes.

High levels of circulating TNF α and other cytokines have also been found in patients with severe sepsis syndrome, and there has been great interest in neutralizing TNF α and interleukin-1 with monoclonal antibodies or receptor antagonists.³⁸ Although these strategies have proved effective in animal models of severe sepsis,^{39,40} clinical trials have shown no significant benefits. There was even a trend towards increased mortality in some groups of patients.^{38,41} However, an ovine Fab fragment anti-TNF polyclonal antibody given before antibiotic therapy, prevented the development of the Jarisch-Herxheimer reaction, associated with explosive release of TNF α and other cytokines, in louse-borne relapsing fever (*Borrelia recurrentis* infection).^{42,43} The efficacy of this polyclonal antibody is now being assessed in adult patients with sepsis syndrome in intensive care units in the USA and with cerebral malaria in Thailand (Looareesuwan S, personal communication).

The most convincing evidence that excessive TNF α release causes cerebral malaria is from genetic studies in the Gambia.⁴⁴ Children homozygous for the TNF-2 allele at the TNF α promoter region of human chromosome 6 showed a 4.0-fold increased risk of cerebral malaria and a 7.7 increased risk of death or sequelae from cerebral malaria. A number of other genes have been implicated in resistance or susceptibility to malaria infection or to its severe manifestations such as cerebral malaria and malarial anaemia (Table II).

Understanding the molecular mechanisms of malaria pathogenicity is not of purely academic interest. It might lead to new interventions in severe disease, such as competitive inhibition of the tissue targets of malaria toxin, TNF α and other cytokines and the receptors involved in cytoadherence or rosetting. These discoveries

TABLE I: CYTOADHERENCE: CANDIDATE LIGANDS ON THE SURFACE OF PARASITIZED ERYTHROCYTES AND RECEPTORS ON CEREBRAL VENULAR ENDOTHELIUM

Erythrocyte surface ligands	
PfEMP-1	(binds to CD36, ICAM-1 ?thrombospondin)
Erythrocyte band 3 anion transporter	(binds to CD36 ?thrombospondin)
Sequesterin	(binds to CD36)
Endothelial receptors	
Thrombospondin	adhesive glycoprotein
CD36	leukocyte differentiation antigen, platelet glycoprotein IV (binds to OKM5)
ICAM-1	cell adhesion (binds to LFA-1)
VCAM-1	cell adhesion (binds to VLA-4)
E-selectin	leukocyte-endothelial adhesion
Chondroitin-sulphate A	glycosaminoglycan

TABLE II: MALARIA RESISTANCE GENES

a, β-globin	Major histocompatibility complex class I & II
Glucose-6-phosphate dehydrogenase deficiency	Spectrin
Blood group 0	Glycophorin A & B
Erythrocyte band 3	Chemokine receptor (Duffy-negative blood group)
Tumour necrosis factorα promoter	

could also stimulate new ideas for “anti-disease” vaccines (see below).

Chemotherapy in the Era of Multi-resistant P falciparum

A crisis in the chemotherapy of severe falciparum malaria has been developing over the last 30 years. Strains of *P falciparum* have become resistant to pyrimethamine and other antifoies, biguanides, chloroquine and most recently, to quinine and mefloquine. Chloroquine resistance reinstated quinine as the drug of choice in the treatment of severe falciparum malaria, but in countries such as Thailand, a progressive increase in the minimal inhibitory concentration of quinine for local strains of *P falciparum* has been observed and individual cases of RIII resistance (complete unresponsiveness of parasitaemia to the drug during 7 days of treatment) have been documented. The increasing doses of quinine required for cure have resulted in greater dose-related toxicity, especially hypoglycaemia, while the familiar unpleasant side effects known as cinchonism, limit patient compliance to extended courses of oral quinine in convalescence.

Qing Hao Su

The most promising group of drugs to replace quinine are the artemisinin derivatives, extracted from the sweet wormwood (*Artemisia annua* Linné). Extracts of this plant, known as *huang hua cao* (yellow flowered herb) were mentioned in prescriptions from the Mawang Dui Hang Dynasty Tomb dating back 2000 years. Their use in malaria dates from the middle of the 4th century AD (Zhouhou Bei Gi Fang-the Handbook of Prescriptions for Emergencies).⁴⁵ Artemisinin (*qing hao su*) was first obtained in crystalline form in China in 1972 and the efficacy of it and its derivatives, artesunate and artemether, was quickly established in China and later in Burma, Thailand and some other countries.^{46,47} However, there have been reports of fatal neurotoxicity associated with lesions in the caudal brain stem in several species of laboratory animals⁴⁸ and only recently, have large randomized studies compared the efficacy and safety of artemether (an oily suspension given by intramuscular injection) with quinine (Table III).⁴⁹⁻⁵¹

These studies, the most powerful carried out in the

TABLE III: ARTEMETHER (BY INTRAMUSCULAR INJECTION) VERSUS QUININE (BY INTRAMUSCULAR INJECTION OR INTRAVENOUS INFUSION) IN CEREBRAL/SEVERE FALCIPARUM MALARIA

Reference	Boele van Hensbroek et al ⁵⁰	Murphy et al ⁴⁹	Hien et al ⁵¹
Country	The Gambia	Kenya	Vietnam
Age range (y)	1-9	0.4-12	15-79
Number	576	160	560
Cerebral	576	160	290
Case fatality %	20.5 (21.5)†	20.2 (11.3)	13 (17)
Cerebral neurol sequelae %	3.3 (5.3)	8.3 (11.1)	1 (0.4)
Parasite clearance 50% (h)	9 (14)″	7.3 (15.5)*	10.3 (11.5)
Fever clearance (h)	30 (33)	32 (32)	127 (90)″
Coma clearance (h)	2 6 (20)*	12 (13)	6 6 (48)*
Convulsions %	38.5 (28)″		

† artemether group (quinine group)

* statistically significant (<0.05)

history of antimalarial chemotherapy, confirmed that artemether was as effective as quinine in reducing case fatality of cerebral and severe falciparum malaria. However, coma was significantly prolonged in the artemether-treated patients in two of these studies. In Gambian children there was an excess of convulsions in the artemether-treated group, and in the Kenyan study, children with metabolic acidosis were less likely to survive when treated with artemether rather than quinine. Both artemether and quinine have advantages and disadvantages (Table IV) but artemether may be the preferred treatment where quinine resistance is suspected, in patients with recurrent quinine-induced hypoglycaemia and in those with “blackwater fever” in which quinine sensitization may be involved. For the early treatment of severe falciparum malaria at the most peripheral levels of the health service, artemisinin and artesunate suppositories have produced very promising results,^{52,53} but cinchona alkaloids and chloroquine may also prove bioavailable and effective when given by this route.^{54,55}

Control Of Malaria Transmission

The traditional methods of malaria control and protection of individuals and populations have been directed against adult and larval mosquitoes and against the malaria parasite (Table V). The success of these methods in some parts of the world, such as peninsular Malaysia and Singapore (see above) is unquestionable and, during the WHO’s global malaria eradication campaign (1955-1969), elimination of malaria, almost to the point of eradication, was achieved in parts of the Indian subcontinent. However, this effort could not be sustained and there has been a massive resurgence of malaria in many parts of the endemic region. Attention has moved from attempted eradication to containment

TABLE IV: ADVANTAGES AND DISADVANTAGES OF ARTEMISININ DERIVATIVES

Advantages

Effective against quinine-resistant parasites
 Resistance not yet observed
 Easy and convenient to administer (daily injection, suppository)
 Clinically safe (does not induce hypoglycaemia)
 ? Special role in pregnancy hypoglycaemia and "blackwater fever" caused by quinine
 Kills young ring forms, rapid parasite clearance^{49,50}

Disadvantages

? Less rapidly effective than quinine (coma,^{50,51} fever⁵¹ clearance)
 ? Less effective in African children with metabolic acidosis⁴⁹
 Neurotoxic in animals⁴⁸ ?in children (convulsions)⁵⁰
 Optimal dosage and pharmacokinetics uncertain
 Insufficient data in pregnancy (fetal toxicity in animals)⁴⁶

TABLE V: MALARIA CONTROL METHODS: PRESENT AND FUTURE

Mosquitoes	kill adults	insecticides
	kill larvae	larvicides, drainage, larvivorous predators etc
	prevent transmission	insecticide-impregnated bed nets repellants [? genetic manipulation]
Parasites	kill asexual parasites	chemoprophylaxis, chemotherapy
	kill gametocytes	[? vaccines]

with a greater emphasis on personal protection and the early diagnosis and treatment of clinical attacks of malaria. Targeting of individuals with a high risk of severe malaria, such as pregnant women and infants in endemic areas, with selective chemoprophylaxis, has been frustrated by the spread of resistance to the acceptably safe drugs such as chloroquine and proguanil.

Insecticide-impregnated mosquito nets

By far the most impressive results of any intervention in recent years have been obtained by the use of insecticide (pyrethroid-permethrin)-impregnated bed nets. At a recent WHO meeting in Brazzaville, reductions in childhood mortality (all causes) of between 20% and 30% were reported. It seems that prevention of malaria transmission by treated bed nets reduces not only malarial mortality but also mortality attributable to other infections such as acute respiratory tract infections (potentiated perhaps by the transient immuno-suppression induced by malaria). The enormous optimism engendered by the success of treated bed nets has been countered by two major concerns. First, can the impressive short term results of this intervention be sustained and, secondly, might the reduction in exposure of young children to malaria infection result, paradoxically, in excess malarial mortality at a later age? Epidemiological studies in Africa have confirmed that, as the intensity of *P falciparum* infection [reflected by the

entomological inoculation rate (EIR)] (number of infective mosquito bites per person per year) increases, the age at which disease occurs, decreases and the overall chances of developing severe disease by the age of 10 years is reduced⁵⁶ (Snow R W, personal communication). In Africa, whose population suffers more than 80% of the global total of malaria deaths, the EIR ranges from 5 to 1000, whereas elsewhere in the world (except in New Guinea), the EIR is almost always less than 1.⁵⁷ In the Congo, some of the highest reported EIRs are associated with low childhood malaria mortality.⁵⁸ It seems possible that if the force of malaria infection were reduced over time, by, for example, the widescale use of insecticide-treated bed nets, the incidence of severe and fatal disease might increase (Snow RW, personal communication). Trape and Rogier⁵⁷ have argued that in tropical Africa, only a large and widespread reduction in transmission, far greater than is currently achievable, could produce a long term reduction in the burden of malaria for the whole community. In the present circumstances, they advocate improvements in health services together with health education to improve antimalarial chemotherapy as the only effective ways of combatting malaria.

The Future

The most optimistic future expectations for the control of malaria must lie in the harnessing of the new techniques of molecular biology. One exciting possibility would be to alter the vectorial ability of key species of anopheline mosquitoes by introducing into the population viable mosquitoes which had been altered genetically so that they could no longer support the sexual cycle of *P falciparum*. This would involve the identification of "refractoriness genes" determining vectorial functions.

Malaria Vaccines

The scale and complexity of the global malaria problem indicates the urgent need for a malaria vaccine. Yet, despite almost two decades of well-funded research, only one candidate vaccine, Patarroyo's SPf66 vaccine, has been tested in the field.^{59,60} The efficacy of this vaccine has been variable and it now seems highly unlikely that it will provide universal protection (Table VI).⁶¹⁻⁶³ *Plasmodium falciparum* poses a number of difficulties for the vaccine developer. Its antigens, exposed to the host's immune system, vary at each stage of the life cycle, display clonal variation to evade immune defences and are enormously diverse, both locally and geographically. Some antigens that are immuno-dominant in natural infections may not induce protective immunity. Practical problems include the fact that the *P falciparum* genome is extremely large, the parasite is culturable only in human erythrocytes and there is no completely satisfactory animal model in which to test

TABLE VI: RESULTS OF SPf66 MALARIA VACCINE TRIALS

Reference	Valero et al ⁶¹	Alonso et al ⁶²	D'Alessandro et al ⁶³
Country	Colombia	Tanzania	The Gambia
Age range(y)	1-45+	1-5	0.5-0.9
Number	1548	586	630
Protective efficacy %	33.6	31.0	8.0
95% confidence interval %	18.8 to 45.7	0 to 52	-18 to 29
P	<0.001	0.046	0.50

the protective efficacy of candidate vaccines.⁵⁹ However, despite these difficulties, vaccines are being developed, directed both at the parasite and against its pathogenic effects in the host (the “anti-disease” vaccines). In its pre-erythrocytic cycle (sporozoite invasion of the hepatocyte and subsequent schizogony) a cytotoxic T-lymphocyte-inducing vaccine might be a possibility.⁶⁴ Other targets are the erythrocytic cycle and gametocytes (“transmission-blocking vaccine”). “Anti-disease” vaccines might be directed against the malaria toxin, released at schizont rupture of the infected erythrocyte (see above); its receptor on the macrophage; or the ligands/receptors involved in cytoadherence or rosetting.

Conclusions

Malaria remains the supremely challenging problem in tropical medicine. Whether *P falciparum* infection is a relatively recent scourge of mankind, since the start of concerted agriculture within the last 5000 years, or a really ancient disease dating from the divergence of hominids from hominoids nine million years ago, is still hotly debated. Treatment of life-threatening disease should improve with better understanding of its pathophysiology, but new antimalarial drugs will be needed to combat antimalarial resistance. The introduction of insecticide-treated bed nets has reduced childhood mortality from malaria and other infections by 20% to 30% in several parts of Africa. The longer term results of this intervention are uncertain. Hope for control of malaria in the future rests on the development of effective vaccines and genetic alterations in the vectorial ability of mosquitoes.

REFERENCES

- Bruce-Chwatt L J, De Zulueta J. The rise and fall of malaria in Europe. A historico-epidemiological study. Oxford: Oxford Univ Press, 1980.
- Dobson M J. Malaria in England: a geographical and historical perspective. *Parassitologia* 1993; 36:35-60.
- Garnham P C C. Malaria parasites and other haemosporidia. Oxford: Blackwell, 1966.
- Waters A I, Higgins D G, McCutchan T F. The phylogeny of malaria: a useful study. *Parasitology Today* 1993; 9:246-50.
- Escalante A A, Barrio E, Ayala F J. Evolutionary origin of human and primate malaria: evidence from the circumsporozoite protein gene. *Mol Biol Evol* 1995; 12:615-26.
- Flint J, Harding R M, Boyce A J, Clegg J B. The population genetics of the haemoglobinopathies. *Baillieres Clin Haematol* 1993; 6:215-62.
- Watson M. Opening address on malaria. In: Hoops A L, Scharff J W, editors. Far Eastern Association of Tropical Medicine. Transactions of the Fifth Biennial Congress held in Singapore, 1923. London: John Bale, Sons & Danielsson, 1924:15-29.
- Watson M. Some pages from the history of the prevention of malaria. *Glasgow Med J* 1935; 123:49,130,202.
- Warrell D A, Molyneux M E, Beales P F, editors. Severe and complicated malaria. 2nd ed. World Health Organisation Malaria Action Programme. *Trans R Soc Trop Med Hyg* 1990; 84:(suppl)1-65.
- Daroff R B, Deller J J, Castl A J, Blocker W W. Cerebral malaria. *JAMA* 1967; 202:119-22,679-82.
- Maegraith B, Fletcher A. The pathogenesis of mammalian malaria. *Adv Parasitol* 1972; 10:49-75.
- Warrell D A, Looareesuwan S, Warrell M J, et al. Dexamethasone proves deleterious in cerebral malaria. A double-blind trial in 100 comatose patients. *N Engl J Med* 1982; 306:313-9.
- Hoffman S L, Rustama D, Punjabi N H, et al. High-dose dexamethasone in quinine-treated patients with cerebral malaria: a double-blind placebo-controlled trial. *J Infect Dis* 1988; 158:325-31.
- Warrell D A, Looareesuwan S, Phillips R E, et al. Function of the blood-cerebrospinal fluid barrier in human cerebral malaria: rejection of the permeability of hypothesis. *Am J Trop Med Hyg* 1986; 35:882-9.
- Looareesuwan S, Warrell D A, White N J, et al. Do patients with cerebral malaria have cerebral oedema? A computed tomography study. *Lancet* 1983; i:434-7.
- Looareesuwan S, Wilairatana P, Krishna S, et al. Magnetic resonance imaging of the brain in patients with cerebral malaria. *Clin Infect Dis* 1995; 21:300-9.
- Newton C R J C, Kirkham F J, Winstanley P A, et al. Intracranial pressure in African children with cerebral malaria. *Lancet* 1991; 337:573-6.
- Newton C R J C. Intracranial hypertension in Kenyan children with cerebral malaria [dissertation]. South Africa: University of Cape Town, 1994:72.
- Lucas S B, Hounnou A, Bell J, et al. Severe cerebral swelling is not observed in children dying with malaria. *Q J Med* 1996; 89:351-3.
- Miller L H. Distribution of mature trophozoites and schizonts of *Plasmodium falciparum* in the organs of *Aotus trivirgatus*, the night monkeys. *Am J Trop Med Hyg* 1969; 18:560-5.
- Luse S A, Miller L H. *P falciparum* malaria ultrastructure of parasitized erythrocytes in cardiac vessels. *Am J Trop Med Hyg* 1971; 20:655-60.
- MacPherson G G, Warrell M J, White N J, et al. Human cerebral malaria. A quantitative ultrastructural analysis of parasitised erythrocyte sequestration. *Am J Pathol* 1985; 119:385-401.
- Warrell D A, White N J, Veal N, et al. Cerebral anaerobic glycolysis and reduced cerebral oxygen transport in human cerebral malaria. *Lancet* 1988; ii:534-8.
- White N J, Warrell D A, Looareesuwan S, et al. Pathophysiological and prognostic significance of cerebrospinal fluid lactate in cerebral malaria. *Lancet* 1985; i:776-8.
- Baruch D I, Pasloske B L, Singh H B, et al. Cloning the *P falciparum* gene encoding PfEMP-1, a malarial variant antigen and adherence receptor on the surface of parasitised human erythrocytes. *Cell* 1995; 82:77-87.
- Su X-Z, Heatwole V M, Wertheimer S P, et al. The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell* 1995; 82:89-100.
- Smith J D, Chitnis C E, Craig A G, et al. Switches in expression of *Plasmodium falciparum var* genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell* 1995; 82:101-10.
- Berendt A R, Simons D L, Tansey J, et al. Intercellular adhesion molecular-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature*

- 1989; 341:57-9.
29. Turner G D H, Morrison H, Jones M, et al. An immunohistochemical study of the pathology of fatal malaria. Evidence of widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *Am J Pathol* 1994; 145:1057-69.
 30. Rogerson S J, Sansanee C C, Ng K, et al. Chondroitin sulfate A is a cell surface receptor for *Plasmodium falciparum*-infected erythrocytes. *J Exp Med* 1995; 182:15-20.
 31. Fried M, Duffy P E. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science* 1996; 272:1502-4.
 32. Carlson J. Erythrocyte rosetting in *Plasmodium falciparum* malaria-with special reference to the pathogenesis of cerebral malaria. *Scand J Infect Dis* 1993; 86(suppl):1-79.
 33. Carlson J, Helmby H, Hill A V S, et al. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* 1990; 336:1457-60.
 34. Kwiatkowski D. The biology of malarial fever. *Baillieres Clin Infect Dis* 1995; 2:371-88.
 35. Grau G E, Taylor T E, Molyneux M E, et al. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989; 320:1586-91.
 36. Kwiatkowski D, Hill A V S, Sambou I, et al. TNF concentration in fatal cerebral, nonfatal cerebral and uncomplicated *Plasmodium falciparum* malaria. *Lancet* 1990; 336:1201-4.
 37. Kwiatkowski D, Molyneux M E, Stephens S, et al. Anti-TNF therapy inhibits fever in cerebral malaria. *Q J Med* 1993; 86:91-8.
 38. Fisher C J, Agosti J M, Opal S M, et al. Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein. *N Engl J Med* 1996; 334:1697-702.
 39. Beutler B, Milsark I W, Cerami A C. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effects of endotoxin. *Science* 1985; 229:869-71.
 40. Tracey K J, Fong Y, Hesse D G, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. *Nature* 1987; 330:662-4.
 41. Abraham E, Wunderink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor α in patients with sepsis syndrome: a randomized, controlled, double-blind, multicenter clinical trial. *JAMA* 1995; 273:934-41.
 42. Negussie Y, Remick D G, De Forge L E, et al. Detection of plasma tumor necrosis factor, interleukins-6, and -8, during the Jarisch-Herxheimer reaction of relapsing fever. *J Exp Med* 1992; 175:1207-12.
 43. Fekade D, Knox K, Hussein K, et al. Prevention of Jarisch-Herxheimer reactions by treatment with antibodies against tumor necrosis factor α . *N Engl J Med* 1996; 335:311-5.
 44. McGuire W, Hill A V S, Allsopp C E M, Greenwood B M, Kwiatkowski D. Variation in the TNF α promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; 371:508-11.
 45. Shen J X, Li G Q. The history of the development of artemisinin derivatives for antimalarial chemotherapy in China. A symposium presentation for the VII International Conference on Infectious Diseases, 12 June 1996, Hong Kong.
 46. Hien T T, White N J. Qinghaosu. *Lancet* 1993; 341:603-8.
 47. Wellcome Trust. Artemisinin. Proceedings of a meeting convened by the Wellcome Trust on 25-27 April 1993. *Trans R Soc Trop Med Hyg* 1994; 88(suppl 1):1-65.
 48. Brewer T G, Grate S J, Peggins J O, et al. Fatal neurotoxicity of arte-ether and artemether. *Am J Trop Med Hyg* 1994; 51:251-9.
 49. Murphy S, English M, Waruiru C, et al. An open randomized trial of artemether versus quinine in the treatment of cerebral malaria in African children. *Trans R Soc Trop Med Hyg* 1996; 90:298-301.
 50. Boele van Hensbroek M, Onyiorah E, Jaffar S, et al. A trial of artemether or quinine in children with cerebral malaria. *N Engl J Med* 1996; 335:69-75.
 51. Hien T T, Day N P J, Phu N H, et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. *N Engl J Med* 1996; 335:76-83.
 52. Hien T T, Arnold K, Vinh H, et al. Comparison of artemisinin suppositories with intravenous artesunate and intravenous quinine in the treatment of cerebral malaria. *Trans R Soc Trop Med Hyg* 1992; 86:582-3.
 53. Looreesuwan S, Wilairatana P, Vanijanonta S, et al. Efficacy and tolerability of a sequential: artesunate suppository + mefloquine, treatment of severe falciparum malaria. *Ann Trop Med Parasitol* 1995; 89:469-75.
 54. Barennes H, Pussard E, Mahaman Sani A, et al. Intra-rectal Quinimax® (a combination of cinchona alkaloids) administered at three different dosages to children with *Plasmodium falciparum* malaria in Niger. *Clin Drug Invest* 1996; 11:154-8.
 55. Antia-Obong O E, Alaribe A A A, Young M U, Bassey A, Etim B V. Chloroquine phosphate suppositories in the treatment of childhood malaria in Calabar, Nigeria. *Curr Ther Res* 1995; 56:928-35.
 56. Snow R W, Marsh K. Will reducing *Plasmodium falciparum* transmission alter malaria transmission among African children? *Parasitol Today* 1995; 11:188-90.
 57. Trape J-F, Rogier C. Combatting malaria morbidity and mortality by reducing transmission. *Parasitol Today* 1996; 12:236-40.
 58. Carme B. Low malaria mortality among children and high rates of *Plasmodium falciparum* inoculation: a Congolese reality in the 1980s. *Parasitol Today* 1996; 12:206-8.
 59. Riley E. Malaria vaccine trials: SPf66 and all that [guest editorial]. *Curr Opin Immunol* 1995; 7:612-6.
 60. Moreno A, Patarroyo M E. Malaria vaccines [guest editorial]. *Curr Opin Immunol* 1995; 7:607-11.
 61. Valero M V, Amador L R, Galindo C, et al. Vaccination with SPf66, a chemically synthesized vaccine, against *Plasmodium falciparum* malaria in Colombia. *Lancet* 1993; 341:705-10.
 62. Alonso P L, Smith T, Armstrong-Schellenberg J R M, et al. Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. *Lancet* 1994; 344:1175-81.
 63. D'Alessandro U, Leach A, Drakeley C J, et al. Efficacy trial of malaria vaccine SPf66 in Gambian infants. *Lancet* 1995; 346:462-7.
 64. Aidoo M, Lalvani A, Allsopp C E M, et al. Identification of conserved antigenic components for a cytotoxic T-lymphocyte-inducing vaccine against malaria. *Lancet* 1995; 345:1003-7.