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# Malaria: deploying a candidate vaccine (RTS,S/AS02A) for an old scourge of humankind

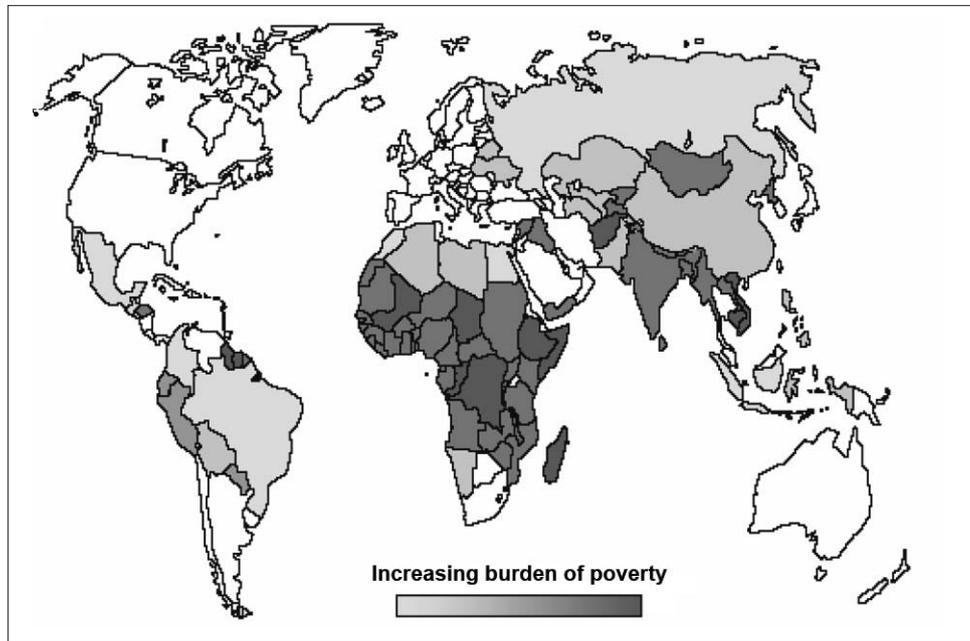
**Summary.** Malaria is an infectious disease caused by the protist *Plasmodium* spp. and it currently kills more than one million people annually. The burden of malaria is concentrated in sub-Saharan Africa, India, and Southeast Asia. The parasite's resistance to commonly used anti-malarial drugs has worsened the situation in the poorest countries. The World Health Organization (WHO) estimates that more than 100 countries suffer from endemic malaria episodes. In addition to numerous control measures and treatments, several vaccines are at different research stages and trials. We have assayed RTS,S/AS02A, a pre-erythrocytic candidate vaccine that has shown promising protection levels in phase IIb trials in Mozambique. The vaccine is directed against the sporozoite form of the parasite, which is injected by the mosquito *Anopheles* spp. The vaccine induces a strong antibody response and stimulates Th1 cells—a subset of helper T cells that participates in cell-mediated immunity. Recent interest by international funding agencies has provided new inputs into initiatives and programs to fight malaria, which, under normal welfare and adequate social development conditions, is a curable disease. [Int Microbiol 2006; 9(2):83-93]

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E-mail: palonso@clinic.ub.es**Key words:** *Plasmodium* spp · *Anopheles* spp. · malaria · pre-erythrocytic malaria vaccine · RTS,S/AS02A malaria vaccine

## Introduction

Over the twentieth century, the worldwide area of land affected by malaria decreased from 54 to 27%. In spite of this reduction, malaria remains a major global concern and one of the most common illnesses affecting humankind [12]. It is estimated that there are between 300 and 500 million clinical cases per year. Data from the World Health Organization (WHO) [28] show that 3000 million people, 50% of the world's population, live in malaria-risk areas. The disease causes about one million deaths per year, 90% of which in Africa, mostly children under five. The disease subsists in

more than 100 countries in different regions of the world, including India, Southeast Asia, and Central and South America, although sub-Saharan Africa is the most strongly affected (Fig. 1). Efforts to reduce poverty and childhood mortality in those vulnerable societies will fail if this devastating disease is not adequately controlled. However, the problem of controlling malaria in those countries is aggravated by inadequate health-care infrastructures and the precarious socioeconomic conditions. The situation has become even more complex over the last few years due to resistance of the parasite (*Plasmodium* spp.) to commonly used anti-malarial drugs, and to the emerging resistance of the vector (the mosquito *Anopheles* spp.) to chemicals for controlling it.



**Fig. 1.** Increasing burden of malaria and poverty. Malaria negatively affects the social and economic development of societies. It is part of the vicious cycle of disease and poverty, since not only does poverty cause disease, but disease causes poverty. (Based on “Tackle malaria today. Give tomorrow a chance”. Multilateral Initiative on Malaria [<http://www.mim.su.se>].)

To achieve a reasonable level of success in fighting malaria, a well-designed attack is necessary using a combination of strategies. Recent political interests and initiatives are providing renewed hope, which is being converted into action through different programs and funding devoted to fighting not only malaria but also several other diseases [7,15]. Those and other approaches are absolutely necessary, as well as the cooperation of the pharmaceutical companies. It has been estimated that a minimum of US\$ 3200 million per year is needed to effectively control malaria, but only a part of that sum is currently available [28].

The malaria literature is extraordinarily abundant. Besides human beings, other species, including birds and primates, are also natural hosts of malaria parasites [22]. This review will briefly provide a look at the historical and socio-economic background of malaria, and offer a perspective of the current initiatives and programs aimed at eradicating the disease. The *modus operandi* of the malaria vector and parasite as well as the features of the tested candidate vaccine RTS,S/AS02A and other tools to prevent and fight the disease will be examined.

## Historical background

Some historians have situated the beginnings of malaria as far back as 10,000 years ago and even earlier. The establishment

of agriculture and farming created favorable conditions for spreading the disease [19,24]. The increase in human population density that followed the shift from a nomadic way of life to a sedentary agricultural one that included the raising of cattle may have provided adequate breeding places for anopheline mosquitoes [14]. Evidences from archaeological deposits in the East Mediterranean date the disease to at least ca. 6000 years ago. Some ancient products used in the treatment of malaria, which were remarkably effective, confirm the antiquity of the disease. A drink prepared with qinghao (*Artemisia annua*) has been used for at least 2000 years in China. The active ingredient is artemisinin, which only recently has been chemically identified. Currently, artemisinin is being considered to replace drugs such as chloroquine, which have become useless due to resistance of the parasite [26].

References to malaria can be found in early Chinese, Caldean, and Hindu writings. In the 4th century BC, malaria was endemic in the Mediterranean basin. Fevers caused by the proximity of humans to swamps and standing water were noted by both the Greeks and the Romans, who established the practice of draining swamps to control periodic fever episodes. Despite the antiquity and morbidity of malaria, it is unclear whether its fatality/lethality ratio was as high as in modern times. During World War I, thousands of soldiers on the Macedonian front were victims of malaria attacks. But, curiously, this was not the case in the battle of Actium (September, 31 BC) that confronted the Romans under the

leadership of Octavian (later Emperor Augustus), with the Alexandrian army led by Mark Anthony and Cleopatra. The battle involved about 400,000 soldiers, but there appear to have been no losses due to malaria, although the disease already existed. What was the reason for this? Some evidence indicates that, by the fifth century AD, the malaria vector had changed from a mild variety to another, much more aggressive one. Therefore, it can be speculated that, had there been a very efficient vector of malaria in Europe during antiquity, Classic culture, with its impact on modern civilization worldwide, would not have developed.

Before discussing the systematic control of malaria, some historical observations are necessary. The malaria parasite was discovered in 1880 by Charles Louis Alphonse Laveran (1845–1922), who found exflagellated gametocytes in a fresh blood film obtained from a malaria patient. The work of Ronald Ross (1857–1932) in India and Gianni Battista Grassi (1854–1925) in Italy was also of great importance [5]. Working independently, both men described the life cycle of the malaria parasite in birds and humans, respectively. In 1897, Ross showed that the mosquito was the vector of the disease based on his observations of developing plasmodia in the mosquito gut. At the same time, Grassi provided evidence of the transmission of malaria from vectors to humans. However, in 1902, only Ross was awarded the Nobel Prize in Physiology or Medicine [11], “for his work on malaria, by which he has shown how it enters the organism and thereby has laid the foundation for successful research on this disease and methods of combating it”. Controversy arose then—and remains to the present day—about whether Ross or Grassi was the first to discover the role of the mosquito in the transmission of malaria. What is clear is that Grassi’s contribution was as relevant as that of Ross, and that the Nobel Commission, and even Ross, ignored his work [5]. In 1907, Laveran was awarded the Nobel Prize for his work on the role of protozoa in causing diseases, which was performed much earlier than that of Ross and Grassi. Taken together, these discoveries allowed the development of new control strategies. The xenobiotic compound dichlorodiphenyltrichloroethane (DDT) was introduced at the time of World War II [12]. The discovery of the properties of this compound, which is very effective in killing insects, earned Paul Müller the Nobel Prize in Physiology or Medicine in 1948. Later, drugs of the chloroquine group were widely and effectively used against the parasite until resistance arose, first in South America and Asia (1960s) and later in Africa (1980s). Significant actions for controlling the disease included the US malaria eradication program, which was set up in 1946 and managed by the Communicable Disease Center (later renamed the Centers for Disease Control, CDC) in Atlanta [www.cdc.gov]. Similar

actions were undertaken in Italy by the Istituto Superiore di Sanità, located in Rome [www.iss.it], which from 1945 onwards has concentrated efforts and resources to fighting malaria.

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## Consequences on social and economical development

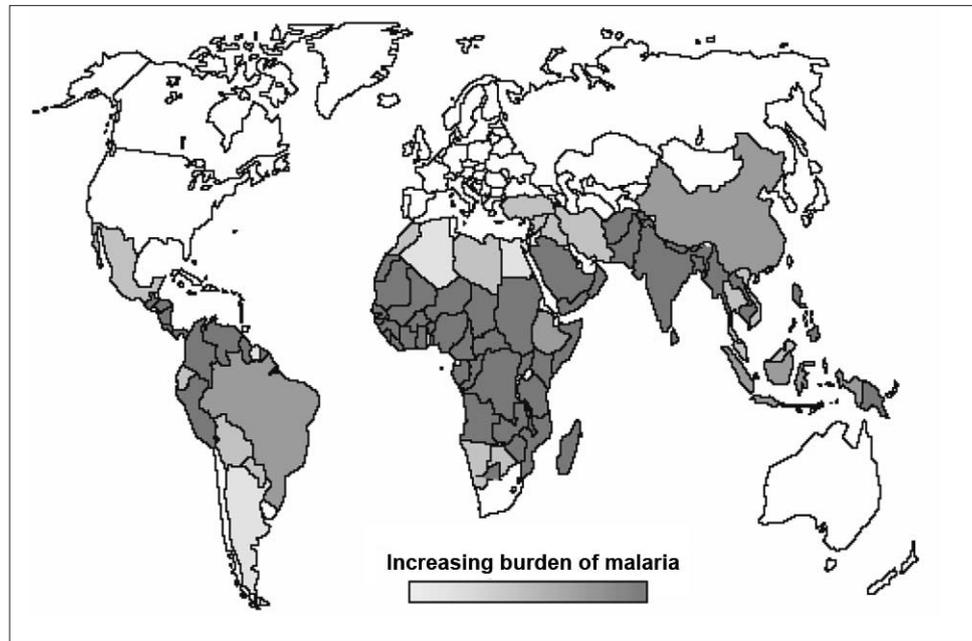
Malaria is an infectious disease that, in addition to its health consequences, has a large and tragic impact on the social and economic development of societies. There is a close relationship between malaria and poverty (Fig. 2). It is important to understand that this is not a linear relationship but a vicious cycle of disease and poverty, since not only does poverty causes disease, but disease causes poverty. Malaria destroys the capacities of individuals and hence the economic development of their countries. Under this enormous burden, local industries, such as those for the production and export of coffee, vegetal oils, copper, cotton, and sugar, cannot become commercially viable. The disease delays the development of sub-Saharan African countries at a cost of at least US\$ 12,000 million per year [17]. The cumulative effect during the last 35 years of this loss in growth means that the gross domestic product of the African continent is 32% lower than it would have been under healthy conditions.

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## Disease, parasite, and vector

The name of the disease derives from the Italian term for bad air (*mala aria*). It has also been known as paludism (from *paludisme* in French, *paludo* is wetland in Latin) and intermittent fever, among other terms. Malaria is diagnosed by clinical symptoms and microscopic examination of blood smears. The accompanying symptoms, fever, shivering, pain in the joints, and headache, quickly disappear after the parasite is killed by anti-malarial drugs. In endemic regions, however, the parasites have developed resistance to several commonly-used drugs. Consequently, patients in those areas require treatment with other, more expensive drugs.

The pathogenic mechanisms involved in the clinical illness proceed along the following steps: (i) cyclic fever, the hallmark of the infection; (ii) anemia; (iii) tissue hypoxia, due to anemia and alterations in the microcirculation of the blood; and (iv) immunopathologic events, as malaria causes an increase in circulating immunoglobulins [3]. Since the signs and symptoms of anemia are non-specific, this disorder commonly goes unrecognized. Hence the goal is a preventive, rather than a curative approach, as this would reduce the impact of poor access to health-care services.



**Fig. 2.** Countries in which malaria is endemic and the current distribution of the disease. Malaria is present from Central America through large areas of South America, Africa, India, Southwest Asia, and the Pacific Islands. The most highly stricken area is sub-Saharan Africa, which accounts for more than 90% of all malaria deaths. Those figures indicate the probability of dying before the age of five. In 2002 the world population exposed to malaria was 48.30%, about 3000 million people. (Based on “Tackle malaria today. Give tomorrow a chance”. Multilateral Initiative on Malaria [<http://www.mim.su.se>].)

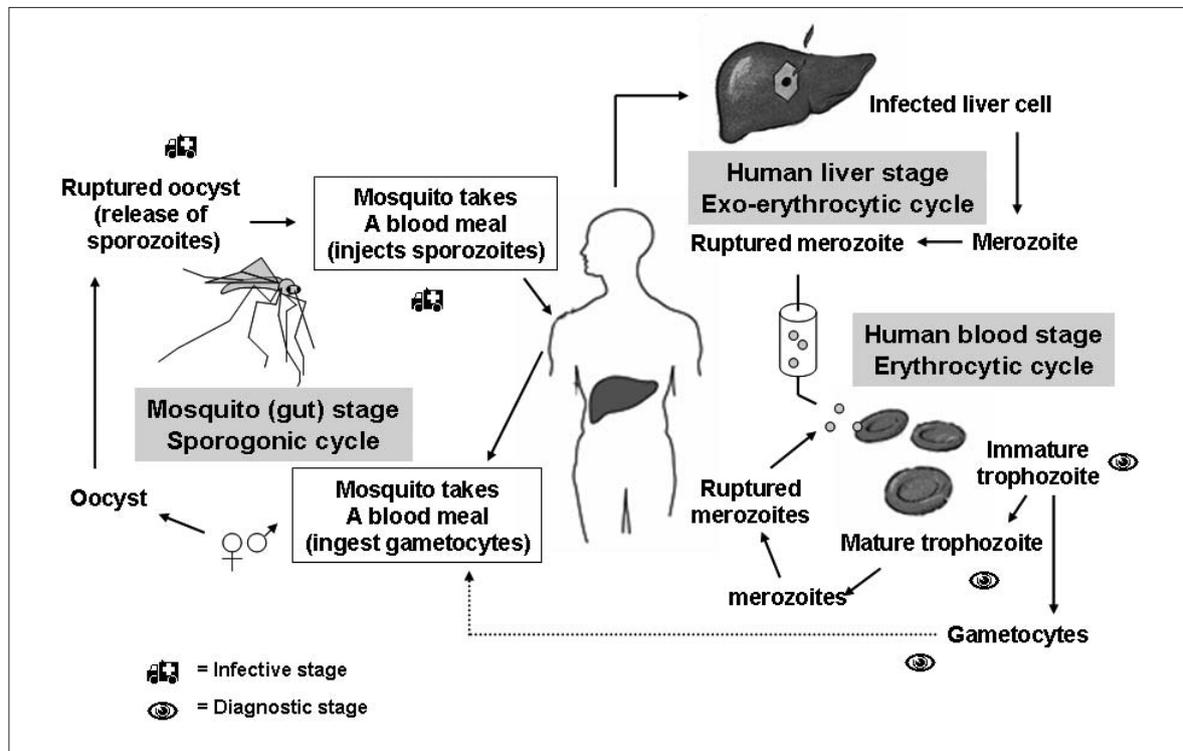
Since 1638, malaria has been treated with quinine, an alkaloid from the bitter bark of the South American quina-quina tree (*Cinchona ledgerian*). The natives of Peru were already aware of the anti-febrile properties of the tree before the fifteenth century, and its use was extended to other South American countries in the treatment of intermittent fevers. The active ingredient, quinine, was first isolated in 1820. In 1945, another drug, chloroquine, a member of the quinolone family, was found to be extremely effective for malaria prophylaxis and treatment, preventing and virtually curing “jungle” fever. However, nowadays, many strains of the parasite have developed resistance to chloroquine and to other synthetic drugs [26]. To treat malaria caused by those strains, quinine is still used. Indeed, before the emergence of resistance, the use of anti-malarial drugs and potent pesticides directed against the vector had been quite successful in controlling malaria in some countries. But the appearance of drug-resistant strains and insecticide-resistant mosquitoes, as well as the severe health and environmental problems caused by the large-scale use of pesticides, prevented the eradication of the disease.

**The parasite.** Malaria parasites have a complex life cycle, comprising sexual and asexual phases [26] that require the

succession of several developmental stages (Fig. 3). The parasite lives in humans for most of its life cycle, in the liver and in erythrocytes. Intracellularly enclosed by a “parasitophorous” vacuole in erythrocytes, the parasite reproduces asexually. In recent years, more than 20 surface antigens have been characterized by molecular techniques in *Plasmodium* spp. An interesting feature of some of those antigens is that, very often, the peptide regions have a repetitive structure [13].

Four species of apicomplexan protozoa cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Aspects such as the molecular structure and cellular localization of putative target antigens, as well as the biology of antigens by transfection of related parasites, have been extensively studied for vaccine development [22]. *P. falciparum* is the causative agent of 90% of infections and is the target of the vaccine trials of the different initiatives and programs. The genome sequence of *P. falciparum* was elucidated in 2002 [8]. The second most important causative agent is *P. vivax*. In spite of the differences affecting appearance and pathogenicity among the four species, their life cycles are basically the same [14].

*P. falciparum* can develop in erythrocytes of people of all ages, and parasitemia can reach very high levels. The infected erythrocytes break, releasing many new parasites (mero-



**Fig. 3.** Life cycle of *Plasmodium*. In the history of humankind, infectious diseases have played significant roles in wars, and have tragically brought about the intermediate or final fate of many conflicts. Those numberless armies of men and other animals, transporting materials and weapons, succumbed in the face of attacks by unexpected and invisible enemies: infectious diseases, with malaria being one of the most frequent. (Based on "Scheme of the life cycle of malaria". Centers for Disease Control and Prevention (CDC) [[http://www.cdc.gov/malaria/biology/life\\_cycle.htm](http://www.cdc.gov/malaria/biology/life_cycle.htm)].)

zoites) that then infect more erythrocytes; ultimately, this leads to the destruction of massive numbers of red blood cells. The characteristic chills and fever, or paroxysm, associated with malaria occur when the parasites are released from the erythrocytes, and since release of the parasites is periodic, paroxysms are also periodic.

Sporozoites from the mosquito salivary gland are injected into the human while the mosquito injects anticoagulant saliva to ensure an even-flowing blood meal. Once in the bloodstream, the sporozoites reach the liver and penetrate hepatocytes, where they remain for 9–16 days, multiplying within the hepatic cells. On release, they return to the bloodstream and penetrate erythrocytes, in which they produce either merozoites or micro- and macrogametocytes, which have no further activity within the human host. Another mosquito arriving to feed on the blood may suck up these gametocytes into its gut, where exflagellation of microgametocytes occurs, and the macrogametocytes are fertilized. The resulting ookinete penetrates the wall of a cell in the midgut of the insect, where it develops into an oocyst. By sporogony, this oocyst produces many sporozoites; when the oocyst ruptures, the sporozoites migrate to the salivary gland of the

vector for injection into another human host. A biting female mosquito transfers about 10% of its sporozoite load into the capillaries or perivascular tissue of the host. The sporozoites must then begin their evasion of host defenses, possibly by "camouflaging" themselves with binding proteins from the host serum. Some sporozoites are destroyed by macrophages or by antigen-specific antibodies in immune individuals, but in non-immune individuals they reach the hepatocytes and initiate schizogony or become hypnozoites, depending on their delay trigger. All sporozoites leave the peripheral circulation within 45 min [27].

**The vector.** Malaria is a vector-borne disease spread by the bite of the female *Anopheles* mosquito, a genus of the group Anophelinae. Although the mosquito is always the vector, out of the 380 species of *Anopheles* only 60 transmit malaria parasites, and only the females do, as the males do not feed on blood. Anophelinae breed in water, like most other mosquitoes. Each species has its preferred breeding grounds and feeding pattern. Usually, breeding sites are forest pools, irrigated fields (rice fields, for instance), lakes, and temporary rainwater puddles. Their sensitivity to insecticides

is highly variable, and adaptation of some anophelian species to urban environments has been reported [16].

Climatic factors, such as temperature, humidity, and rainfall, are key determinants in the distribution of malaria. In tropical and subtropical areas, the climatic conditions are optimal to allow the survival and multiplication of the mosquitoes. To transmit malaria effectively, female *Anopheles* must survive long enough after they have become infected through a blood meal from an infected human. Temperature is most significant, since below 20°C the parasite cannot complete its growth cycle inside the mosquito. Collections of water following rainfall are also breeding sites where *Anopheles* eggs are deposited and larvae develop into adults in a process that approximately takes 9–12 days.

The Global Malaria Eradication Campaign, an important program led by WHO, was started in 1955 and its aim was the worldwide eradication of malaria. However, those efforts were gradually abandoned from 1969 to 1976 due to the realization that the objective was unlikely to be ever achieved. Although large areas of the world were successful in controlling the disease, this was not the case for developing countries. The resistance of the vector to DDT and the resistance of strains of *Plasmodium falciparum* to chloroquine severely impaired the WHO program.

## Strategic programs and initiatives

Anti-malarial chemoprophylaxis in endemic areas of Africa was shown to reduce malaria morbidity, but it was abandoned due to the growing threat posed by resistance of the parasite. This remains an acute problem for drugs that have been used in the treatment of the infection and is a prominent factor explaining the increase in mortality [26]. Chloroquine is ineffective in most parts of Africa and the efficacy of sulfadoxine-pyrimethamine, which replaced it, is decreasing due to the appearance of resistant strains. Drug resistance causes treatment failure and can lead to death and the further, rapid spreading of resistance. New hope has been offered by artemisin derivatives, which are effective in combination with other anti-malarials. Nevertheless, the success of this approach has been limited by the poor supply of high-quality artemisin derivatives and by their high cost. The WHO currently recommends artemisin-class combination therapies (ACTs) for the treatment of malaria, since the benefits of combined therapy include a delay in the appearance of resistance [2]. However, the high price of ACTs remains problematic.

The deployment of existing “weapons” on a vastly greater scale is required to fight malaria, together with the improvement of those weapons and the development of new

ones. This is in fact true for most infectious diseases, which mainly occur in poor regions of the world (21). In-depth knowledge not only of the pathogenesis of malaria but also of the economic and social conditions of those countries where the disease is endemic is necessary to design effective treatment strategies. Vaccines are well-suited for resource-poor settings, and insecticide-treated bednets with long-acting insecticides are suitable for rural zones in Africa. Also, funds are needed to provide drugs, logistics, and health-care services in most endemic areas.

Currently, three major tools are used to control malaria: (i) control of mosquitoes, (ii) reduction of human–vector contact, and (iii) prevention and treatment of the disease with drugs [15]. Vector control includes indoor residual spraying, management to eliminate breeding sites, and treatment to eliminate mosquito larvae with appropriate larvicides. Nevertheless, while those practices have effectively saved millions of lives worldwide, their high cost and complexity make them difficult to implement in rural areas of sub-Saharan Africa. The reduction of human-vector contact through insecticide-treated bednets has much better acceptance and suitability; but, although they are inexpensive and effective, less than 2% of Africans sleep under them. Thus, the future of malaria control requires the development of vaccines and the availability of effective and inexpensive drugs. As part of the search for new means of control, international initiatives like the Intermittent Preventive Treatment in infants (IPTi) [<http://www.ipti-malaria.org>] are offering new approaches.

**IPTi** is a promising strategy in the fight against malaria. Infants receive an anti-malarial drug three times during their first year of life. This practice has been shown to reduce malaria and anemia in infants less than 1 year of age by up to 60% [21], and it seems that IPTi could become a major tool for malaria control in Africa. IPTi can be delivered through the Expanded Programme on Immunization (EPI): [<http://www.wpro.who.int/sites/epi/overview.htm>], which is one of the best-functioning systems of regular health contact with young children in Africa.

Besides the aforementioned, some other actions and programs currently working on malaria are:

**Malaria Vaccine Initiative (MVI)** was created in 1999 by a grant from the Bill & Melinda Gates Foundation. MVI works with government, industry, and academic partners on five continents within PATH (Programme for Appropriate Technology in Health), a world-wide nonprofit organization: [<http://www.malariavaccine.org>]. Its mission is to accelerate the development of malaria vaccines and ensure their availability for developing countries.

The candidate RTS,S/AS02A vaccine is being developed and tested under the auspices of **The Global Health Program**,

which is also financed by the Bill & Melinda Gates Foundation: [<http://www.gatesfoundation.org/GlobalHealth>].

**Roll Back Malaria (RBM)** is a global partnership to develop and coordinate strategies and interventions against malaria: [<http://www.rbm.who.int>]. It aims at halving malaria deaths by 2010, and halving them again by 2015.

**The Global Fund to Fight AIDS, Tuberculosis and Malaria** is a partnership between governments, civil society, the private sector, and affected communities to support interventions against all three diseases: [<http://www.theglobalfund.org>].

**The Multilateral Initiative on Malaria (MIM)** supports international collaboration and co-operation in scientific research on malaria: [<http://www.mim.su.se>].

These initiatives and programs, among others, are working alone or in combination to offer new hopes of achieving the objective of controlling malaria. Nevertheless, the financial and human efforts and resources need to be continuous.

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## The RTS,S/AS02A vaccine

Vaccine development is a long process that takes years of clinical testing and trials until licensing and public availability are reached. Attempts to develop a malaria vaccine began more than 50 years ago. Some of the developed vaccines showed promising results [9,23,25], such as the candidate vaccine developed in Colombia by Manuel Patarroyo, Spf66. Nevertheless, a lack of consistency with other trials [6,18], the failure to prevent malaria in infants [1], and difficulties in product availability and reproducibility stopped further development of this vaccine [10].

The partnership formed between MVI-GSK (produced by GlaxoSmithKline), the Centro de Investigaç o em Sa de da Manhiça (CISM), the Mozambique Ministry of Health, the Bill & Melinda Gates Foundation, and the Hospital Cl nic de Barcelona has been involved in the development and assay of the candidate vaccine RTS,S/AS02A, which was specifically designed to prevent infection by *P. falciparum*. The vaccine is directed against the sporozoite, the form of the parasite that is injected by mosquitoes. After immunization, antibodies and white blood cells are produced that can prevent the sporozoite from either surviving or developing further in the liver.

Our team has successfully tested RTS,S/AS02A in a phase IIb trial that was conducted in children age 1–4 years in Mozambique, where malaria is endemic. The results were clearly the best that have ever been obtained with a candidate malaria vaccine. The findings were very encouraging: the vaccine induced a strong antibody response, stimulated Th1 cellular immunity, and showed efficacy against clinical malaria. However, further trials will be needed to prove that

the vaccine is safe and effective before a license can be granted. This goal will probably be achieved by 2010.

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## Development of the vaccine

We have been involved in the development of RTS,S/AS02A since 2000. The vaccine's target is the protein of the circumsporozoite on the sporozoite of *P. falciparum*. The repetitive sequences of the protein have long been considered as a target for a potential vaccine. RTS,S is a hybrid molecule expressed in yeast by recombination, in which the circumsporozoite protein, central tandem repeat, and carboxyl-terminal region are fused to the N terminal of the S-antigen of hepatitis B virus in a particle that also includes unfused S-antigen [3]. It is assumed that the adjuvant used in the vaccine, AS02A, which is an oil in water emulsion with the immunostimulant monophosphoryl lipid A, has been essential to improving the viability of this vaccine.

The relevance of an adjuvant—an area of intensive study—lies in the fact that pairing a malaria antigen with the adequate vaccine adjuvant greatly contributes to the ability of the vaccine to trigger a strong immune response. Other trials, including those with RTS,S, have used conserved circumsporozoite protein fragments as a vaccine, but without success. The change came with the addition of this new adjuvant. It was known that this protein induced good titers of antibodies and cellular responses, as measured by the production of  $\gamma$ -interferon and by cytotoxic reactions. Protection evidence came from experimental challenge models in a trial conducted in the USA. In that study, the development of infection was monitored in healthy volunteers who had been previously immunized with the vaccine and who were subsequently challenged with the parasite.

Good profiles of safety and immunogenicity of the product were confirmed in phase I studies in Gambian children and adults [4] and in Mozambican children. This initial success allowed us to start a phase IIb, proof-of-concept efficacy trial in Mozambique. This trial estimated the immunogenicity, safety, and efficacy of the vaccine as determined by the prevention of malaria episodes [3]. The trial, results and comments were published in *The Lancet* in October 2004 [3]. Here we include some additional comments, results, and procedures of that trial based on the lectures and interviews we gave after publication of our work. It is our intention to attract the interest of the general public, institutions, and scientific community in the fight against malaria.

One of the greatest problems with malaria, which accounts for the extreme difficulty of developing a vaccine, is that we do not yet understand how individuals develop

immunity against the disease. Since the work of Koch on Java Island at the end of the 19th century, it has been known that adults who survive malaria infection acquire a highly effective immunity, but the mechanisms involved and how they operate remain unknown, as is the role of indirect protective measures. Tishkoff et al. [24] stated that, throughout human evolution, genetic defense mechanisms have arisen in regions where malaria is prevalent, and that most human genes that reduce the risk of malaria infection are expressed in erythrocytes. Nonetheless, little is known about the immunity that offers protection against malaria, nor is it at all clear which proteins are responsible for protection. Animal models, including rats, chimpanzees, and rhesus monkeys, are not useful for studying malaria in humans. Thus, the only way to assess whether a vaccine is effective is by conducting clinical trials in natural conditions. In hepatitis B, for instance, it is known that people who have a certain threshold level of antibodies against the viral surface antigen are protected. Thus, for hepatitis B, individuals must be immunized and then after 20 or 30 days antibody titers must be measured. The same procedure is used for diphtheria, tetanus, pneumococcus, and several other infectious diseases, but not malaria.

### The geographical and sociological context

Mozambique is one of the poorest countries in the world. Manhiça, in the southern part of the country, is a district of Maputo province. The vaccine trial was carried out in the CISM, where the demography department is in charge of geographically delimitating the study area, mapping it, geopositioning each house using GPS, and registering the population in a census. We often start our papers saying that, in Africa, most people are born and die without ever being registered; and this is true. There are no direct measures of mortality. In the study area, we registered the population, which currently is around 70,000. Births, deaths, emigrations, immigrations, pregnancies, and changes of residence were registered periodically in our census, which provided a powerful research tool (Fig.4).

In the Manhiça region, malaria transmission takes place throughout the year. *P. falciparum* is the most common species and *Anopheles funestus* is the main vector. The entomological inoculation rate for 2002 was 38 infective bites per person/year. In Ilha Josina, 55 km north of Manhiça, we estimated that people received between one and two bites per day. Those figures reflect the high rates of transmission, which are also evidenced by the fact that malaria is the main cause of hospitalization and death in children under the age

of five. The situation is quite representative of what happens in sub-Saharan Africa and in most of the African continent. In Manhiça and Ilha Josina, we undertook the last proof-of-concept trial in children age 1–4 years. The primary objective was to evaluate the efficacy of the vaccine as measured by the number of clinical episodes of malaria caused by *P. falciparum*. Clinical episodes were defined by an axillary temperature of  $\geq 37.5^{\circ}\text{C}$  and parasitemia (presence of the asexual stage of *P. falciparum* in the peripheral blood at a concentration higher than 2500 parasites per microliter). Since not everybody infected with the parasite presents with a clinical episode of malaria, we looked for indicators that better defined clinical episodes when the prevalence of infection is high. This was important because, in the absence of specificity, the results become biased towards zero.

### The vaccine trial

The trial was designed to assess the efficacy at two stages in the life cycle of *P. falciparum* and the pathogenesis of malaria: clinical disease and infection. Each outcome was measured simultaneously in two cohorts based in different sites: Manhiça (cohort 1) and Ilha Josina (cohort 2). The study was a phase IIb, double-blind, randomized controlled trial. Three intramuscular doses of either RTS,S/AS02 vaccine or a control vaccine (not placebo) were administered at 0, 1, and 2 months, that is with a 30-day interval. As control vaccine, hepatitis B was administered to children older than 24 months. Children younger than 24 months received pneumococcal vaccine (2 doses) and *Haemophilus influenzae* vaccine (1 dose). This procedure ensured that the study remained double-blind. The trial began in April 2003 and the double-blind phase ended on May 30, 2004. The opening of the code and the analysis were done during the first week of August 2004, according to an analytical plan previously decided, closed, and submitted to the U.S. Food and Drug Administration (FDA). The results were published in *The Lancet* in October 2004 [3].

Cohort 1 (Manhiça), with 1600 children age 1–4 years, received three vaccine doses with intervals of 30 days. Cohort 2 (Ilha Josina), with 417 children, also received the vaccine at 30-day intervals, but peripheral parasitemia was cleared with anti-malarial drugs between the second and third dose in order to assess the efficacy of the vaccine in reducing new infections. Thus, the two groups allowed us to ascertain the answers to the following questions: What is the efficacy of the vaccine against clinical episodes of malaria (passive surveillance)? What is its efficacy against new infections (detected through a combination of active and passive sur-



**Fig. 4.** Malaria-affected population, from the mid nineteenth century to the dawn of the twenty-first century. Left, *La Malaria* (1850–1851), oil canvas by Antoine August Ernest Hébert (1817–1908). Right, waiting for the doctor at the health facilities in Vilha Josina Island, Manhiça district, Mozambique.

veillance)? Once the correct random allocation was checked, after the code was broken, and the comparability of the two groups was ensured, any difference found could be attributed to the vaccination and not to the introduction of confounding factors (Table 1).

## The state of the art: the vaccination results

**Severe adverse events.** These were defined as any hospital admission by any cause and their numbers were higher in the control group (249) than in the RTS,S/AS02A group (180). Fifteen children died, 10 in the control group and 5 in the RTS,S/AS02A group. Assuming the mortality rate at this age in that location, between 35 and 40 deaths would have been expected. A reduction in mortality often occurs during a study due to the fact that the participants are being closely followed. In the control group, four deaths were specifically attributed to malaria. Also, there were three episodes of cerebral malaria in the control group but none in the vaccine group.

**Immunogenicity.** Anti-circumsporozoite (the part of the *P. falciparum* protein included in the vaccine) antibodies and antibodies against the hepatitis B surface antigen were evaluated. Geometric mean titers of anti-circumsporozoite antibodies for children younger or older than 24 months were obtained. The same was done for antibodies against surface antigen of hepatitis B. It was remarkable that there was good production of antibodies against the conserved region of the circumsporozoite. Production increased from 0.3 at baseline to 273 one month after the third dose in children younger than

24 months, and from 0.3 to 158 in children older than 24 months. There was some evidence that antibody titers were greater in small children, as the vaccine seems to be more immunogenic in children younger than 24 months. Although antibody kinetics fell, six months after the third dose the high titers persisted. High levels of anti-hepatitis B surface antigen antibodies were also produced. Antibodies increased from 63 at baseline to 51,000 one month after the third dose in children less than 24 months and from 9 to 11,000 in children older than 24 months, which again shows greater immunogenicity in children younger than 24 months. Another interesting result was that the antibody titers against hepatitis B surface antigen induced by RTS,S/AS02A vaccine were higher than those induced by the existent hepatitis B vaccine.

**Efficacy.** In Manhiça, during the first 6 months of follow up, 123 first clinical episodes of malaria occurred in the RTS,S/AS02A group, compared to 159 in the control group (Table 1). Incidence rates were 0.38 versus 0.52, respectively, which gives an estimation of vaccine efficacy of 29.9%, (confidence intervals 11.0–44.8,  $p < 0.004$ ). Vaccine efficacy was assessed with Cox regression models; Schoenfeld residuals and time-dependent Cox models were used to investigate the proportional hazards assumption. Consistent with this efficacy estimate at the cross-sectional survey 6 months after the third dose, the prevalence of infection was 37% lower in the RTS,S/AS02A group than in the control group.

Estimates of RTS,S/AS02A efficacy with respect to other endpoints, including first episode of fever and any level of *P. falciparum* parasitemia, first episode of fever or history of fever and *P. falciparum* parasitemia, first episode of fever and *P. falciparum* parasitemia with more than 15,000 para-

Table 1. Vaccine efficacy

	Control vaccine			RTS,S/AS02A			Vaccine efficacy (95% CI)	<i>p</i>
	Events	Pyear	Rate	Events	Pyear	Rate		
Clinical malaria (cohort 1)								
First episode of fever and parasitaemia > 2500 per µl	159	302.9	0.52	123	321.6	0.38	29.9% (11.0 to 44.8)	0.004
First episode of fever and parasitaemia > 0 per µl	176	300.5	0.59	137	318.1	0.43	28.6% (10.4 to 43.1)	0.004
First episode of fever or history of fever and parasitaemia > 0 per µl	251	284.3	0.88	188	309.0	0.61	33.8% (19.7 to 45.3)	<0.0001
First episode of fever and parasitaemia > 15 000 per µl	138	307.08	0.45	104	324.5	0.32	31.7% (11.5 to 47.2)	0.004
First episode of fever and parasitaemia 100 000 per µl	44	324.9	0.14	40	335.3	0.12	16.4% (-29.1 to 45.9)	0.419
Multiple episodes of fever and parasitaemia > 2500 per µl	190	330.1	0.58	153	341.0	0.45	27.4% (6.2 to 43.8)	0.014
Malaria infection (cohort 2)								
First episode of parasitaemia > 0 per µl	166	25.9	6.42	157	45.0	3.49	45.0% (31.4 to 55.9)	<0.0001

## Notes:

Pyear = person-year at risk.

Vaccine efficacy estimates adjusted by age at baseline, bednet use at baseline, distance from health facility, and geographical region.

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sites/microliter, were most of them around 30%, and all of them were statistically significant. The efficacy of the vaccine against episodes of severe malaria was assessed. Severe malaria, defined according to WHO criteria, included a composite of severe anemia (hematocrit < 15%), cerebral malaria (Blantyre coma score < 2), generalized convulsions, prostration, hypoglycemia (< 2.2 mmol/dl), acidosis, or circulatory collapse. In the control group, 26 cases of severe malaria were reported versus 11 in the RTS,S/AS02A group, which provides an efficacy of 57.7% ( $p < 0.019$ ). Exploratory analysis led to an estimate of vaccine efficacy in children under 24 months of 76.9% ( $p < 0.018$ ). Finally, no correlation was found between titers of antibodies to circumsporozoite and protection.

In Ilha Josina, where transmission is very intense, the time to first infection was determined and resulted in an estimated efficacy of 45% in the prevention of new infections (confidence intervals 31.4–55.9,  $p < 0.0001$ ). In summary, the RTS,S/AS02A malaria vaccine was shown to be safe and well-tolerated when administered for the first time to children 1–4 years old living in endemic zones. The vaccine showed a high degree of immunogenicity against both circumsporozoite protein and hepatitis B. The efficacy in delaying time to first infection was 45%. Vaccine efficacy for the first clinical episode was 30%, and for severe malaria 58%. Children younger than two years presented higher immunogenicity and efficacy. All our data suggest that efficacy lasts at least during the first 6 months.

Trial participants are still under surveillance to evaluate

whether efficacy is maintained during the following year. [The following phase of the vaccination assay was performed as initially scheduled and the results confirmed the levels of protection by at least 18 months. Published in *The Lancet*, Nov. 15, 2005, online edition.]

Clearly, delaying time to new infections means that not only is there a reduction of new infections but also of new episodes of mild and complicated malaria. This is a novelty that opens unexpected, important doors. Nevertheless, an immunological surrogate to correlate with protection is lacking. From the public health point of view, these are very important results. Due to the extraordinary antigenic complexity and cycle of the malaria parasite, it is naive to think there will be a malaria vaccine with 100% efficacy. Nevertheless, the availability of a vaccine with nearby 60% efficacy, if administered in combination with other available control tools, could play a critical role in controlling the disease.

For many years, the difficulty or even impossibility to develop a vaccine against malaria has been well known. All researchers involved in the fight against this disease agree that it is an strenuous task. But there is no place for hopelessness. In view of our results, we can conclude that a vaccine against malaria can be developed, although continuous efforts and resources, as well as a high level of confidence are needed to pursue promising trials and initiatives.

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## La malaria: preparación de una vacuna (RTS,S/AS02A) para un antiguo azote de la humanidad

**Resumen.** La malaria, una enfermedad infecciosa causada por el protista *Plasmodium* spp. causa anualmente más de un millón de muertes. Las regiones más afectadas son África subsahariana, India y el sudeste asiático. La resistencia del parásito a los fármacos antimaláricos más comunes ha empeorado la situación en los países más pobres. La Organización Mundial de la Salud (OMS) calcula que son más de 100 los países donde la enfermedad es endémica. Además de las numerosas medidas de control y de los tratamientos a los afectados, varias vacunas se encuentran en diferentes fases de prueba. Nuestro grupo ha ensayado la RTS,S/AS02A, una vacuna candidata pre-eritrocítica que ha dado niveles de protección esperanzadores en ensayos de fase IIb en Mozambique. La vacuna está dirigida contra el esporozoito, la forma del parásito inyectada por el mosquito *Anopheles* spp. La vacuna induce una fuerte producción de anticuerpos y células Th1 (el tipo de células T que intervienen en la inmunidad mediada por células). El reciente interés de organizaciones internacionales patrocinadoras ha supuesto un renovado estímulo a iniciativas y programas para combatir la malaria, una enfermedad curable en condiciones adecuadas de desarrollo social. [*Int Microbiol* 2006; 9(2):83-93]

**Palabras clave:** *Plasmodium* spp. · *Anopheles* spp. · malaria · vacuna antimalaria pre-eritrocítica · vacuna antimalaria RTS,S/AS02A

## A malária: preparação de uma vacina (RTS,S/AS02A) para um antigo flagelo da humanidade

**Resumo.** A malária, uma doença infecciosa causada por o protista *Plasmodium* spp. causa anualmente mais de um milhão de mortes. As regiões mais afectadas são África subsaariana, Índia e o sudeste asiático. A resistência do parasita aos fármacos antimaláricos de uso mais comum piorou a situação nos países mais pobres. A Organização Mundial da Saúde (OMS) calcula que são mais de 100 os países onde a doença é endémica. Além das numerosas medidas de controle e dos tratamentos aos afectados, estão a desenvolver-se vacinas, cujo ensaio se encontra em diferentes fases. O nosso grupo ensaiou a RTS,S/AS02A, uma vacina candidata pre-eritrocítica que deu níveis de protecção esperanzadores em ensaios de fase IIb em Moçambique. A vacina está dirigida contra o esporozoito, a forma do parasita inyectada pelo mosquito *Anopheles* spp. A vacina induz uma forte produção de anticorpos e células Th1 (o tipo de células T que intervêm na imunidade mediada por células). O interesse recente de organizações internacionais patrocinadoras supôs um estímulo renovado a iniciativas e programas para combater a malária, uma doença curável em condições adequadas de desenvolvimento social. [*Int Microbiol* 2006; 9(2):83-93]

**Palavras chave:** *Plasmodium* spp. · *Anopheles* spp. · malária · vacina antimalária pre-eritrocítica · vacina antimalária RTS,S/AS02A