Summary

- Malaria is very common; a very important cause of mortality and morbidity in the tropics
- Five parasites: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and since 2007; *P. knowlesi* (in Southeast Asia)
- Transmission via female Anopheles mosquitoes which bite at night
- Symptoms: fever and body-ache; sometimes atypical or “chronic” (anaemia, splenomegaly)
- Risk of complicated presentations, mainly with P. falciparum (severe anaemia, kidney failure, cerebral malaria)
- Infections often asymptomatic in semi-immune people (generally low parasitaemia)
- Clinical diagnosis not reliable.
- Often clinical over-diagnosis of malaria and under-diagnosis of other disorders in endemic areas
- Diagnosis via thick smear, thin smear, rapid antigen-detection, DNA-based methods
- Treatment of *P. malariae*: chloroquine
- Treatment of *P. vivax* and *P. ovale:* chloroquine or if possible with primaquine (hypnozoites, G6PD).
- Resistance of *P. vivax* to chloroquine is rising in several areas
- Increasing multidrug resistance of *P. falciparum,* including resistance to artemisinin derivatives
- Combination treatment of *P. falciparum* infection is strongly advised:
  - a) ACT: artemisinin combination treatment (e.g. artemether + lumefantrine; = Co-Artem, Riamet).
  - b) Quinine + (doxycycline or clindamycin)
  - c) Atovaquone + proguanil (Malarone)

- Individual prevention via pyrethroid-impregnated bed net ± chemoprophylaxis; stand-by emergency treatment (self-medication) for certain travelers?
- Population protection via vector control, but increasing resistance of mosquitoes to various insecticides
- First malaria vaccine undergoing phase 4 studies in children of endemic countries

Malaria in humans

Malaria is the common name for diseases caused by infection with single-celled parasites of the genus Plasmodium. Among the parasites of the genus Plasmodium five species have been identified which regularly cause disease in humans:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium ovale
- Plasmodium malariae
- Plasmodium knowlesi

However, in 2017 several malaria outbreaks in Brazil were caused by *P. simium,* a malaria species closely related to *P. vivax* that was previously considered to be a monkey-specific malaria parasite.

Human malaria parasites have a restricted host-specificity. They don’t develop disease in rabbits, rats or mice but need to be maintained either in human volunteers or in primates. A common used less-than-optimal substitute is to perform experiments on primate, rodent or avian malaria parasites in their natural host. Most animal models are inadequate and, while they can help the researcher in answering specific questions, any extrapolation to human disease has to be considered with extreme caution. For example dexamethasone was considered to be useful in severe malaria caused by P. knowlesi in rhesus monkeys, but was found to be harmful in humans.

Historical note

Discovery of the parasite

Malaria has been with humanity since millennia. The most famous historical case of falciparum malaria is probably King Tutankhamen, the boy pharaoh from Old Egypt, in whose 3,000-year-old mummy the parasite was demonstrated. Although usually

associated with the tropics malaria was endemic in North America and large parts of Europe until the middle of the 20th century. Malaria transmission occurred in Belgium, the Netherlands, Sweden, Finland and the United Kingdom. It was a significant impediment for the European nations during the colonial period. In Northern Europe, only *P. vivax* and *P. malariae* occurred. In Southern Europe, malaria was due to infection with *P. falciparum, P. vivax* and *P. malariae*.

In 1880 the French army doctor Charles Louis Alphonse Laveran discovered malaria parasites in fresh blood from malaria patients in the coastal town of Bone (Annaba), Algeria.

Transmission

The transmission of malaria had for long been a mystery. One of the researchers was the Briton Sir Ronald Ross. He left for India with a personal mission to prove transmission via insects. In 1897, after three years of hard work, he demonstrated parasites in mosquitoes which had bitten patients. Later he also demonstrated transmission of avian malaria via mosquitoes. He was able to describe the complete development of the parasite in the mosquito and also demonstrated that transmission took place via the bite of the mosquito (and not via the presence of dead mosquitoes in drinking water, as his mentor Patrick Manson had initially thought).

Life Cycle

After the cause and transmission of malaria became known, it was logical to assume that the parasites inoculated via a mosquito bite would directly penetrate red blood cells. This wrong idea was proposed in 1903 by Fritz Schaudinn, a distinguished German microscopist. It was based on faulty observation and due to his authority, it entered some textbooks. It was known that when blood from a patient with active malaria was inoculated into a healthy volunteer, the volunteer would develop malaria and would become infectious nearly instantaneously. However, when a volunteer was inoculated via a mosquito bite, the blood was not infective for 6 days (in case of *P. falciparum*) to 9 days (*P. vivax*).

Why? This was a vexing problem which took decades to answer. It was by very careful

animal experiments with *P. cynomolgi*, a primate malaria species, that the puzzle was solved. *Shortt and Garnham* collected a large number of infected mosquitoes, mashed them to pulp and injected the lot (including sporozoites) into monkeys. After waiting a period, they killed the animals and searched the various organs and tissues. The parasites (with a different shape) were found in the liver. They had to support their hypothesis of the existence of a pre-erythrocyte stage with a species of human malaria. They used *P. vivax* and a human volunteer. This man was inoculated IV with sporozoites isolated from 200 mosquito salivary glands. A week later, the volunteer was operated on and a piece of liver tissue was obtained. The parasites were present in the liver. A year later they obtained a strain of *P. falciparum*, infected 770 mosquitoes and inoculated another human volunteer. About 6 days later a liver biopsy was taken and again the parasite was found.

Pyrotherapy

In 1927 Julius Wagner-Jauregg won the Nobel prize for his discovery of malaria pyrotherapy for treatment of late stage neurosyphilis. To induce repeated spikes of high fever in patients with progressive paralysis, he inoculated them with blood from patients who were suffering from tertian malaria (*Plasmodium vivax*). Although not without risk, this treatment proved to be very successful.

Life cycle

When a mosquito lands on the skin, it attempts to pierce a small blood vessel with its proboscis in order to suck blood. To prevent the blood from coagulating the mosquito first injects some saliva. Besides vasodilating agents this saliva contains anticoagulants. However, the saliva may also contain micro-organisms. When a human is bitten by an Anopheles infected with malaria, parasites (sporozoites) [Gr. sporos = seed] are introduced into the human body. On average 10-20 sporozoites are injected per bite, although this number can be higher, e.g. 100.

A certain protein of the parasite, (the circumsporozoite protein, CSP), plays an important role in the penetration of the sporozoite into a liver cell (cf. Mosquirix vaccine). Sporozoites

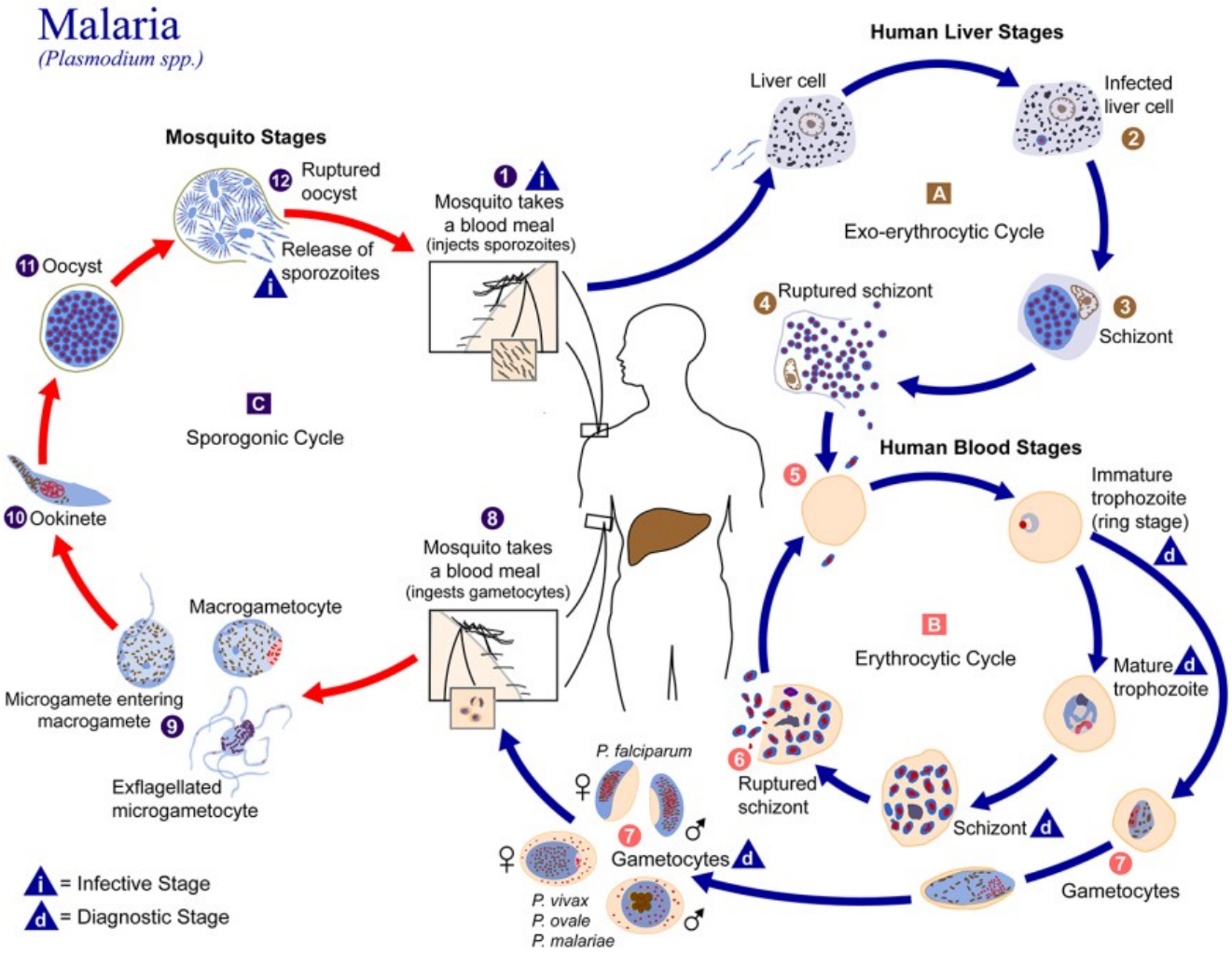
reproduce asexually in liver cells, by schizogony [Gr. schizo = split, divided]. This is called exo-erythrocytic or pre-erythrocytic reproduction. The form of the parasite produced in this way is called a liver schizont. The multinuclear schizont splits into many thousands of small offspring (merozoites) [Gr. meros = part]. Every successful sporozoite can produce some 20,000 merozoites.

After some time the infected liver cells burst and the merozoites enter the blood stream. While the parasites are reproducing in the liver, there are no symptoms. Neither the sporozoites, nor the liver forms are sensitive to most of the drugs used in malaria prophylaxis (atovaquone/proguanil is an exception). The minimal required time from infection to the appearance of the first merozoites, is the prepatent period. The incubation period is somewhat long because signs and symptoms do not appear until the parasitaemia is sufficiently advanced. Of note, merozoites in blood are usually too small to be seen by microscopy.

In the case of *P. vivax* and *P. ovale* only some of the infected liver cells burst. The parasites in the liver cells which do not burst (hypnozoites) [Gr. hypnos = sleep] may remain viable for years and are responsible for new attacks of the disease if reactivated. The trigger which reactivates the hypnozoites is not known. The existence of hypnozoites in *P. vivax* was only formally demonstrated in 1985 via fluorescence microscopy. Reactivation of these “sleeping” forms explains delayed exacerbations of the disease after treatment with chloroquine and other antimalarial drugs. They kill the blood forms, but not the liver forms. Hypnozoites are not present in *P. falciparum* and probably not in *P. malariae* (although this is controversial). This is important for treatment because hypnozoites are not sensitive to chloroquine, quinine, mefloquine or artemisinin. Accidental inoculation with infected blood (blood containing trophozoites) may lead to infection, e.g. transfusion malaria or malaria via shared contaminated syringes by drug users. Since the infection in these cases is not transmitted by sporozoites, there are no liver forms. Liver forms are also absent in congenital malaria. This is important for treatment (no primaquine for congenital malaria with *P. vivax* or *P. ovale*). The chronic nature of infections with *P. malariae* is traditionally explained by assuming that the parasite can induce a very low parasitaemia (or hidden erythocytic schizontes) for many years, which is below the detection threshold of normal diagnostic methods.

In the red blood cell the parasite feeds on haemoglobin. The form of the parasite when present in the red blood cell is now known as a trophozoite (Gr. trophe = nutrition). The young parasite possesses a digestive vacuole with lysosomal enzymes. This vacuole contains proteinases (plasmepsin and falcipain). The vacuole can be clearly seen in a blood smear and explains the ring shape of the young parasite. The breakdown of haemoglobin results in an iron-containing pigment: hemozoin. The vacuole disappears as the parasite becomes older. The trophozoites will once more reproduce asexually and lead to the formation of a multinuclear parasite (schizont). The latter divides to form merozoites. Each schizont produces 8 to 24 merozoites, depending on the species, within a time span of 48 hours (*P. falciparum*, *P. vivax*, *P. ovale*), 72 hours (*P. malariae*) or 24 hours (*P. knowlesi*). The infected red blood cells burst after a while so that once more merozoites appear in the blood from where they will penetrate new erythrocytes within a few seconds. This bursting (lysis) of the red blood cells is accompanied by a bout of fever. If the development is synchronous (all parasites being at the same stage of development) the fever will follow a typical pattern (see below). This is, however, unusual: asynchrony is more common than synchrony, especially early in infections. The development from merozoite to schizont takes place in the peripheral blood and all stages can be observed. In *P. falciparum* usually only very young forms (ring forms) can be observed in the peripheral blood because older parasites (and schizonts) adhere to the endothelium of blood vessels in deep organs (e.g. the brain).

After a few days some of the merozoites transform into male or female gametocytes. These are necessary for sexual reproduction of the parasite (which only occurs in the mosquito). Gametocytes are responsible for transmitting the disease but do not themselves cause symptoms. Adult *P. falciparum* gametocytes are not sensitive to chloroquine and quinine, while those of *P. vivax*, *P. ovale* and *P. malariae* are sensitive. This means that following adequate treatment of *P. falciparum* there may still be gametocytes in the blood, and this may continue for several weeks. This does not mean that the treatment has failed. One interesting hypothesis is that chloroquine might significantly increase the gametocytemia of chloroquine-resistant *P. falciparum,* resulting in an increased infectivity for *Anopheles*. This could, therefore, contribute to the rapid spread of chloroquine resistance.



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Glucose metabolism and LDH

The trophozoite has no carbohydrate reserves and needs to consume glucose continually. The glucose metabolism in infected red blood cells is 50-100 times higher than that in non-infected cells. This probably contributes to the hypoglycaemia which is often seen in severe infections.

The parasite have mitochondria, but these play a minor role in the provision of energy (the last word on this has not yet been said). Glucose is converted by anaerobic glycolysis to pyruvate and then to lactate. This latter step, as in humans, is catalyzed by the enzyme

lactate dehydrogenase (LDH). The parasite's LDH is clearly different from that of humans and forms the basis of a diagnostic test (see below).

Geographical distribution

Many lay people regard malaria as a purely tropical disease. However, the distribution of malaria used to be world-wide. Today, it still occurs in some 100 countries. The situation varies from region to region. Until 1938 there was still *P. vivax* malaria ("polderkoorts") in Belgium, and in the Netherlands as late as 1958, although there was an unexplained (possibly autochthonous) case of *P. malariae* infection in a child in Zeeland in 1969. The WHO declared the Netherlands officially malaria-free only in 1970. It is chiefly the pollution of surface waters which makes reproduction of *Anopheles* mosquitoes difficult.

Yet some *Anopheles* persist and can transmit malaria. *Anopheles atroparvus* is able to transmit *Plasmodium vivax* malaria but cannot transmit *Plasmodium falciparum*. *Anopheles plumbeus* can transmit tropical falciparum malaria. In the last century there were important changes in the lifestyle of humans, resulting in less human/mosquito contact. Effective therapy was available. All these factors mean that malaria has disappeared in Northwest Europe. Cases in Western countries are generally dealt with swiftly and satisfactorily and one person with malaria very rarely leads to the infection of others. Chronic large scale reintroduction of the disease in Europe is thus improbable, although with the combination of the current economic crisis with its plummeting health budgets, the massive influx of tropical migrants refugees and global climate change, makes this possibility more real at present than in the last decades of the 20th Century. To maintain an infectious disease, it is necessary for one infectious case to lead to one other infectious case, otherwise the disease will die out in the area. One would need sufficient gametocyte carriers and vectors to ensure the continuation of the disease.

Malaria is a very important public health problem in most tropical countries although the incidence rate of malaria declined globally between 2010 and 2018 from 71 to 57 cases per 1000 population at risk. In 2018, an estimated 228 million cases of malaria occurred worldwide, compared with 251 million cases in 2010. In that same year 405.000 people died of malaria mainly young children in Africa. Most lethal infections are due to Plasmodium falciparum. Six countries cause more than half of all malaria cases worldwide: Nigeria (25%),

DRC (12%), Ivory Coast, Mozambique and Niger (4% each). Of note, the decrease in incidence seems to stagnate these very last few years in sub-Saharan Africa.

P. falciparum is the most common form in sub-Saharan Africa (99,7% of malaria cases in this region),, tropical South America and Southeast Asia. The parasite occurred previously in the Mediterranean basin.

P. vivax has the widest distribution area (previously as far as London, Norway, Denmark, New York, southern Canada and even Siberia). In 1922 the number of cases in Texas was estimated at 500,000. It is the most common form in certain regions (e.g. Maghreb, Middle East countries, parts of China, Argentina). P. vivax preferentially penetrates young red blood cells (reticulocytes). In 1976 Miller discovered that P. vivax uses the Duffy blood group antigens (Fya and Fyb) as receptors to penetrate red blood cells. People who do not have this protein on their red blood cells cannot be infected with P. vivax. These antigens do not occur in the majority of humans in West Africa [phenotype Fy (a-b-)]. As a result, P. vivax does not occur in West Africa, or occurs in low numbers (and could be systematically missed). Duffy blood group negative erythrocytes are, in vitro, also resistant to infection with P. knowlesi (monkey malaria).

P. ovale is found chiefly West Africa, less elsewhere in Africa and sporadically in the Far East.

P. malariae is not very common but can be found anywhere. Often confused with P. knowlesi.

P. knowlesi is known from Malaysia (including Borneo), The Philippines, Vietnam, Thailand and recently Myanmar. The vector is present in India (Kerala) and Sri Lanka, but in these areas there is no known zoonotic reservoir. *P. knowlesi* infections are often confused with P. malariae.

Malaria can only persist naturally when climatic conditions are suitable for the vector(s) and for the development of the parasites in the vector. Increased rainfall and higher temperatures may make larger areas favourable for malaria transmission in the future. Tropical P. falciparum requires a minimum temperature of 18°C, while tropical P. vivax strains require a minimum of 16°C. The European strain of P. vivax which persisted in the high North was uniquely adapted, with summer temperatures being sufficiently high for P. vivax

development in the mosquito. Infection of patients occurred in autumn (September / October) when mosquitoes started to enter homes looking for shelter. The P. vivax parasites in humans had a very long incubation period of 6 to 9 months. A patient infected with these northern strains of P. vivax would remain asymptomatic during winter until the following spring. This clearly differs from tropical P. vivax dynamics. In Southern Europe P. falciparum used to be common in Portugal, Spain, Italy and Greece. It is likely that the strain of this parasite was genetically different from tropical strains.

However for malaria to become re-established, a sizable parasite reservoir (gametocyt carriers) must be present. This has not happened in other circumstances, such as after World War II, when great numbers of people (patients and gametocyt carriers) returned from tropical areas. In the current health system and socioeconomic situation in Europe, it is likely that patients will be treated early, diminishing the reservoir and lessening the threat of new epidemics. Small outbreaks and some local transmission might occur from time to time, but large reinvasion of the North European landmass is unlikely. South Europe would have a somewhat larger risk, as reflected by the outbreak of autochthonous *P. vivax* cases in Greece in 2012.

Epidemiological classification – stable versus unstable malaria

There is no completely satisfactory epidemiological classification of malaria. Stable malaria means that the clinical disease is characterized by preferentially affecting children and achieving a protective “immunity” in adults. Stability does not mean that there can be no variation in transmission. In some regions seasonal malaria occurs. In other areas there is unstable malaria: transmission differs greatly from year to year and occasionally epidemics occur. Then the disease also occurs in older persons. This is important in many respects; as irregular control of malaria may lead to changes in the immune status of the population. Sometimes malaria may appear again in a region after a long absence. For example: in 1972 the disease was eradicated in South Korea following an intensive eradication campaign with case detection and vector control. In 1993 one case of P. vivax was observed. There then followed 22 cases in 1994, in 1995 there were 107 cases, 356 in 1996 and more than 1600 in 1997. In 1995 all cases were still limited to the border area with North Korea, but in 1996 there was also transmission outside the demilitarized zone. After entomological surveys had

shown that *Anopheles sinensis* was the chief vector, measures were taken to control the disease.

Vector, *Anopheles* mosquitoes

Malaria is transmitted via the bite of infected female *Anopheles mosquitoes.*

Malaria is transmitted by *Anopheles* mosquitoes. This applies to the malaria of all mammals. Avian malaria is chiefly transmitted by Culicinae. There are some 400 *Anopheles* species, 40 of which are good vectors while 28 are poor vectors. *Anopheles* mosquitoes are active at night. They do not buzz much and are not easily noticed. The world's most important vector is *Anopheles gambiae,* an anthropophagic and endophilic freshwater mosquito which flourishes preferentially in moist regions. It typically breeds in exposed sunlit and often transient aquatic habitats such as pools, puddles, and irrigation channels. *Anopheles* mosquitoes are good flyers: they can cover several kilometres in one night. This is of course of great importance for their control. Endophagic (bite inside the house) mosquitoes will often rest on walls after a blood meal. Residual insecticides which are applied there will kill the vector.

How do mosquitoes find their prey?

Mosquitoes are attracted by an increasing CO_2 gradient. The warmth of the skin, lactic acid and moisture (breath) play a part over short distances. Every animal produces several volatile substances in its skin, breath, faeces and urine. A number of the substances (kairomones) are used by the mosquito to find its prey. The details are complex. *Anopheles gambiae* prefers to land on the feet, while *A. atroparvus* prefers to bite the face.

Vector control

Malaria vector control is primarily based on the use of insecticides. Appropriate monitoring of vector resistance to insecticides is an integral component of planning and evaluation of insecticide uses in malaria control programmes. Pyrethroids and DDT, two important insecticides used for vector control, block the nerve-impulse conduction by preventing a sodium channel from closing after an action potential. An important mechanism that confers resistance to pyrethroids and DDT, known as knockdown resistance or kdr, was first described in the housefly *Musca domestica*. It has been reported that a single mutation in the sodium channel sequence is the molecular basis of kdr in *Musca domestica*. The gene has also been characterized for *Anopheles gambiae*. PCR tests have been developed for the detection of the kdr-mutation in *A. gambiae*.

Physiopathology

The incubation period may be short (minimum 7-9 days for *P. falciparum*) to very long (several years for *P. ovale*). In falciparum malaria the parasitaemia can be very high: up to 80% of erythrocytes may contain parasites, but even 5% is enough to result in severe disease. These situations may be life-threatening. The other malaria parasites produce much lower parasitaemia (especially *P. ovale*). They do cause severe illness but are rarely life-threatening. *P. knowlesi* infections mimics severe P. malariae infections.

The rupture of the red blood cells (haemolysis) is accompanied by fever, muscle pain and general malaise. Massive haemolysis may cause kidney failure. Parasitized red blood cells are removed by the spleen. Splenomegaly will result. Anaemia occurs due to the destruction of

erythrocytes, suppression of the bone marrow and excess activity of the enlarged spleen (hypersplenism). In severe falciparum malaria, there is activation of blood coagulation system along with thrombocytopenia, even before widespread DIC and coagulation failure occur. In falciparum malaria there will often be a drop in glycaemia that can be corrected by administration of glucose.

The details of how cerebral malaria happens, are not clear at present, and various researchers have different opinions. More than 100 years ago, the Italian pathologists Bignami and Marchiafava reported on the sequestration of parasitized red blood cells in the brains of people who died of cerebral malaria. Erythrocytes which contain schizonts of *P. falciparum*, develop small knobs on their cell membranes. These consist, among other things, of a histidine-rich protein, *P. falciparum* erythrocyte membrane protein 1 and rifins. Rifins are clonally variant proteins encoded by rif genes ("repetitive interspersed family") and are expressed at late ring or early trophozoite stage on the infected red cell surface. Their high copy number, sequence variability, and red cell surface location indicate an important role in host-parasite interaction. The knobs have an overall negative charge, allowing non-specific attraction to positive endothelial ligands, but specific molecular adhesion also play a part. With these knobs the infected cells cling to the walls of the capillaries and to the vascular endothelium of the post-capillary venules in the brain. The low local O2 pressure and high CO2 pressure are optimal for further maturation of the parasite. Infected red blood cells are less easily distorted and more rigid than normal erythrocytes. This impedes the blood flow, which can lead to cerebral malaria. Other organs may also be affected for example the placenta and the intestines (resulting in abdominal pain and diarrhoea). Red blood cells which contain schizonts of *P. malariae*, also develop knobs on their membranes, but these cells do not adhere to the vascular endothelium. When post mortem cerebral sequestration was compared with the peripheral parasitaemia, there were about 26 times more infected red blood cells in the brain microvasculature than in the peripheral blood if there were free-mixing. More blood vessels in the cortex and cerebellum than in the brain stem are affected. Some researchers found more sequestration in white matter than in cortex. Coma requires sequestration, but sequestration itself is not enough to provoke cerebral malaria. The rapid reversible nature of cerebral malaria led to the hypothesis that soluble neuroactive mediators might play a role in the pathogenesis possibly involving reversible disturbances of the blood brain barrier and biochemical disruption of normal metabolism.

There are two groups of parasites in *P. falciparum* infections: (1) the young forms in the peripheral blood which can easily be observed in a thin blood smear and (2) the mature group which is attached to small blood vessels and which cannot be seen. Falciparum schizonts are rarely found in peripheral blood but these are important for the development of cerebral malaria. The whole mechanism of cerebral malaria has not to date been fully explained. As well as the attachment of parasitized red blood cells to the vessel walls (cytoadherence) other mechanisms possibly also play a part. Normal red blood cells sometimes attach to parasitized cells, which impairs the microcirculation. All kinds of released chemical substances (cytokines, oxygen radicals, etc.) may also play a part. Cytokines such as tumour necrosis factor (TNF-a) increase the expression of receptor molecules on the endothelium and will contribute to the cytoadherence and flow obstruction which characterize falciparum malaria. This mechanism is similar to the release of TNF-a by endotoxins in Gram-negative septicaemia.

Increased brain volume was seen in children who died from cerebral malaria but was uncommon in those who did not die from the disease; this suggests that raised intracranial pressure may contribute to a fatal outcome.

Carriers of the sickle cell anaemia gene (heterozygotes for haemoglobin S) have relative protection against severe infection with *P. falciparum* and thus have a survival advantage (in homozygous patients, malaria may be fatal and the disease itself tends to kill patients before the reproductive age). The same advantage probably applies to persons deficient in G6PD. This may explain why these two conditions are so common in Africa. In Papua New Guinea ovalocytosis is common. These red blood cells have an oval shape and cannot be penetrated by *P. falciparum* parasites. Heterozygotes are thus protected against *P. falciparum* (homozygosity is not compatible with life).

In West Africa, haemoglobin C is rather frequent. People with haemoglobin AC or CC can be infected with *Plasmodium falciparum* and can develop substantial parasitaemia. The presence of Hb C therefore does not protect against infection itself. Haemoglobin C might protect against the lethal effects of *P. falciparum* malaria by reducing cytoadherence of parasitized erythrocytes.

Haemoglobin E (chiefly Southeast Asia) does not protect against *P. falciparum* infections

itself.

While circulating in human blood *P. falciparum* exhibits antigenic variation. On the surface of the infected red blood cell a certain protein is expressed: *the P. falciparum* erythrocyte membrane protein 1 (PfEMP-1). The parasite can make many variants of this protein. By interchanging which variant of PfEMP-1 is present, the parasite can evade the immune response to these immune dominant antigens. These proteins are thought to be the major virulence factor found on the surface of infected red blood cells, directly contributing to the pathogenic nature of the infection and placing these genes at the centre of a disease responsible for several million deaths in developing countries. Although there are many var gene copies, only a single var gene is expressed at any given moment (i.e. there is mutually exclusive expression). Over the course of an infection, expression switches from one var gene to another, resulting in antigenic variation of the parasite population and a persistent infection which is difficult to clear by the human immune system.

Antigenic variation has important implications for the development of vaccines. The repertoire of proteins which are expressed in the *Anopheles* mosquito is far less pronounced probably because the vector has no adaptive immune system.

A large case-control study of malaria in West African children showed that a human leukocyte class I antigen (HLA-Bw53) and an HLA class II haplotype (DRB1*1302-DQB1*0501), common in West Africans but rare in other racial groups, are independently associated with protection from severe malaria. In this population they account for as great a reduction in disease incidence as sickle-cell trait. These data support the hypothesis that the extraordinary polymorphism of major histocompatibility complex genes as well as other genes has evolved primarily through natural selection by infectious pathogens.

Malaria is very often accompanied by thrombocytopenia, the causes of which seem to be multiple and not completely known. The severity of the thrombocytopenia correlates with the parasitaemia and the clinical severity of infection.