

## Chapter 15

### Malaria

Malaria has been and still is the cause of much human morbidity and mortality. Although the disease has been eradicated in many temperate zones, it continues to be endemic throughout much of the tropics and subtropics. Forty percent of the world's population lives in endemic areas. Epidemics have devastated large populations and malaria poses a serious barrier to economic progress in many developing countries. There are an estimated 300-500 million cases of clinical disease per year with 1.5-2.7 million deaths. Some of the earliest known medical writings from China, Assyria, and India accurately describe the malaria-like intermittent fevers. Hippocrates is generally credited with the first description of the clinical symptoms in 500 BC, more than 2000 years before the parasite and life cycle were described (Table 15.1).

**Table 15.1 Some historical highlights in the description of malaria and the parasite.**

<b>Year</b>	<b>Person</b>	<b>Described:</b>
500 BC	Hippocrates	clinical symptoms
1880	Laveran	blood stage parasite
1898	Ross	mosquito transmission
1948	Garnham	liver stage of life cycle

Malaria is caused by members of the genus *Plasmodium*. *Plasmodium* species are apicomplexa (see Chapter 11) and exhibit a heteroxenous life cycle involving a vertebrate host and an arthropod vector. Vertebrate hosts include: reptiles, birds, rodents, monkeys and humans. *Plasmodium* species are generally host and vector specific in that each species will only infect a limited range of hosts and vectors. Four distinct species infected humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. In addition, there have been some reports of humans naturally infected with the simian parasites *P. simiovale* and *P. knowlesi*. However, these cases appear to be relatively rare in comparison to the four human parasite species and in the case of *P. knowlesi* restrict to some parts of Malaysia. The four human parasite species differ in regards to their morphology, details of their life cycles, and their clinical manifestations.

#### Life Cycle

Human and other mammalian *Plasmodium* species are transmitted by anopheline mosquitoes. The parasite is injected with the saliva during mosquito feeding and first undergoes a round of merogony in the liver followed by multiple rounds of merogony in the erythrocytes (Figure 15.1). Gametogony begins within the erythrocytes of the vertebrate host and is completed within the mosquito where sporogony takes place. This life cycle exhibits the general features of other apicomplexan parasites characterized by

asexual replication and the formation of invasive stages with typical apical organelles (Chapter 11).

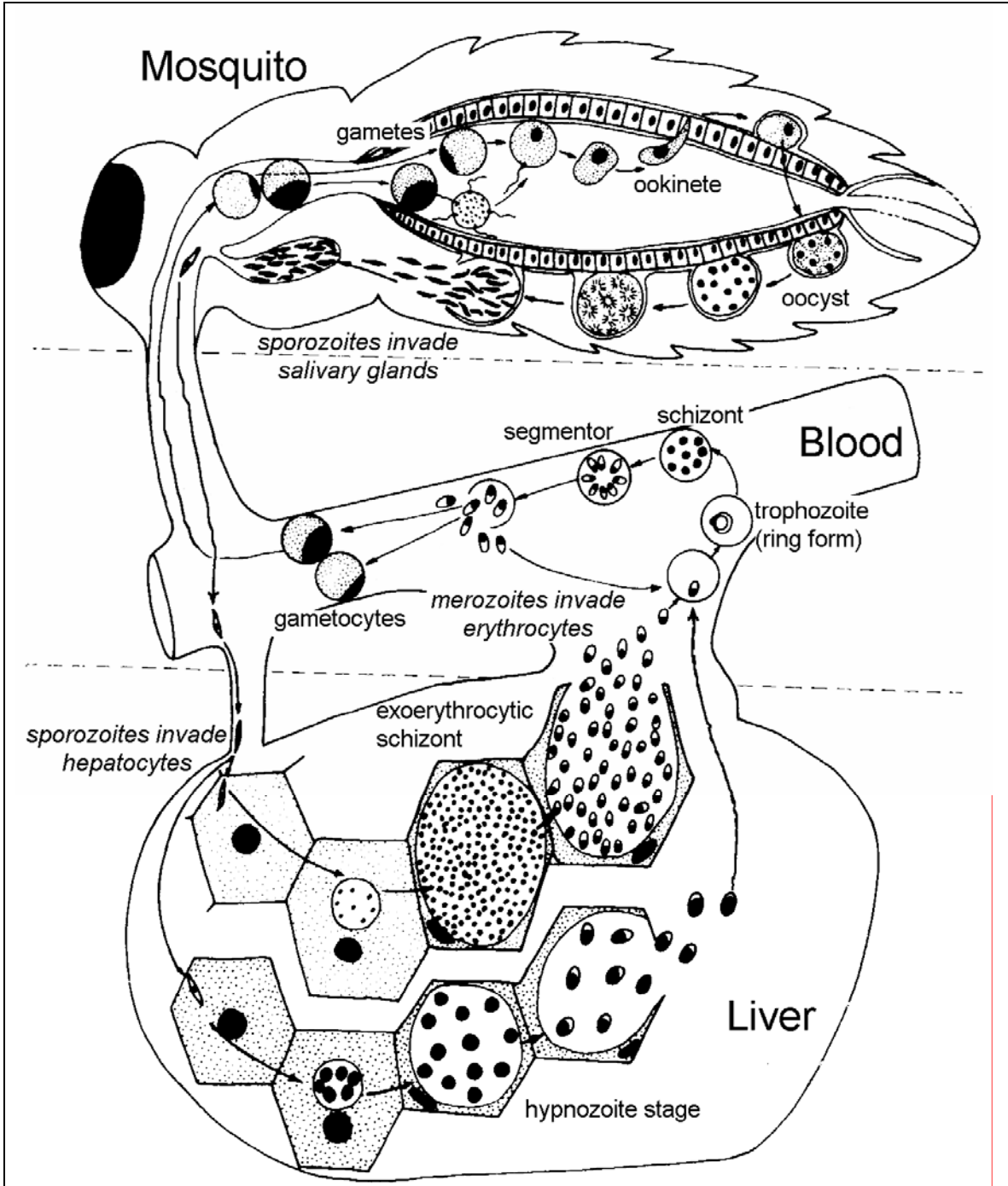


Figure 15.1. Life cycle of the malaria parasite. The infection in humans is acquired when sporozoites are injected with the saliva during mosquito feeding. The sporozoites invade liver cells and under an exoerythrocytic schizogony resulting in the production of merozoites. In *P. vivax* and *P. ovale* some of the exoerythrocytic schizonts undergo a dormant period known as the hypnozoite stage. Merozoites are released from the infected liver cells and invade erythrocytes and undergo an erythrocytic stage schizogony

producing more merozoites which can reinvade new erythrocytes. Alternatively, some of the merozoites will undergo gametocytogenesis and produce either macrogametocytes or microgametocytes. The gametocytes are infective for the mosquito and when ingested will produce macrogametes and microgametes. The zygote resulting from the fusion of the gametes develops into an ookinete which will penetrate the gut epithelial cells and develop into an oocyst and undergoes sporogony. The resulting sporozoites invade the salivary glands and are infective for the human host.

Liver Stage. The infection is initiated when sporozoites are injected with the saliva during mosquito feeding. The sporozoites enter the circulatory system and within 30-60 minutes will invade a liver cell. The sporozoites gain access to the hepatocytes by first invading and traversing a Kupffer cell. Host cell entry, as in all apicomplexa, is facilitated by the apical organelles (Chapter 11). After exiting the Kupffer cell, the sporozoite can traverse several hepatocytes before developing into an exoerythrocytic (or pre-erythrocytic) schizont. Schizogony refers to an asexual replicative process in which the parasite undergoes multiple rounds of nuclear division without cytoplasmic division followed by a budding, or segmentation, to form progeny called merozoites (Chapter 11). The merozoites are released into the circulatory system following rupture of the host hepatocytes. Recent observations suggest that the merozoites are released as a membrane-bound aggregate, called a meroosome, from the dying hepatocytes and the meroosomes are delivered to the blood stream. This presumably provides protection from phagocytosis of the free merozoites by the Kupffer cells of the liver.

In *P. vivax* and *P. ovale* some of the sporozoites do not immediately undergo asexual replication, but enter a dormant phase known as the hypnozoite. This hypnozoite can reactivate and undergo schizogony at a later time resulting in a relapse. Relapse has a specific meaning in regards to malaria and refers to the reactivation of the infection via hypnozoites. Recrudescence is used to describe the situation in which parasitemia falls below detectable levels and then later increases to a patent parasitemia. Interestingly, strains isolated from temperate regions tend to exhibit a longer latent period between the primary infection and the first relapse than strains from tropical regions with continuous transmission. This suggests that the hypnozoite stage provides a means for the parasite to survive during periods in which mosquitoes are not available for transmission.

Blood Stage. Merozoites released from the infected liver cells invade erythrocytes. The merozoites recognize specific proteins on the surface of the erythrocyte and actively invade the cell in a manner similar to other apicomplexan parasites. After entering the erythrocyte the parasite undergoes a trophic period followed by an asexual replication. The young trophozoite is often called a ring form due to its morphology in Giemsa-stained blood smears (Figure 15.2). As the parasite increases in size this 'ring' morphology disappears and it is called a trophozoite. During the trophic period the parasite ingests the host cell cytoplasm and breaks down the hemoglobin into amino acids. A by-product of the hemoglobin digestion is the malaria pigment, or hemozoin. (See Box 15.1) These golden-brown to black granules have been long recognized as a distinctive feature of blood-stage malaria parasites.

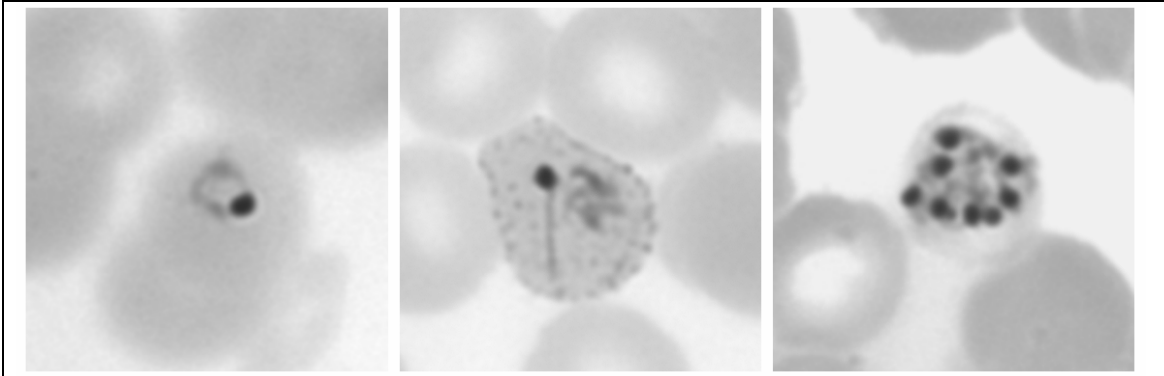


Figure 15.2. Blood stages of the malaria parasite. Ring stage of *P. falciparum* (left). Trophozoite of *P. vivax* (center). Note the stippling known as Schuffner's granules within the infected erythrocyte. Multinucleated schizont stage of *P. malariae* (right).

[Box 15.1]

Nuclear division marks the end of the trophozoite stage and the beginning of the schizont stage. Erythrocytic schizogony consists of 3-5 rounds (depending on species) of nuclear replication followed by a budding process. Late stage schizonts in which the individual merozoites become discernable are called segmenters. The host erythrocyte ruptures and releases the merozoites. These merozoites invade new erythrocytes and initiate another round of schizogony. The blood-stage parasites within a host usually undergo a synchronous schizogony. The simultaneous rupture of the infected erythrocytes and the concomitant release of antigens and waste products accounts for the intermittent fever paroxysms associated with malaria. Blood stage schizogony in *P. falciparum* differs from the other human malarial parasites in that trophozoite- and schizont-infected erythrocytes adhere to capillary endothelial cells and are not found in the peripheral circulation. This sequestration is associated with cerebral malaria and other complications.

Sexual Stage. As an alternative to schizogony some of the parasites will undergo a sexual cycle and differentiate into either micro- or macrogametocytes. The factors involved in the induction of gametocytogenesis are not known. However, commitment to the sexual stage occurs during the asexual erythrocytic cycle that immediately precedes gametocyte formations since the daughter merozoites from a particular schizont will develop into either all asexual forms or all sexual forms. Gametocytes do not cause pathology in the human host and will disappear from the circulation if not taken up by a mosquito.

Gametogenesis, or the formation of micro- and macrogametes, is induced when the gametocytes are ingested by a mosquito. After ingestion by the mosquito, the microgametocyte undergoes three rounds of nuclear replication. These eight nuclei then become associated with flagella that emerge from the body of the microgametocyte. This process is readily observable by light microscopy due to the thrashing flagella and is called exflagellation. The macrogametocytes mature into macrogametes. However, at the

morphological level this is much less dramatic than the exflagellation exhibited by the microgametocytes.

Exflagellation occurs spontaneously when infected blood is exposed to air. Critical factors involved in the induction of this gametogenesis are a decrease in temperature and a decrease in the dissolved carbon dioxide and the subsequent increase in pH to above 8.0 (Table 15.2). This somewhat mimics the environmental changes experienced by the gametocytes in that there will be a change to ambient temperature and the gut of the mosquito exhibits a pH of approximately 7.8 as compared to a pH of 7.4 for blood. In addition, a mosquito-derived exflagellation factor has also been identified as xanthurenic acid, a metabolite from insects. Xanthurenic acid lowers the permissive pH for exflagellation to below 8.0 and is probably a biological cue for the parasite to undergo gametogenesis.

**Table 15.2. Factors associated with exflagellation (i.e., gametogenesis).**

- occurs spontaneously after exposure to air
  - ↓ temperature (2-3°C)
  - ↓ pCO<sub>2</sub>
  - ↑ pH (8-8.3)
- mosquito-derived exflagellation factor lowers permissive pH
- MEF = xanthurenic acid

The highly mobile microgametes will seek out and fuse with macrogametes. Within 12-24 hours the resulting zygote develops into an ookinete. The ookinete is a motile and invasive stage which can transverse both the peritrophic membrane and the midgut epithelium of the mosquito. Transversing the peritrophic membrane probably involves secretion of chitinases. Penetration of the midgut epithelium involves invading and exiting several epithelial cells before emerging on the basal side of the epithelium. The invasion process is similar to other apicomplexa except that the ookinete does not have rhoptries and does not form a parasitophorous vacuole after invading the host cell.

Sporogony. After reaching the extracellular space between the epithelial cells and the basal lamina, the ookinete develops into an oocyst. Oocysts undergo an asexual replication, called sporogony, which culminates in the production of several thousand sporozoites. This generally takes 10-28 days depending on species and temperature. Upon maturation the oocyst ruptures and releases the sporozoites which cross the basal lamina into the hemocoel (body cavity) of the mosquito.

These sporozoites are motile and have an ability to specifically recognize the salivary glands. After finding the salivary glands the sporozoites will invade and transverse the salivary gland epithelial cells and come to lie within its lumen. These sporozoites will be expelled into the vertebrate host as the mosquito takes a blood meal, and thus reinitiate the infection in the vertebrate host. Although the hemocoel and salivary gland sporozoites are morphologically similar, they are functionally distinct. Salivary gland sporozoites efficiently invade liver cells, but cannot re-invade the salivary glands, whereas the hemocoel sporozoites are inefficient at invading liver cells.

In summary, the malaria parasite exhibits a life cycle with typical apicomplexan features (Chapter 11). There are three distinct invasive stages: sporozoite, merozoite and ookinete. All are characterized by apical organelles and can invade or pass through host

cells. Two distinct types of merogony are observed. The first, called exoerythrocytic schizogony, occurs in the liver and is initiated by the sporozoite. The resulting merozoites then invade erythrocytes and undergo repeated rounds of merogony called erythrocytic schizogony. Some of the merozoites produced from the erythrocytic schizogony will undergo gamogony. *Plasmodium* gamogony is described in two phases: gametocytogenesis occurring in the bloodstream of the vertebrate host, and gametogenesis taking place in the mosquito gut. The gametes fuse to become a zygote which first develops into an ookinete and then becomes an oocyst where sporogony takes place.

### Clinical Manifestations

The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stage parasites. Tissue schizonts and gametocytes cause little, if any, pathology. *Plasmodium* infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occurring at either 48 or 72 hour intervals. The severity of the attack depends on the *Plasmodium* species as well as other circumstances such as the state of immunity and the general health and nutritional status of the infected individual. Malaria also develops into a chronic disease which has a tendency to relapse or recrudesce over months or even years.

Symptoms of malaria usually start to appear 10-20 days after the bite of an infected mosquito. The prepatent and incubation periods vary according to species (Table 15.3). The prepatent period is defined as the time between sporozoite inoculation and the appearance of parasites in the blood and represents the duration of the liver stage and the number of merozoites produced. The incubation period tends to be a little longer and is defined as the time between sporozoite inoculation and the onset of symptoms. Symptoms will appear when the blood stage parasitemia reaches sufficient levels and is determined in part by the number of merozoites produced per exoerythrocytic schizont and the maturation time. Sometimes the incubation periods can be prolonged for several months in *P. vivax*, *P. ovale*, and *P. malariae*. Mechanical transmission of infected blood via blood transfusions or sharing syringes will result in a shorter incubation period since there will be no liver stage. There is also an increased risk of fatality with mechanically-transmitted *P. falciparum*. The lack of the liver stage infection as a result of mechanical transmission also precludes relapses in *P. vivax* or *P. ovale* infections.

**Table 15.3. Exoerythrocytic schizogony and prepatent and incubation periods.**

	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>
Prepatent period (days)	6-9	8-12	10-14	15-18
Incubation period (days)	7-14	12-17	16-18	18-40
Merozoite maturation (days)	5-7	6-8	9	12-16
Merozoites produced	40,000	10,000	15,000	2000

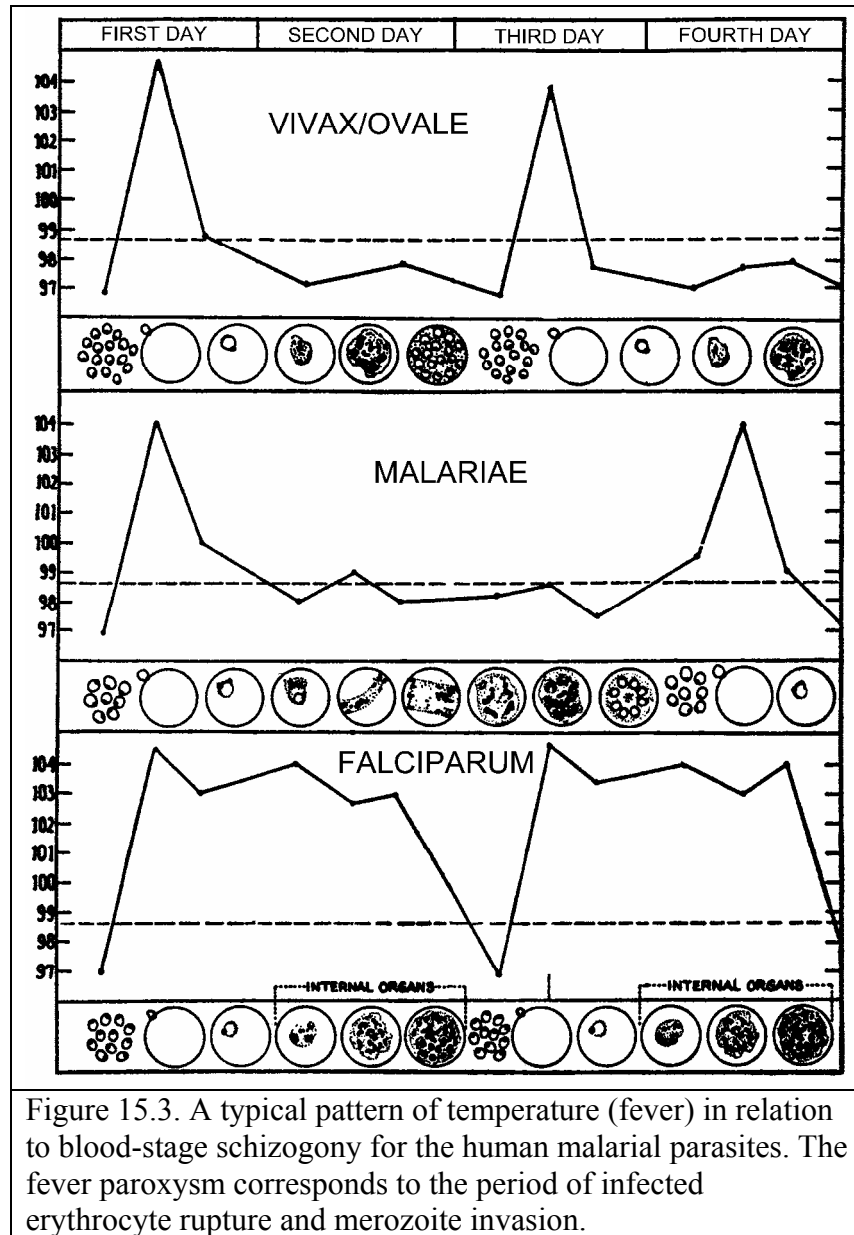
Malaria is characterized by febrile attacks. All four species can exhibit non-specific prodromal symptoms a few days before the first febrile attack. These prodromal symptoms are generally described as flu-like and include: headache, slight fever, muscle pain, anorexia, nausea and lassitude. The symptoms tend to correlate with increasing

numbers of parasites in the blood. These prodromal symptoms will be followed by febrile attacks also known as the malarial paroxysms. These paroxysms will exhibit periodicities of 48 hours for *P. vivax*, *P. ovale*, and *P. falciparum*, and a 72-hour periodicity for *P. malariae*. Initially the periodicity of these paroxysms may be irregular as the broods of merozoites from different exoerythrocytic schizonts synchronize. This is especially true in *P. falciparum* which may not exhibit distinct paroxysms, but exhibit a continuous fever, daily attacks or irregular attacks (eg., 36-48 hour periodicity). Patients may also exhibit splenomegaly, hepatomegaly, slight jaundice, and hemolytic anemia during the period in which the malaria paroxysms occur.

<b>Table 15.4. Malarial Paroxysm</b>		
<b>cold stage</b>	<b>hot stage</b>	<b>sweating stage</b>
<ul style="list-style-type: none"> <li>• feeling of intense cold</li> <li>• vigorous shivering</li> <li>• lasts 15-60 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• intense heat</li> <li>• dry burning skin</li> <li>• throbbing headache</li> <li>• lasts 2-6 hours</li> </ul>	<ul style="list-style-type: none"> <li>• profuse sweating</li> <li>• declining temperature</li> <li>• exhausted and weak → sleep</li> <li>• lasts 2-4 hours</li> </ul>

The malarial paroxysm (Table 15.4) will usually last 4-8 hours and begins with a sudden onset of chills in which the patient experiences an intense feeling of cold despite having an elevated temperature. This is often referred to as the cold stage, or rigor, and is characterized by a vigorous shivering. Immediately following this cold stage is the hot stage. The patient feels an intense heat accompanied by severe headache. Fatigue, dizziness, anorexia, myalgia, and nausea will often be associated with the hot stage. Next a period of profuse sweating will ensue and the fever will start to decline. The patient is exhausted and weak and will usually fall asleep. Upon awakening the patient usually feels well, other than being tired, and does not exhibit symptoms until the onset of the next paroxysm.

The periodicity of these paroxysms is due to the synchronous development of the malarial parasite within the human host. In other words, all of the parasites within a host are at approximately the same stage (i.e., ring, trophozoite, schizont) as they proceed through schizogony. The malarial paroxysm corresponds to the rupture of the infected erythrocytes and the release of merozoites (Figure 15.3). The 72 hour periodicity in *P. malariae* is due to its slower growth and maturation during blood-stage schizogony. Studies in *P. vivax* have demonstrated a correlation between fever and serum tumor necrosis factor-alpha (TNF- $\alpha$ ) levels (Figure 15.4). Presumably antigens or toxins are released when the infected erythrocyte ruptures and lead to the production of TNF- $\alpha$  and the febrile attacks. It has been suggested that this presumptive toxin might be glycosylphosphatidylinositol derived from parasite proteins.



Typically the acute infection characterized by the febrile illness and paroxysms is controlled and a chronic infection is established. The malarial paroxysms become less severe and irregular in periodicity as the host develops immunity and the infection becomes chronic characterized by lower parasitemias. Typically the chronic phase is characterized by intermittent episodes of fever associated with higher levels of parasitemia. This immunity, however, is not a sterilizing immunity in that the infection persists and individuals can exhibit relapses or recrudescences or become reinfected.

The species exhibit different ranges of symptoms. The severity of the paroxysms and duration of the symptoms varies according to species (Table 15.5). In general, the severity of the disease correlates with the average and maximum parasitemia exhibited by the various species. *P. falciparum* is capable of producing a severe and lethal infection, whereas the other species are rarely mortal. Patients infected with *P. vivax*, especially for the first time, can be quite ill. However, *P. vivax* rarely causes complications or results in death. Relapses due to the activation of *P. vivax* hypnozoites can occur for several years. *P. ovale* is the most benign in that the paroxysms tend to be mild and of short duration and relapses seldom occur more than one year after the initial infection. *P. malariae* generally produces a mild disease, but the initial paroxysms can be moderate to severe. It is the most chronic, though, and recrudescences have been documented several decades after the initial infection. This chronicity is sometimes associated with renal complications, which are probably due to the deposition of antigen-antibody complexes in the glomeruli of the kidney.

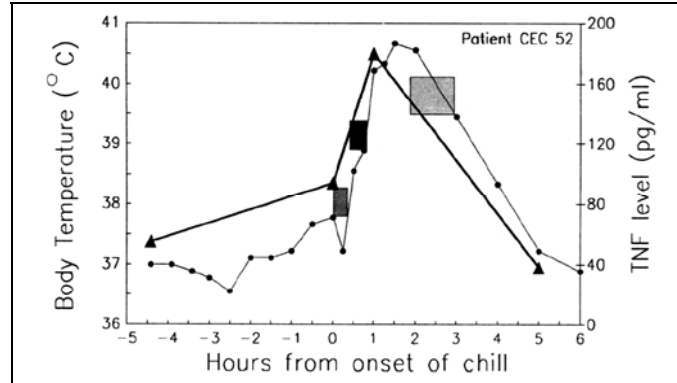


Figure 15.4. Correlation between fever and TNF- $\alpha$  levels during *P. vivax* infection. Body temperature (circles) and serum TNF- $\alpha$  levels (triangles) were measured during the malarial paroxysm. The hatched box denotes the period of intense shivering, the black box the hot stage and the gray box denotes profuse sweating. Reprinted with permission from Karunaweera et al (1992) Proc. Natl. Acad. Sci. 89:3200.

**Table 15.5. Disease Severity and Duration**

	<b>vivax</b>	<b>ovale</b>	<b>malariae</b>	<b>falciparum</b>
Severity of Initial Paroxysms	moderate to severe	mild	mild to moderate	severe
Average Parasitemia (per mm <sup>3</sup> )	20,000	9,000	6,000	50,000-500,000
Maximum Parasitemia (per mm <sup>3</sup> )	50,000	30,000	20,000	2,500,000
Symptom Duration (untreated)	3-8+ weeks	2-3 weeks	3-24 weeks	2-3 weeks
Maximum Infection Duration (untreated)	5-8 years*	12-20 months*	20-50+ years	6-17 months
Anemia	++	+	++	++++
Other Complications			renal	cerebral

\*Includes relapses from the hypnozoite stage.

In contrast to the other three species, *P. falciparum* can produce serious disease with mortal consequences. This increased morbidity and mortality is due in large part to the high parasitemias associated with *P. falciparum* infections. These potentially high parasitemias are due in part to the large number of merozoites produced and the ability of *P. falciparum* to invade all erythrocytes. In contrast, *P. vivax* and *P. ovale* prefer reticulocytes (i.e., immature erythrocytes), whereas *P. malariae* prefers senescent erythrocytes. The parasitemia can also rapidly increase due to the cytoadherence and sequestration (see below) of *P. falciparum*. This sequestration in the tissues also minimizes removal of infected erythrocytes by the spleen and can be viewed as an avoidance of the immune system. In addition, the cytoadherence to the endothelial cells of the capillaries allows for a more efficient erythrocyte invasion since the blood flow is much slower. The low oxygen tensions found in the deep tissues is also metabolically favorable for parasite growth.

**Table 15.6. Indicators of severe malaria and poor prognosis.**

<b>Manifestation</b>	<b>Features</b>
cerebral malaria	unrousable coma not attributable to any other cause
severe anemia	hematocrit <15% or hemoglobin <50 g/l in the presence of parasite count >10 000/ $\mu$ l
respiratory distress	defined by labored breathing and pulmonary edema that can progress to an acute respiratory distress syndrome
renal failure	low urine output and high serum creatinine despite adequate volume repletion
circulatory collapse (shock)	systolic blood pressure <70 mm hg in patients with cold clammy skin
acidemia/acidosis	arterial pH <7.25 or plasma bicarbonate <15 mmol/l
hypoglycemia	whole blood glucose concentration <2.2 mmol/l (<40 mg/dl)
impaired consciousness	impaired consciousness less marked than unrousable coma, can localize a painful stimulus
repeated generalized convulsions	$\geq 3$ convulsions observed within 24 hours
prostration or weakness	patient unable to sit or walk, with no other obvious neurological explanation
abnormal bleeding and/or coagulation	spontaneous bleeding from gums, nose, gastrointestinal tract, or laboratory evidence of disseminated intravascular coagulation
malarial hemoglobinuria	need to exclude hemoglobinuria due to antimalarial medications and to G6PD deficiency
jaundice	bilirubin >43 $\mu$ mol/l (> 2.5 mg/dl)
hyperparasitemia	>5% parasitized erythrocytes or >250 000 parasites/ $\mu$ l (interpreted in light of immune status and prior exposure)
hyperpyrexia	core body temperature >40°C

Severe falciparum malaria. Approximately 10% of falciparum malaria cases will develop into complicated or severe disease with a mortality of 10-50%. The high parasitemia and sequestration can result in various complications and severe malaria encompasses a complex syndrome affecting many organs resulting in biochemical and hematological abnormalities (Table 15.6). Patients will often exhibit several of these manifestations either simultaneously or sequentially. Any of these complications can develop rapidly and progress to death within hours or days. The three most common syndromes associated with severe malaria and most often correlated with death are: cerebral malaria, severe anemia, and respiratory distress.

Cerebral malaria is characterized by an impaired consciousness and other neurological symptoms (Table 15.7). Patients typically present with fever for several days followed by a loss of consciousness. The onset of cerebral malaria can be gradual or rapid. The presenting symptoms are usually severe headache followed by drowsiness, confusion, and ultimately an unrousable coma. Convulsions, vomiting, and respiratory distress are also frequently associated with cerebral malaria, especially in children. The neurological manifestations are believed to be due to the sequestration of the infected erythrocytes in the cerebral microvasculature.

**Table 15.7. Cerebral Malaria**

- complication of severe falciparum malaria
- a diffuse encephalopathy with loss of consciousness
- consciousness ranges from stupor to coma
- onset can be gradual or rapid
- unresponsive to pain, visual, and verbal stimuli
- associated with sequestration in cerebral microvasculature

The severe anemia is due in part to the destruction of erythrocytes during blood-stage schizogony. In addition, non-infected erythrocytes are destroyed at higher rates due to complement-mediated lysis and phagocytosis resulting from immune complex deposition and complement activation. Furthermore, there is a decreased production of erythrocytes during infection. Severe anemia is more common in children from highly endemic areas due to repeated or chronic infections.

More recently it has been recognized that metabolic acidosis as manifested by respiratory distress has emerged as a central feature of severe malaria and is a better predictor of death than cerebral malaria or severe anemia. The first signs of lung injury are rapid and difficult breathing and are indicative of pulmonary edema. This pulmonary edema can progress to an acute respiratory distress syndrome and even respiratory failure.

### **Pathogenesis**

Pathology associated with all malarial species is related to the rupture of infected erythrocytes and the release of parasite material and metabolites, hemozoin (i.e., malaria pigment) and cellular debris. In addition, there is an increased activity of the reticuloendothelial system as evidenced by macrophages with ingested infected and normal erythrocytes and hemozoin. In particular the liver and spleen are often enlarged during malaria. Except for *P. falciparum*, the pathology associated with malaria tends to

be benign with little mortality. As discussed above, proinflammatory cytokines, and especially TNF- $\alpha$ , are believed to play a role in the disease manifestations. Higher levels of TNF- $\alpha$  and other proinflammatory cytokines are also associated with severe anemia, cerebral malaria, and respiratory distress. However, it is not clear the precise role proinflammatory and anti-inflammatory immune responses play in the resolution of the disease and its pathogenesis.

Cytoadherence and sequestration are also believed to contribute to the pathology of severe falciparum malaria. Sequestration refers to the cytoadherence of trophozoite- and schizont-infected erythrocytes to endothelial cells of deep vascular beds in vital organs, especially brain, lung, gut, heart and placenta. As discussed above, this sequestration provides several advantages for the parasite and contributes to the high parasitemias. In addition, this cytoadherence and sequestration can have pathological effects on the specific organs, most notably the brain.

Receptor-ligand interactions between protein ligands expressed on the surface of trophozoite and schizont-infected erythrocytes and various receptors found on endothelial cells mediate cytoadherence (see next section). The parasite ligands are focused in electron-dense protuberances, or 'knobs', on the surface of the infected erythrocyte. Among human *Plasmodium* species, knobs are restricted to *P. falciparum* and thus suggest that the knobs play a role in cytoadherence. In addition, there is also a good correlation between animal *Plasmodium* species which express knobs and exhibit sequestration. Electron microscopy also shows that the knobs are contact points between the infected erythrocyte and the endothelial cell.

Pathophysiology of cerebral malaria. Early observations of the pathology of cerebral malaria suggested a relationship between large numbers of infected erythrocytes in the microvasculature and the development of the syndrome (Figure 15.5). Initially it was assumed that the cytoadherence would lead to a mechanical blockage (i.e., cerebral ischemia) and subsequently hypoxia. In addition, the parasite exhibits a high level of glycolysis which could cause localized metabolic effects such as hypoglycemia and lactic acidosis. The hypoxia and metabolic effects would then lead to coma and subsequent death.

However, long-term neurological sequelae among survivors of cerebral malaria are lower than would be expected for ischemia. In addition, the coma associated with cerebral malaria is rapidly reversible upon treatment and the phenomenon of sequestration occurs in all *P. falciparum* infections and is not limited to cerebral malaria cases. Thus, other factors likely contribute to the development of cerebral malaria. For example, cytokines, such as TNF- $\alpha$ , or short-lived intermediates, such as nitric oxide, are speculated to be responsible for the pathology. In this cytokine theory, malarial antigens stimulate the production of TNF- $\alpha$  and other cytokines which could then induce nitric oxide or have other pathological effects. Nitric oxide is known to affect neuronal function and, it could also lead to intracranial hypertension through its vasodilator activity. It is unlikely, though, that the systemic release of cytokines would cause coma and there is minimal lymphocyte infiltration or inflammation associated with the blocked capillaries (Figure 15.5). Thus questions have been raised in regards to whether these mediators

would reach sufficiently high local concentrations in the brain. At this time the exact mechanisms leading to the development of cerebral malaria are not known.

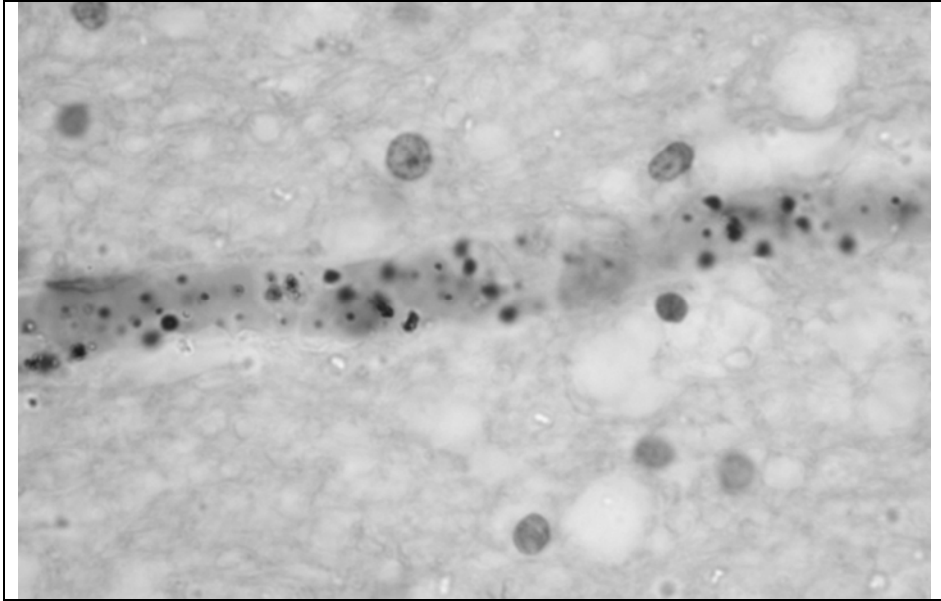


Figure 15.5. Brain section from autopsy of cerebral malaria patient. A capillary is filled with infected erythrocytes which are adhered to the brain endothelial cells with relatively little inflammation.

The sequestration hypothesis and cytokine theory for the pathophysiology of cerebral malaria are not mutually exclusive, and both phenomenon are likely to be involved. For example, parasite exo-antigens, which are released at erythrocyte rupture, are known to stimulate macrophages to secrete TNF- $\alpha$ . TNF- $\alpha$  up regulates the expression of adhesion molecules such as ICAM1 on the surface of brain endothelial cells. This would lead to increase binding of infected erythrocytes and amplify the effects whether they are due to vascular blockage, soluble mediators, metabolic effects, or a combination (Figure 15.6). TNF- $\alpha$  and other cytokines also stimulate the production of nitric oxide. Nitric oxide is known to affect neuronal function and it could also lead to intracranial hypertension through its vasodilator activity. The pathophysiology of cerebral malaria is not completely understood, but likely involves multiple factors and complex interactions between the host and parasite.

Rosetting. Rosetting is another adhesive phenomenon exhibited by *P. falciparum*-infected erythrocytes which may play a role in pathogenesis. Some infected erythrocytes bind to multiple uninfected erythrocytes resulting in a large clump of erythrocytes, or a rosette. Some studies have shown an association between the rosetting phenotype and severe malaria. These clumps could restrict microvascular flow in a similar fashion as cytoadherence to endothelial cells and thus contribute to the pathology.

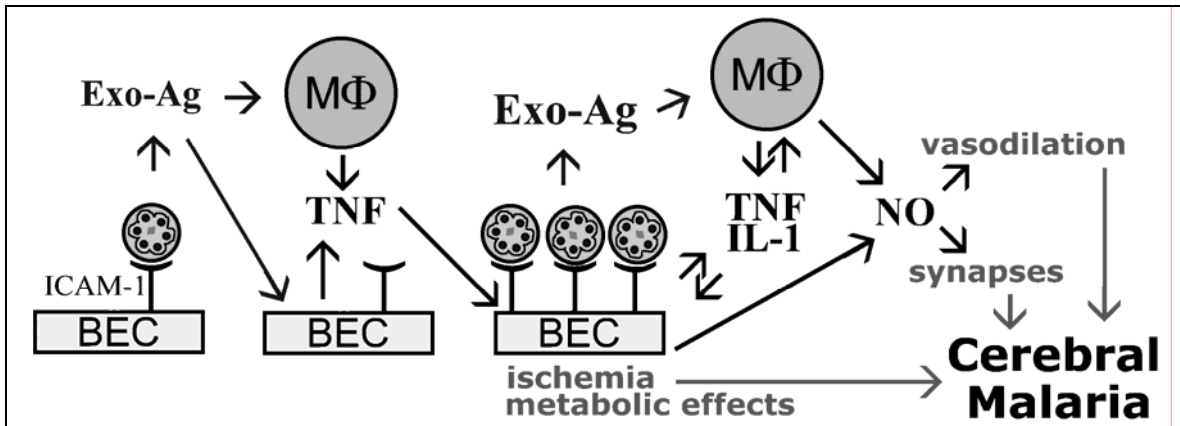


Figure 15.6. A schematic model depicting some possible mediators of cerebral malaria. The cytoadherence of infected erythrocytes to brain endothelial cells (BEC) and the release of exo-antigens could stimulate the BEC and immune effector cells such as macrophages (MΦ) to secrete cytokines. These cytokines, such as tumor necrosis factor- $\alpha$  (TNF), would lead to an increased expression of possible endothelial cell receptors (eg., ICAM-1) and promote an increase cytoadherence of infected erythrocytes. Large numbers of bound infected erythrocytes could lead to vascular blockage and hypoxia and have localized metabolic effects (eg., hypoglycemia, lactic acidosis). TNF- $\alpha$  is also known to stimulate nitric oxide (NO) which can affect neuronal function by interfering with neurotransmission and causing vasodilation.

### Modification of the Host Erythrocyte by the Malarial Parasite

Another aspect of the pathophysiology of malaria is the alteration of the host erythrocyte by the parasite. During the trophic period the parasite induces many changes in the erythrocyte which affect its structure and function. For example, the malarial parasite is a rapidly growing organism that exhibits a high metabolic rate and has a large demand for small molecular metabolites that will serve as precursors for the synthesis of nucleic acids, proteins and lipids. Thus, the host erythrocyte with its comparatively low level of metabolism and limited transport capabilities poses some potential problems for the actively growing parasite. The infected erythrocyte exhibits a substantial increase in its permeability to low molecular weight solutes as compared to the uninfected erythrocyte. Much of this increase in permeability can be attributed to a new permeation pathway induced by the parasite with characteristics quite distinct from the transporters of the host erythrocyte.

Ultrastructural alterations of the host erythrocyte have also been noted. For example, caveola-vesicle complexes are found on the surface of *P. vivax*-infected erythrocytes and electron-dense protrusions, or 'knobs', are found on the surface of *P. falciparum*-infected erythrocytes. [figure?] The function of the caveola-vesicle complexes is not known, but they are responsible for the Schüffner's granules seen in stained thin blood smears. In addition, membrane bound compartments such as Maurer's clefts are found within the cytoplasm of the infected erythrocyte. The knobs of *P. falciparum* play an important role in the cytoadherence and sequestration of the infected

erythrocyte. This sequestration allows the parasite to avoid the spleen and contributes to the survival of the parasite as well as plays a major role in the morbidity and mortality associated with falciparum malaria.

Parasite proteins are associated with knobs. The knobs are induced by the parasite and several parasite proteins are associated with the knobs. Two proteins which probably participate in knob formation are the knob-associated histidine rich protein (KAHRP) and erythrocyte membrane protein-2 (PfEMP2). Neither KAHRP nor PfEMP2 are exposed on the outer surface of the erythrocyte, but are localized to the cytoplasmic face of the host membrane (Figure 15.7). Their exact roles in knob formation are not known, but may involve reorganizing the submembrane cytoskeleton. A polymorphic protein, called PfEMP1, has also been localized to the knobs and is exposed on the host erythrocyte surface. PfEMP1 probably functions as a ligand which binds to receptors on host endothelial cells.

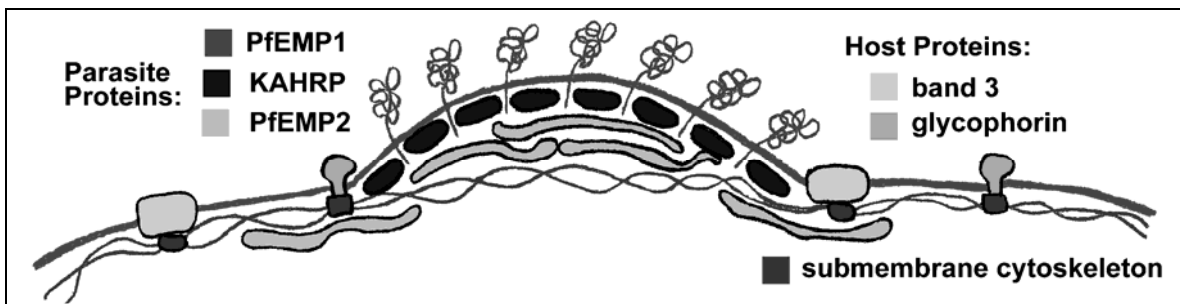
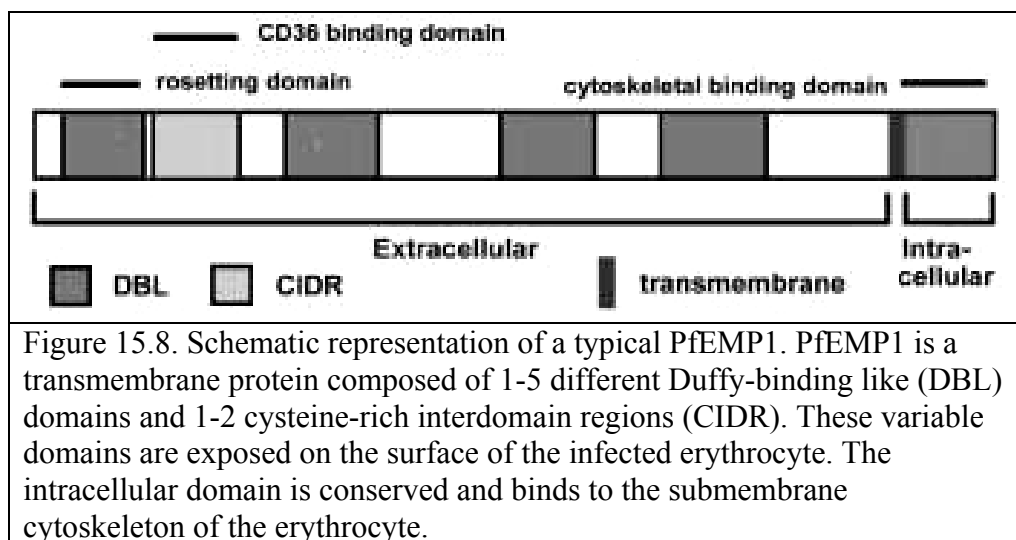


Figure 15.7. Schematic representation of knob structure. Parasite proteins are associated with the knobs found on the surface of infected erythrocytes. These proteins probably cause a reorganization of the submembrane cytoskeleton of the erythrocyte and are important in the cytoadherence of the infected erythrocyte to host endothelial cells.

PfEMP1 is a member of the *var* gene family. The 40-50 *var* genes exhibit a high degree of variability, but have a similar overall structure (Figure 15.8). PfEMP1 has a large extracellular N-terminal domain, a transmembrane region and a C-terminal intracellular domain. The C-terminal region is conserved between members of the *var* family and is believed to anchor PfEMP1 to the erythrocyte submembrane cytoskeleton. In particular, this acidic C-terminal domain may interact with the basic KAHRP of the knob as well as spectrin and actin of the submembrane cytoskeleton. The extracellular domain is characterized by 1-5 copies of Duffy-binding like (DBL) domains. These DBL domains are similar to the receptor-binding region of the ligands involved in merozoite invasion (see Chapter 11). The DBL domains exhibit a conserved spacing of cysteine and hydrophobic residues, but otherwise show little homology. Phylogenetic analysis indicates that there are five distinct classes (designated as  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and  $\epsilon$ ) of DBL domains. The first DBL is usually a DBL $\alpha$  type and this is followed by a cysteine-rich interdomain region (CIDR). A variable number and types of DBL domains in various orders make up the rest of the extracellular domain of PfEMP-1.



The various members of the *var* gene family bind to different host receptors. Several possible endothelial receptors (Table 15.8) have been identified by testing the ability of infected erythrocytes to bind in static adherence assays. A wide range of proteins, proteoglycans and complex carbohydrates can serve as receptors. Many of the receptors have roles in adhesion of cells to the extracellular matrix or to other cells. For some of the receptors the binding has been mapped to particular domains of PfEMP1. For example, CD36, an 88 kDa integral membrane protein found on monocytes, platelets and endothelial cells binds to the CIDR domain (Figure 15.8). Similarly, the different classes of DBL domains bind to different endothelial cell receptors. For example, DBL $\alpha$  binds to the receptors associated with rosetting; DBL $\beta$  binds to intracellular adhesion molecule-1 (ICAM1); and DBL $\gamma$  binds to chondroitin sulfate. Thus, the different PfEMP1 molecules will exhibit different phenotypes in regards to receptor binding activity depending upon their domain structure and organization.

**Table 15.8. Possible Cytoadherence Receptors Identified by In Vitro Binding Assays.**

Host Receptor	PfEMP1 Domain	Comments
CD36	CIDR	
intracellular adhesion molecule-1 (ICAM1)	DBL $\beta$	Implicated in cerebral malaria
chondroitin sulfate A	DBL $\gamma$	Receptor in the placenta
heparan sulfate		
hyaluronic acid		
E-selectin		
thrombospondin		
complement receptor-1	DBL $\alpha$	Rosetting receptor
blood group A antigen	DBL $\alpha$	Rosetting receptor
glycosaminoglycan	DBL $\alpha$	Rosetting receptor

Different parasite isolates exhibit different phenotypes in regards to which receptors are recognized due to the particular *var* gene being expressed. For example, most parasite isolates bind to CD36 which is likely due to the CIDR domain being relatively conserved and present on all PfEMP1 molecules. However, CD36 has not been detected on endothelial cells of the cerebral blood vessels and parasites from clinical isolates tend to adhere to both CD36 and ICAM1. ICAM1 is a member of the immunoglobulin superfamily and functions in cell-cell adhesion. Binding of infected erythrocytes to ICAM1 is speculated to play a role in the development of cerebral malaria (see above). Similarly, chondroitin sulfate A has been implicated in the cytoadherence within the placenta and may contribute to the adverse affects of *P. falciparum* during pregnancy.

Antigenic variation. Although sequestration offers many advantages to the parasite, the expression of antigens on the surface of the infected erythrocyte provides a target for the host immune system. The parasite counters the host immune response by expressing antigenically distinct PfEMP1 molecules on the erythrocyte surface, thus allowing the parasite to evade the immune system. Normally, though major changes in the sequence of a protein will result in structural changes that will affect the function of the protein--especially in the case of receptor-ligand interactions which are generally considered highly specific. Several studies have indicated that the expression of different PfEMP1 genes is correlated with different receptor-binding phenotypes. Thus, the cytoadherence function is preserved through its ability to recognize multiple receptors (Figure 15.9). This allows the parasite to avoid clearance by the host immune system, but yet maintain the cytoadherent phenotype.

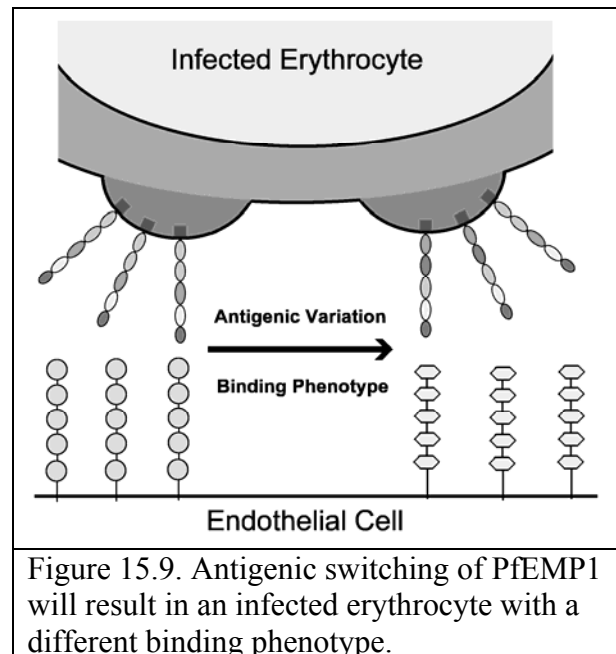


Figure 15.9. Antigenic switching of PfEMP1 will result in an infected erythrocyte with a different binding phenotype.

This antigenic switching may occur as frequently as 2% per generation in the absence of immune pressure. The exact molecular mechanism of antigenic switching is not known. Experimental evidence indicates that the mechanism is not associated with gene rearrangements into specific expression-linked sites as found in African trypanosomes (Chapter 8). However, as in the case of African trypanosomes, only a single *var* gene is expressed at a time (i.e., allelic exclusion). The non-expressed genes are kept silent by proteins which bind to the promoter region. A gene can become activated by repositioning to a particular location in the nucleus and is associated with chromatin modification. This expression spot can only accommodate a single active gene promoter. Thus the *var* promoter is sufficient for both the silencing and the mono-allelic transcription of a PfEMP1 allele.

The expression of a particular PfEMP1 will result in a parasite with a distinct cytoadherent phenotype and this may also affect pathogenesis and disease outcome. For example, binding to ICAM1 is usually implicated in cerebral pathology. Therefore, parasites expressing a PfEMP1 which binds to ICAM1 may be more likely to cause cerebral malaria. In fact, higher levels of transcription of particular *var* genes are found in cases of severe malaria as compared to uncomplicated malaria. Similarly, a higher proportion of isolates which bind to chondroitin sulfate are obtained from the placenta as compared to the peripheral circulation of either pregnant women or children. Furthermore, placental malaria is frequently associated with higher levels of transcription of a particular *var* gene which binds chondroitin sulfate. This phenomenon is not restricted to the placenta in that there is a dominant expression of particular *var* genes in the various tissues (Figure 15.10). This tissue specific expression of particular *var* genes implies that different tissues are selecting out different parasite populations based on the particular PfEMP1 being expressed on the surface of the infected erythrocyte.

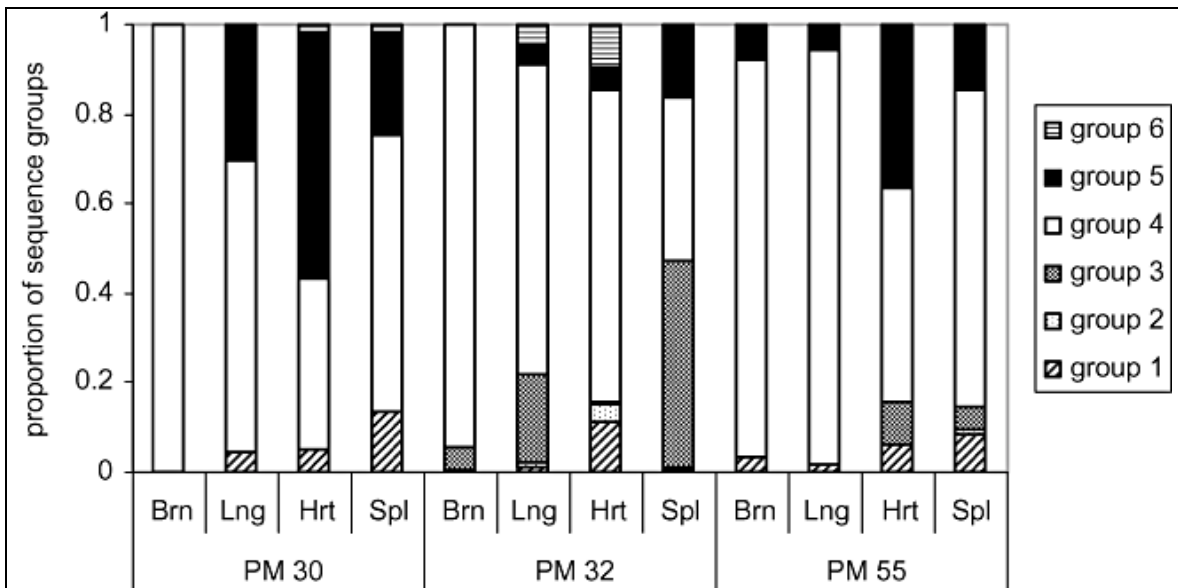


Figure 15.10. Frequency of PfEMP1 types in organs of malaria patients. The organs of patients dying from severe malaria were analyzed for transcripts of *var* genes. The various types of PfEMP1 were grouped according to their sequences at positions of limited variability. Different types of PfEMP1 predominated in the various organs. From Montgomery et al (2007) Differential *var* gene expression in the organs of patients dying of falciparum malaria. Molecular Microbiology 65, 959-967. Printed with permission from Jacqui Montgomery of the College of Medicine, Blantyre, Malawi and Wiley-Blackwell Publishing.

## Immunity

Persons living in endemic areas do develop immunity against malaria. Almost always a person will exhibit symptoms during their initial exposures to malaria. Symptoms associated with subsequent exposures to malaria are usually less severe, though. The immunity against malaria is slow to develop and requires multiple

exposures. In highly endemic areas only young children are at a high risk of developing severe falciparum malaria whereas older children and adults are essentially protected from severe disease and death. However, this immunity is not a sterilizing immunity in that persons can still become infected. In addition the immunity is short lived and in the absence of repeated exposure the level of immunity decreases. For example, previously semi-immune adults will often develop severe malaria upon returning to an endemic area after being in a non-endemic area for 1-2 years. This state of partial immunity in which parasitemia is lowered, but not eliminated, and parasitemia is better tolerated is sometimes referred to as premunition. Premunition refers to an immunity that is contingent upon the pathogen being present.

Immunity against the blood stage parasite. The immune response could be directed at either the pre-erythrocytic or erythrocytic stages of the parasite's life cycle. However, the erythrocytic stage of the life cycle is probably the most important in terms of clearing the parasite and lessening the disease. Due to the lack of HLA molecules on the surface of the parasite or the erythrocyte it is usually assumed that antibody will play a key role in blood-stage immunity. Possible effector mechanisms for antibody include: blocking erythrocyte invasion by merozoites, antibody-dependent cellular killing mediated by cytophilic antibodies, or increased clearance of infected erythrocytes due to binding of antibodies to parasite antigens exposed on the erythrocyte surface. All of these will result in lower parasitemia. The relative importance of these various mechanisms is not clear and probably immunity probably requires the generation of antibodies against numerous targets. This, along with antigenic variation and polymorphisms in many *Plasmodium* antigens, could explain the slow development of immunity.

The observation that asymptomatic individuals can exhibit high levels of parasitemia has led to the concept of 'anti-disease immunity'. This would be in addition to the 'anti-parasite' immunity discussed above which results in lower parasitemias. Severe malaria and death are correlated with TNF- $\alpha$  and other proinflammatory cytokines. As discussed for the paroxysms and cerebral malaria, antigens or toxins released by the infected erythrocyte could stimulate the production of proinflammatory cytokines. Antibodies against these exo-antigens could possibly neutralize their toxic effects and thus lead to an anti-disease immunity. People from endemic areas develop antibodies specific for the parasite glycosylphosphatidylinositol, a proposed parasite toxin, and some studies show a correlation between these antibodies and improved clinical outcomes.

Vaccine development. Because of the difficulties in controlling malaria by other means there is much interest in developing a vaccine against malaria. Currently there is no available vaccine, but there is a substantial research effort directed at identifying vaccine candidates and testing potential vaccines for safety and efficacy. The complex life cycle and biology of the parasite provide several potential targets (Table 15.9). For example, vaccination against the sporozoite or exoerythrocytic stage could prevent infection. However, the induced immunity would need to be highly effective since the escape of a single sporozoite would lead to a blood-stage infection and disease. Vaccines targeted against merozoites or the infected erythrocyte would lower parasitemia by interfering with merozoite invasion or increasing the elimination of infected erythrocytes. Such a vaccine could potentially alleviate much of the pathogenesis associated with

malaria even if it were not complete effective. In addition, infection may serve to boost the immune response. It may be possible to vaccinate against the disease by immunizing against potentially toxic antigens. Antibodies neutralizing antigens or toxins that stimulate a proinflammatory immune response may lessen some of the pathogenesis associated with malaria. Sexual stages of the parasite such as gametocytes and gametes could also be targeted. Antibodies directed against gamete antigens can prevent infection of the mosquito and sporogony. Such a vaccine would be altruistic in that it would not protect the individual against disease, but protect others in the community by lowering the transmission.

**Table 15.9. Potential Vaccine Strategies**

Target	Protection	Mechanism
sporozoite	anti-infection	prevent or eliminate liver stage
merozoite	anti-parasite	decrease efficiency of merozoite invasion
infected erythrocyte	anti-parasite	increase clearance of infected erythrocytes
exoantigens	anti-disease	lower production of inflammatory cytokines
sexual stages	anti-transmission	eliminate gametes or prevent infection of mosquitoes

## Epidemiology and Transmission

Malaria is primarily a disease of the tropics and subtropics and is widespread in hot humid regions of Africa, Asia and South and Central America. Currently malaria is endemic in more than 100 countries and 40% of the world's population lives in areas at risk for infection. The disease was also previously common in many temperate areas including the United States (see Box 15.2) and northern Europe, which are now virtually free of autochthonous transmission. However, many areas which previously had malaria under control are now experiencing resurgences.

[Box 15.2]

The four human malarial species exhibit an overlapping geographical distribution. *P. vivax* and *P. falciparum* are the most commonly encountered species and both are found throughout tropical and subtropical areas. In addition, to being found in tropical and subtropical areas, *P. vivax* is endemic in some temperate areas. *P. falciparum* is the predominant species in sub-Saharan Africa, whereas *P. vivax* is less common in Africa. *P. malariae* exhibits a similar distribution as *P. falciparum*, but is not as extensive and exhibits a more spotty distribution. *P. ovale* also exhibits a patchy distribution and is most prominent in western Africa. Mixed infections are common in many endemic areas.

Stable versus unstable malaria. The epidemiology of malaria can be viewed in terms of being stable (or endemic) or unstable (or epidemic). Stable malaria refers to a situation in which there is a measurable incidence of natural transmission over several years and this incidence remains somewhat constant. This would also include areas which experience seasonal transmission. Different areas can experience different levels of

incidence rates and this is often denoted by: hypoendemic, mesoendemic, hyperendemic, and holoendemic (Table 15.10). Persons living in highly endemic areas usually exhibit a high level of immunity and tolerate the infection well. Symptomatic disease is usually confined to children with relatively low levels of disease in the adult population despite high rates of infection. Paradoxically, the risks of severe disease, especially in children, tend to be lower among populations with the highest transmission intensities, and the highest disease risks are among populations exposed to low-to-moderate levels of transmission.

**Table 15.10. Endemicity of Malaria**

<b>Endemicity</b>	<b>Definition</b>	<b>Prevalence</b>
Hypoendemic	Areas with low levels transmission of malaria	≤ 10%
Mesoendemic	Usually small rural communities with varying intensity of malaria.	11-50%
Hyperendemic	Areas with intense, but seasonal, transmission of malaria	51-75%
Holoendemic	Areas with a high degree of year long malaria transmission.	≥ 75%

Unstable, or epidemic, malaria refers to an increase in malaria in areas of low endemicity or to outbreaks in areas previously without malaria or among non-immune persons. Morbidity and mortality can be quite high in these non-immune populations. These outbreaks can often be attributed to changes in human behavior or effects on the environment. For example, human migration and resettlement can either introduce malaria into an area of hypoendemicity or expose a previously non-immune population to endemic transmission. Changes in the ecology caused by natural disasters or public works projects, such as building roads or dams, can also impact malaria transmission and lead to epidemics.

Host and parasite factors influencing the transmission of malaria. The epidemiology of malaria is quite complex and highly dependent on the local conditions. The intricate interactions between parasite, host, and vector are the major factors in this epidemiological complexity. For example, as with all vector transmitted diseases, the parasite must be able to establish a chronic infection within the host to maximize the opportunities for transmission. This is especially true in the case of seasonal transmission and in areas of low endemicity. And in general malaria infections are characterized by an initial acute phase followed by a longer relatively asymptomatic chronic phase. This is due in part to the ability of the parasite to avoid complete clearance by the immune system. For example, *P. falciparum* exhibits an antigenic variation that allows it to stay one step ahead of the immune system. In addition, *P. vivax* and *P. ovale* exhibit the hypnozoite stage and are capable of relapses. This allows the parasite to maintain the infection within the human host even after the blood stage of the infection has been cleared. The relative long interval between relapses in some *P. vivax* isolates probably explains its ability to maintain transmission cycles in some temperate climates.

In regards to the host, humans are the only significant reservoir for the parasite and sustained transmission depends upon maintaining a pool of infected individuals and

contact between humans and anopheline mosquitoes. Several factors influence the susceptibility of humans to infection. Obviously the immune status of the individual and their prior experience with malaria will influence the course of the infection. Pregnant women, especially during the first pregnancy, are more susceptible to falciparum malaria as illustrated by a higher prevalence of infection and higher parasitemias. In addition, certain genetic diseases and polymorphisms have been associated with decrease infection or disease (Table 15.11). For example, individuals lacking the Duffy blood-group antigen are refractory to *P. vivax*. A large proportion of the populations in western Africa are Duffy negative, thus accounting for the low levels of *P. vivax* in west Africa. This innate resistance led to the identification of the Duffy antigen as the erythrocyte receptor for merozoite invasion (see Chapter 11).

**Table 15.11. Human Genetics and Innate Resistance.**

<b>Polymorphism</b>	<b>Comment</b>
Duffy-negative	Erythrocytes resistant to <i>P. vivax</i> infection
ovalocytosis	Erythrocytes resistant to merozoite invasion
sickle-cell anemia	Heterozygous individuals exhibit lower parasitemias and less severe disease
thalassemia	Erythrocytes unfavorable for <i>P. falciparum</i> development
G6PD deficiency	Erythrocytes not able to handle increased oxidative stress associated with parasite metabolism

Several inherited erythrocyte disorders are found predominantly in malaria endemic areas and at frequencies much higher than expected. This has led to speculation that these disorders confer some protection against malaria. For example, southeast Asian ovalocytosis is due to a mutation in an erythrocyte membrane protein called band 3. This mutation causes the erythrocyte membrane to become more rigid and more refractory to merozoite invasion. The mechanisms by which the other diseases might confer protection against malaria are not known. In most cases it is presumed or speculated that the combination of the defect and infection leads to premature lysis or clearance of the infected erythrocyte. For example, glucose-6-phosphate dehydrogenase deficient erythrocytes would have an impaired ability to handle oxidative stress. The additional oxidants produced as a result of parasite metabolism and the digestion of hemoglobin (Box 15.1) may overwhelm the infected erythrocyte and lead to its destruction before the parasite is able to complete schizogony. Sickle cell anemia and thalassemia are also speculated to make the infected erythrocyte more susceptible to oxidative stress.

Vectorial Capacity. The potential of the mosquito to serve as a vector depends on the ability to support sporogony, mosquito abundance, and contact with humans, which are all influenced by climatic and ecological factors (Table 15.12). The ability to support sporogony is largely dependent upon species in that not all species of *Anopheles* are susceptible to *Plasmodium* infection. Temperature and mosquito longevity are other key factors affecting the parasite's interaction with the vector. Development of *P. falciparum* requires a minimum temperature of 20°C, whereas the minimum temperature for the other species is 16°C. Temperature also affects the time of development in that the

duration of sporogony is substantially shorter at higher temperatures. A shorter duration of sporogony increases the chances that the mosquito will transmit the infection within its lifespan.

**Figure 15.12. Factors influencing vectorial capacity.**

<b>Sporogony</b>	<b>Mosquito Density</b>	<b>Human Contact</b>
<ul style="list-style-type: none"> <li>• temperature</li> <li>• mosquito longevity</li> <li>• mosquito species</li> </ul>	<ul style="list-style-type: none"> <li>• temperature</li> <li>• altitude</li> <li>• rainfall</li> <li>• breeding places</li> </ul>	<ul style="list-style-type: none"> <li>• anthropophilic</li> <li>• indoor vs. outdoor</li> <li>• feeding time</li> </ul>

Mosquito density and feeding habits also influence the transmission of malaria. Mosquito density is affected by temperature, altitude, rainfall and the availability of breeding places, whereas human-mosquito contact will be influenced by the mosquito behavior. For example, the degree to which a particular mosquito species is anthropophilic will influence the probability of the mosquito becoming infected and then transmit the infection to another human. These anthropophilic tendencies are not necessarily absolute in that many zoophilic mosquitoes will switch to humans if densities reach high levels or the preferred animal source is diminished. The preferred feeding time and whether the mosquito feeds predominantly indoors or outdoors will influence the transmission dynamics. For example, outdoor feeding mosquitoes are more likely to find a human blood meal in the early evening than those feeding late at night when most people are inside. The behavior of the mosquito also needs to be considered in control activities.

**Prevention and Control**

Strategies for preventing and controlling malaria involve three different general approaches: reduce human-mosquito contact, reduce vector density, and reduce the parasite reservoir (Table 15.13). Prevention of malaria in individuals will generally involve the reduction of human-mosquito contact through the use of bed nets, repellents, protective clothing, and mosquito avoidance. This can also include reducing the number of mosquitoes in houses through the use of screens and insecticides. Chemoprophylaxis is another strategy used primary for persons visiting an endemic area for a relatively short period of time. However, chemoprophylaxis only suppresses parasitemia and does not prevent infection. Furthermore, chemoprophylaxis should not be used on a mass scale to control malaria since it promotes the development of drug resistance.

**Table 15.13. Prevention and Control Strategies**

<ul style="list-style-type: none"> <li>• reduce human-mosquito contact               <ul style="list-style-type: none"> <li>○ impregnated bed nets</li> <li>○ repellents, protective clothing</li> <li>○ screens, house spraying</li> </ul> </li> <li>• reduce vector density               <ul style="list-style-type: none"> <li>○ environmental modification</li> <li>○ larvicides/insecticides</li> <li>○ biological control</li> </ul> </li> <li>• reduce parasite reservoir               <ul style="list-style-type: none"> <li>○ case detection and treatment</li> <li>○ chemoprophylaxis</li> </ul> </li> </ul>
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Control activities at the community level can utilize approaches which directly reduce human-mosquito contact as well as approaches which reduce the total number of mosquitoes in an area. Such approaches include the reduction in mosquito breeding grounds (eg., environmental modification), target the larva stages with chemical or biological agents, and massive insecticide spraying for the adult mosquitoes. Biological control methods include the introduction of fish which eat the mosquito larvae or bacteria (eg., *Bacillus thuringiensis*) which excrete larval toxins. Case detection and treatment is another potential control method. Identifying and treating infected persons, especially asymptomatic individuals, will reduce the size of the parasite reservoir within the human population and can lower transmission rates. However, this can be a relatively expensive approach.

Malaria control is complex and multifactorial. These various approaches are not mutually exclusive and can be combined. Many of the successful control programs include both measures to control mosquitoes and treatment of infected individuals. There is no standard method of malaria control that has proven universally effective. The epidemiologic, socioeconomic, cultural and infrastructural factors of a particular region will determine the most appropriate malaria control. Some of the factors which need to be considered include: infrastructure of existing health care services and other resources; intensity and periodicity (eg., seasonality) of transmission; mosquito species (ecological requirements, behavioral characteristics, insecticide sensitivity, etc); parasite species and drug sensitivities; cultural and social characteristics of the population; and presence of social and ecological change.

The control of malaria in tropical Africa has been particularly problematic because of the high transmission rates and the overall low socio-economic level. Several studies have shown that insecticide treated bed nets reduce the morbidity and mortality associated with malaria. Introducing bed nets do not require large promotional programs and their use is readily accepted in most areas. This may be in part due to the reduction in mosquito nuisance biting. Some questions have been raised in regards to the economic sustainability of bed net programs. It is necessary to re-treat the bed nets with insecticide periodically and the bed nets need to be repaired and replaced as they become torn and wear out. In addition, some have raised concerns about the long-term benefits of bed nets since they reduce exposure, but do not eliminate it. This reduction in exposure may delay the acquisition of immunity and simply postpone morbidity and mortality to older age groups.

### **Diagnosis and Species Identification**

Malaria is suspected in persons with a history of being in an endemic area and presenting symptoms consistent with malaria, such as fever, chills, headache and malaise. These symptoms, especially in the early stages of the infection, are non-specific and often described as flu-like. As the disease progresses, the patient may exhibit an enlarged spleen and/or liver and anemia. Diagnosis is confirmed by microscopic demonstration of the parasite in blood smears. Thick blood smears are generally more sensitive for the detection of parasites, whereas thin smears are preferable for species identification. If parasites are not found on the first blood smear it is recommended to make additional

smears every 6-12 hours for as long as 48 hours. The level of parasitemia can also be quantified by counting the percent infected erythrocytes in thin smears or converting the ratio of parasites to white blood cells detected in thick smears to parasites per microliter.

Rapid diagnostic tests based on immunochromatography, or ‘dipsticks’, are also available. These tests are based on detecting parasite proteins within blood samples. Several commercially available tests are available. Most are based on the detection of parasite lactate dehydrogenase or the histidine rich protein-2 of *P. falciparum* combined with parasite aldolase. Most of the tests will distinguish *P. falciparum* from the other three species, but cannot distinguish between those three species. Molecular methods using DNA probes or PCR can also be used for diagnosis. However, these are usually used in epidemiological studies.

Distinguishing features of blood stage parasites. The blood-stage parasites of human *Plasmodium* species exhibit differences in their morphology and modify the host erythrocyte differently (Table 15.14 and Figure 15.11). These differences can be used to distinguish the four species. The typical features of a *P. falciparum* infection are that only ring forms are observed in the circulation since the mature forms sequester. In addition, the gametocytes of *P. falciparum* are elongated and crescent shaped and readily distinguished from the gametocytes of the other species. The ring stages of *P. falciparum* tend to be slightly smaller than the other species and are generally more numerous.

**Table 15.14. Key Morphological Differences Between Human *Plasmodium* Species in Thin Blood Smears.**

<i>falciparum</i>	<i>vivax</i>	<i>ovale</i>	<i>malariae</i>
<ul style="list-style-type: none"> <li>• numerous rings</li> <li>• smaller rings</li> <li>• no trophozoites or schizonts</li> <li>• crescent-shaped gametocytes</li> </ul>	<ul style="list-style-type: none"> <li>• enlarged erythrocyte</li> <li>• Schüffner's dots</li> <li>• 'ameboid' trophozoite</li> </ul>	<ul style="list-style-type: none"> <li>• similar to <i>P. vivax</i></li> <li>• compact trophozoite</li> <li>• fewer merozoites in schizont</li> <li>• elongated erythrocyte</li> </ul>	<ul style="list-style-type: none"> <li>• compact parasite</li> <li>• merozoites in rosette</li> </ul>

The most distinctive features of *P. vivax* are the enlarged infected erythrocytes and the appearance of granules, called 'Schüffner's dots', over the erythrocyte cytoplasm. These granules are manifestation of caveola-vesicle complexes that form on the erythrocyte membrane. *P. ovale* also exhibits Schüffner's dots and an enlarged erythrocyte, making it difficult to distinguish from *P. vivax*. The growing trophozoite of *P. vivax* often has an ameboid appearance and the schizonts can have more than 20 merozoites. Whereas, *P. ovale* trophozoites are more compact than *P. vivax* and the mature schizonts have fewer merozoites. *P. ovale* also has more of a tendency to form elongated host erythrocytes. *P. malariae* is characterized by a compact parasite and does not alter the host erythrocyte or cause enlargement. Mature schizonts will typically have 8-10 merozoites that are sometimes arranged in a rosette pattern with a clump of pigment in the center.

	vivax	ovale	malariae	falciparum
Ring Stage				
Trophozoite				
Schizont				
Segmenter				
Gametocytes				

Figure 15.11. Key morphological features of human malaria parasites. The ring forms of all four species are very similar and difficult to distinguish. The presence of a large number of rings in the absence of trophozoite and schizont stages, as well as multiply-infected erythrocytes, is highly suggestive of *P. falciparum*. Erythrocytes infected with *P. vivax* and *P. ovale* are enlarged and exhibit Schüffner's dots as the rings mature into trophozoites. The typical number of merozoites produced per schizont is: *P. vivax* 14-20 (up to 24), *P. ovale* 6-12 (up to 18), *P. malariae* 8-10 (up to 12), and *P. falciparum* 16-24 (up to 36).

### Chemotherapy and Drug Resistance

Several antimalarial drugs are available. Many factors are involved in deciding the best treatment for malaria. These factors include the parasite species, the severity of disease, the patient's age and immune status, the parasite's susceptibility to the drugs (i.e., drug resistance), and the cost and availability of drugs. Therefore, the exact

recommendations will often vary according to geographical region. In addition, the various drugs act differentially on the different life cycle stages (Table 15.15).

**Table 15.15. Selected Antimalarial Drugs**

<b>Drug Class</b>	<b>Examples</b>
Fast-acting blood schizontocide	chloroquine (+ other 4-aminoquinolines), quinine, quinidine, mefloquine, halofantrine, antifolates (pyrimethamine, proguanil, sulfadoxine, dapsone), artemisinin derivatives
Slow-acting blood schizontocide	doxycycline (+ other tetracycline antibiotics)
Blood + mild tissue schizontocide	proguanil, pyrimethamine, tetracyclines
Tissue schizontocide (anti-relapsing)	Primaquine, tafenoquine
Gametocidal	primaquine, artemisinin derivatives, 4-aminoquinolines (limited?)
Combinations	Fansidar <sup>®</sup> (pyrimethamine + sulfadoxine), Maloprim <sup>®</sup> (pyrimethamine + dapsone), Malarone <sup>®</sup> (atovaquone + proguanil), artemisinin combination treatment

Fast-acting blood schizontocides, which act upon the blood stage of the parasite, are used to treat acute infections and to quickly relieve the clinical symptoms. Chloroquine is generally the recommended treatment for patients with *P. vivax*, *P. ovale*, *P. malariae*, and uncomplicated chloroquine-sensitive *P. falciparum* infections. After its introduction near the end of World War II, chloroquine quickly became the drug of choice for the treatment and prevention of malaria. Not only is chloroquine an effective drug--probably due to its site of action in the food vacuole and its interference with hemozoin formation (Box 15.1)--but it is also relatively non-toxic and cheap. Side effects may include pruritus (i.e., itching), nausea, or agitation. Patients infected with either *P. vivax* or *P. ovale*, and that are not at a high risk for reinfection, should also be treated with primaquine (a tissue schizontocide). Primaquine is effective against the liver stage of the parasite, including hypnozoites, and will prevent future relapses. The combination of chloroquine and primaquine is often called 'radical cure'.

Severe, or complicated, falciparum malaria is a serious disease with a high mortality rate and must be regarded as life threatening, and thus requires urgent treatment. Treatment typically requires parenteral drug administration (i.e., injections) since the patients are often comatose or vomiting, and thus cannot take the drugs orally. Parenteral formulations are available for chloroquine, quinine, quinidine and artemisinin derivatives. The artemisinin derivatives are generally the preferred choice, but are not yet approved everywhere. For example, in the United States quinine and quinidine are the approved drugs for severe malaria. Patients need to be continuously monitored for hematocrit, parasitemia, hydration levels, hypoglycemia, and signs of drug toxicity and other complications during the course of treatment. A switch to oral administration should be made as soon as the patient is able. Most deaths due to severe malaria occur at or close to home in situations where the patients cannot be taken to the hospital.

Artemisinin suppositories which can be administered by village health workers have also been developed and have proved to be safe and effective.

The efficacy of chloroquine is greatly diminished by the wide spread chloroquine resistance of *P. falciparum* and the emergence of chloroquine-resistant *P. vivax* (Box 15.3). If chloroquine therapy is not effective, or if in an area with chloroquine-resistant malaria, common alternative treatments include: mefloquine, quinine in combination with doxycycline, or Fansidar®. Derivatives of artemisinin (dihydroartemisinin, artesunate and artemether) are increasingly used in Asia and Africa and are now recommend as the first line of treatment by the World Health Organization. These drugs were originally derived from the wormwood plant (*Artemisia annua*) and have been used for a long time in China as an herbal tea called quinhaosu to treat febrile illnesses. To prevent the high recrudescence rates associated with artemisinin derivatives and to slow the development of drug resistance it is recommended that treatment be combined with an unrelated anti-malarial. Drugs used in combination with artemisinin include mefloquine, lumefantrine, Fansidar®, and amodiaquine.

[Box 15.3]

Chemoprophylaxis. Chemoprophylaxis is especially important for persons from non-malarious areas who visit areas endemic for malaria. Such non-immune persons can quickly develop a serious and life-threatening disease. As in the case of treatment there is no standard recommendation and the choices for chemoprophylaxis are highly dependent upon the conditions associated with the travel and the individual person. Chemoprophylaxis requires the use of non-toxic drugs since these drugs will be taken over extended periods of time. Generally the patient will start to take the drug before traveling and then continue taking the drug during the stay in the endemic area and continue taking the drug after returning. This is to insure the drug is maintained at sufficient levels throughout out the visit and to protect against any infection obtained during the visit. Unfortunately, many of the effective and non-toxic drugs (eg, chloroquine, pyrimethamine, proguanil) are of limited use because of drug resistance. Another strategy is presumptive (or 'standby') treatment to be used in conjunction with prophylaxis. In this case a person either forgoes prophylaxis or takes chloroquine or another relatively non-toxic drug for prophylaxis and carries a drug like Fansidar®, mefloquine, or quinine, which they will take if they start to exhibit symptoms associated with malaria.

The use of mefloquine for malaria chemoprophylaxis is somewhat controversial. Mefloquine is efficacious at preventing malaria with a single does per week, thus offering advantages to drugs that need to be administered daily. At this dosage mefloquine is tolerated by most individuals. However, some people experience neuropsychiatric adverse affects such as sleep disturbances and nightmares. This could be exacerbated by stress associated with international travel. Randomized, blinded and controlled trials indicate that neuropsychiatric adverse affects are only slightly higher with mefloquine than with other anti-malarials.

Killing the exoerythrocytic stage (i.e., liver stage) would prevent the blood infection and is known as causal prophylaxis. This is highly desirable in that it limits the amount of time the prophylactic drug needs to be taken before and after travel to an endemic area. The only currently available drug for causal prophylaxis is primaquine. However, malaria prophylaxis is not an approved use of primaquine and should only be prescribed for prophylaxis on a case-by-case basis. For example, for persons who frequently have trips of short duration to highly endemic areas and that the person does not exhibit glucose-6-phosphate dehydrogenase deficiency. Tafenoquine is currently undergoing field evaluation for its use in causal prophylaxis.

**Table 15.16. Proteins and mutations involved in drug resistance.**

<b>Protein<sup>a</sup></b>	<b>Subcellular Location</b>	<b>Primary Function</b>	<b>Major drugs affected</b>	<b>Major polymorphisms<sup>b</sup></b>
CRT	food vacuole	transporter	chloroquine	K76T
MDR1	food vacuole	transporter	mefloquine, quinine (?)	amplification, D86Y <sup>c</sup>
DHFR	cytoplasm	folate metabolism	pyrimethamine, proguanil	S108N, N51I, C59R, I164L
DHPS	cytoplasm	folate metabolism	sulfadoxine, dapsone	A437G, K540E, A581G
Cytochrome b	mitochondria	electron transport	atovaquone	Y268S/N/C
ATPase6	endoplasmic reticulum	calcium transport	artemisinins	S769N

<sup>a</sup>CRT = chloroquine resistance transporter; MDR1 = multi-drug resistance (P-glycoprotein homologue); DHFR = dihydrofolate reductase; DHPS = dihydropterote sythetase; ATPase6 = sarco/endoplasmic reticulum calcium-dependent ATPase orthologue. <sup>b</sup>Polymorphisms associated with drug resistance where the number refers to amino acid (i.e., codon) and the first letter is the wild type residue and the second letter is the polymorphism(s) associated with resistance. <sup>c</sup>Associated with increased sensitivity to mefloquine and dihydroartemisinin, but an decreased sensitivity to chloroquine.

**Drug resistance.** Drug resistance, and in particular, chloroquine resistance is a major public health problem in the control of malaria. Drug resistance is defined by a treatment failure and can be graded into different levels depending on the timing of the recrudescence following treatment. Traditionally these levels of drug resistance have been defined as sensitive (no recrudescence), RI (delayed recrudescence), RII (early recrudescence), and RIII (minimal or no anti-parasite effect). Drug resistance by this protocol is determined by monitoring patients for parasitemia for 28 days following standard drug treatment. Because of the difficulties in using this protocol in endemic areas a modified protocol based on clinical outcome was introduced by the World Health Organization in 1996. In this protocol the level of resistance is expressed as adequate clinical response (ACR), late treatment failure (LTF), or early treatment failure (ETF). ACR is defined as the absence of parasitemia (irrespective of fever) or absence of clinical symptoms (irrespective of parasitemia) during 14 days following treatment. LTF is the

reappearance of symptoms or the presence of parasitemia during days 4-14 of follow-up. ETF is the persistence of clinical symptoms or the presence of parasitemia during the first three days of follow-up.

Either protocol can be used to determine drug resistance, but the clinical outcome protocol is more practical in areas of intense transmission where it may be difficult to distinguish re-infection from recrudescence and where parasitemia in the absence of clinical symptoms is common. In addition, the clinical outcome protocol requires a shorter period of hospitalization. There are also in vitro tests that can determine the efficacy of the drugs against *P. falciparum* grown in culture. The in vivo and in vitro tests do not always correspond since host immunity and other factors can affect the in vivo outcomes. For some drugs the mechanism of resistance is known and specific genetic polymorphisms are associated with drug resistance (Table 15.16). For these drugs it is possible to screen for particular polymorphisms using molecular markers. In some cases the protein involved in resistance is the target of the drug and reflects decreased affinity of the drug for the target. In other cases the protein involved in resistance is not the target of drug action. For example, chloroquine resistance correlates with specific mutations in a gene for a transporter protein which presumably exports the drug out of the food vacuole (Box 15.3).

**Table 15.17. Factors Contributing to Development and Spread of Drug Resistance**

<b>Factor</b>	<b>Comments</b>
self-treatment	Individuals may only take the drug until symptoms clear or will take lower doses to save money.
poor compliance	Individuals may not complete the full course of treatment because of drug side effects.
mass administration	The widespread use of a drug in an area of intense transmission increases drug pressure by exposing a larger parasite population to the drug.
long drug half-life	Drugs that are slowly eliminated will lead to a longer exposure of the parasite to subtherapeutic drug concentrations.
transmission intensity	High levels of transmission may allow re-infection while drugs are at sub-therapeutic levels.

Drug resistance develops when parasites with decreased sensitivities to antimalarial drugs are selected under drug pressure. Decreased drug sensitivity can be conferred by several mechanisms and reflects genetic mutations or polymorphisms in the parasite population. The drug-resistance parasites will have a selective advantage over the drug-sensitive parasites in the presence of drug and will be preferentially transmitted. Major factors in the development of drug resistance are the use of sub-therapeutic doses of drugs or not completing the treatment regimen (Table 15.17). The lower drug levels will eliminate the most susceptible parasites, but those which can tolerate the drug will recover and reproduce. Over time this will lead to a continued selection for parasites which can tolerate even higher doses of the drug. It is crucial to maintain an adequate concentration of the drug for a sufficient time to completely eliminate the parasites from any given individual.

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## Summary and Key Concepts

- Malaria is major human disease caused by apicomplexan parasites of the genus *Plasmodium* and is found throughout tropical and subtropical regions.
- Four distinct *Plasmodium* species with different biological and epidemiological characteristics as well as different disease etiologies infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*.

- The parasite is transmitted to humans by anopheline mosquitoes and exhibits a complex life cycle in both the human host and the vector.
- During one stage of its life cycle the parasite infects erythrocytes and this blood stage is responsible for the clinical manifestations associated with the disease.
- Infection with the malaria parasite causes an acute febrile illness which is most notable for its periodic fever paroxysms coinciding with the rupture of the infected erythrocyte and release of merozoites.
- *P. falciparum* can cause a severe and fatal disease involving multiple organs. The most notable manifestations of severe malaria are: cerebral malaria, severe anemia, and acute respiratory distress syndrome.
- The higher morbidity associated with *P. falciparum* is due in part to the potentially high levels of parasitemia associated with *P. falciparum* infections and the cytoadherence of infected erythrocytes to endothelial cells within various organs.
- Cytoadherence is mediated by ligands expressed on parasite induced structures located on the surface of *P. falciparum*-infected erythrocytes which bind to various receptors found on endothelial cells.
- Immunity against malaria is slow to develop and requires repeated exposures to the parasite.
- The epidemiology and transmission of malaria varies according to the intricate interactions between the human host, mosquito vector and parasite in any particular location.
- Control and prevention measures should consider the epidemiologic, socioeconomic, cultural and infrastructural factors of a particular region. Generally control and prevention will involve reducing mosquito-human contact, reducing vector density, treatment or chemoprophylaxis, or some combination of approaches.
- The choice of antimalarial drugs for treatment or chemoprophylaxis depends on the parasite species, the severity of disease, drug resistance, and the cost and availability of drugs. Currently artemisinin derivatives are generally the recommend first line of treatment, but use is often restricted by cost or availability.
- Widespread and increasing drug resistance to antimalarial drugs is leading to increased morbidity associated with malaria as well as limiting the ability to control the disease and its spread.

### Box 15.1. The food vacuole and the digestion of hemoglobin.

The malaria parasite takes up the host erythrocyte cytoplasm and breaks down the hemoglobin into amino acids. These amino acids are then used for the synthesis of parasite proteins or possibly as an energy source. During the early ring stage the parasite takes up the host cell stroma by pinocytosis. As the parasite matures, it develops a special organelle, called the cytochrome, for the uptake of host cytoplasm. The hemoglobin-containing vesicles fuse to form a food vacuole. The food vacuole is an acidic compartment (pH 5.0-5.4) that contains protease activities. In this regard the food vacuole resembles a lysosome.

Several distinct protease activities, representing three of the four major classes of proteases, have been identified in the food vacuole (1). The digestion of hemoglobin probably occurs by a semi-ordered process involving the sequential action of different proteases (Figure 15.1A). Aspartic acid proteases designated as plasmepsins make the initial cleavages of the native hemoglobin causing it to dissociate into large globin fragments and free heme. The globin fragments are then further digested by plasmepsins and falcipains, cysteine proteases, into peptides. A metalloprotease designated as falcilysin then digests these peptides into smaller peptides of 6-8 residues. A dipeptidyl aminopeptidase will then generate dipeptides which are then converted into amino acids by aminopeptidases. Some of the smaller peptides may also be transported into the cytoplasm and converted to amino acids through the action of amino peptidases in the cytoplasm. Pfmdr-1, a member of the ATP-binding cassette transporter family, is localized to the food vacuole membrane and is believed to function in this capacity.

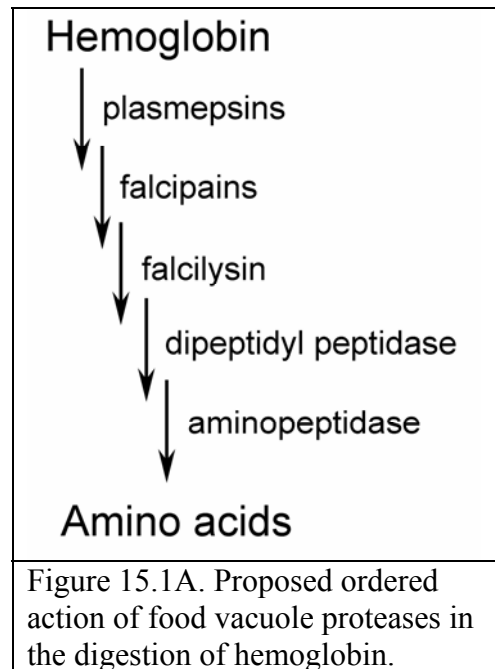


Figure 15.1A. Proposed ordered action of food vacuole proteases in the digestion of hemoglobin.

Digestion of hemoglobin also releases heme. Free heme is toxic due to its ability to destabilize and lyse membranes, as well as inhibiting the activity of several enzymes. Some of this free heme can be degraded in oxidative processes. However the majority of the heme is sequestered into hemozoin or the malarial pigment (2). The deposition of hemozoin has long been known as a characteristic feature of malaria, even before the parasite was discovered. X-ray crystallography and spectroscopic analysis indicates that hemozoin has the same structure as  $\beta$ -hematin which is a heme dimer. These dimers interact through hydrogen bonds to form crystals of hemozoin. Therefore, pigment formation is best described as a biocrystallization, or biomineralization, process. Recently a protein that may catalyze the formation of hemozoin has been described (3). Lipids may also participate in the process.

Chloroquine and other 4-aminoquinolines inhibit pigment formation, as well as the heme degradative processes, and thereby prevent the detoxification of heme. The free heme destabilizes the food vacuolar membrane and other membranes and leads to the death of the parasite. The fact that the biocrystallization of heme is a unique process to the parasite and not found in the host accounts for the high therapeutic index of such drugs in the absence of drug resistance. Many other anti-malarials target the food vacuole indicating the importance of this organelle and its various functions (Figure 15.1B) to the survival of the parasite.

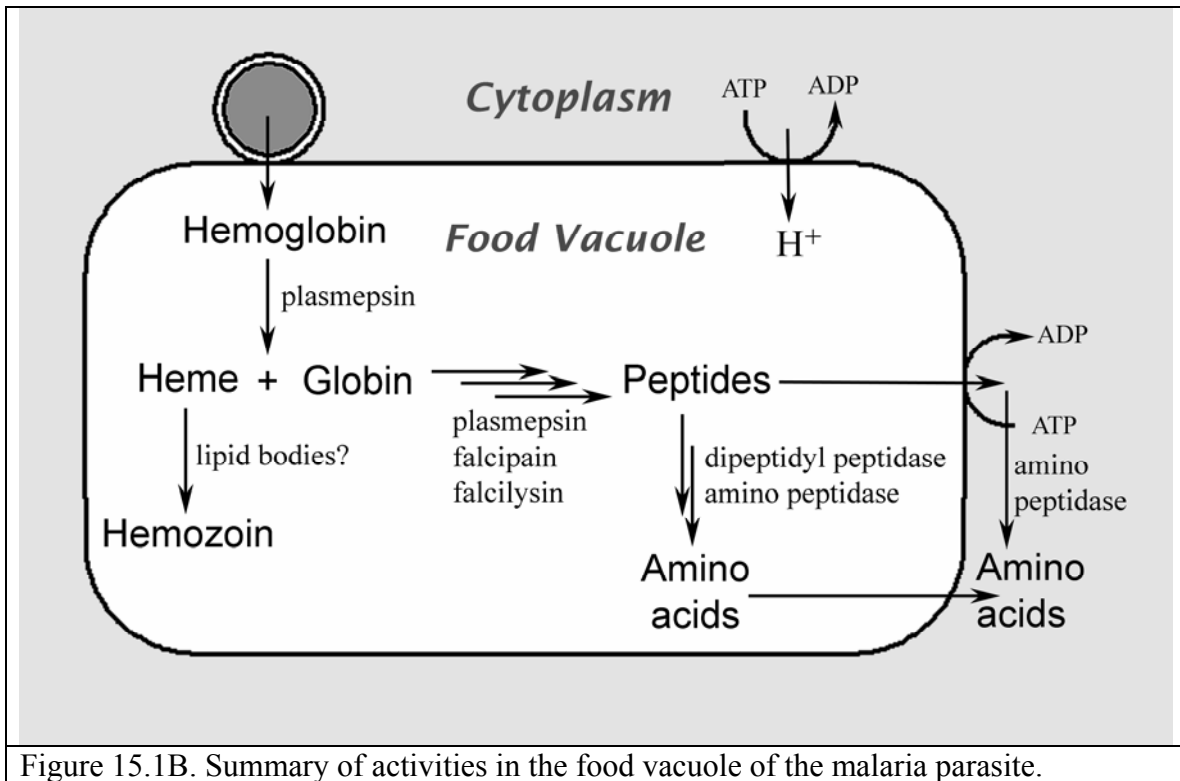


Figure 15.1B. Summary of activities in the food vacuole of the malaria parasite.

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### Box 15.2. Malaria in the United States.

Malaria was previously more widespread in temperate areas including North America and Europe. It is believed that malaria was introduced to the Americas by the European colonists (*P. vivax* and *P. malariae*) and African slaves (*P. falciparum*) during the sixteenth and seventeenth centuries.

**Table 15.2A. Factors leading to a decline in malaria in the United States.**

- population shift from rural to urban areas
- improved socioeconomic conditions
- drainage of breeding grounds
- availability of quinine
- mosquito control activities

Malaria became endemic in many parts of the United States excluding deserts and mountainous areas and the incidence probably peaked around 1875. A population shift from rural to urban areas, drainage of swamps to create farmland, improved housing and nutrition, better socioeconomic conditions and standards of living, greater access to medical services, and the availability of quinine for treatment all contributed to the decline in the prevalence of malaria even before the introduction of specific control measures (Table 15.2A). Some control activities, such as case detection and treatment, larviciding and house spraying, were introduced during the 1940's and led to the eradication of malaria in the United States. Since the 1950's nearly all cases of malaria in the U.S. have been imported. The major factors contributing to this eradication appear to be a population shift from rural to urban areas and an increase in the standard of living, which resulted in improved housing, better nutrition, and greater access to medical services.

The vast majority of malaria cases diagnosed in the United States are acquired by persons while traveling to countries where malaria is endemic. However, there have been several outbreaks of autochthonous malaria transmission in the United States (1, 2). More than 80% of the cases were *P. vivax*. In some cases, the outbreaks were associated with large numbers of immigrants suggesting that infected persons can import the infection and then transmit it to local *Anopheles*. Many of these outbreaks were associated with unusually hot and humid weather, which may increase anopheline survival and decrease the duration of the sporogonic cycle, thus allowing for the development of infective sporozoites.

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### **Box 15.3 Genes associated with chloroquine resistance.**

Chloroquine resistant *P. falciparum* were first detected in Colombia and at the Cambodia-Thailand border during the late 1950's. During the 1960's and 1970's, resistant parasites spread through South America, Southeast Asia, and India. Resistance was first reported in east Africa in 1978 and spread throughout the continent during the 1980's. Chloroquine resistant *P. vivax* was first reported in 1989 in Papua New Guinea and is now found in several foci in southeast Asia and perhaps South America.

It is generally accepted that the site of action of chloroquine is in the food vacuole and the mechanism of action is the inhibition of hemozoin formation (Box 15.1). Chloroquine concentrates up to several thousand-fold in the food vacuole of the parasite. Possible mechanisms for this selective accumulation of chloroquine in the food vacuole are: 1) protonation and ion trapping of the chloroquine due to the low pH of the food vacuole; 2) active uptake of chloroquine by a parasite transporter(s); and/or 3) binding of chloroquine to a specific receptor in the food vacuole. The exact contributions of these three postulated mechanisms are not clear.

Chloroquine resistance is associated with a decrease in the amount of chloroquine that accumulates in the food vacuole. The mechanism for this decreased accumulation is controversial. Some studies have shown that the decrease in drug accumulation is due to an increase in drug efflux. Whereas other studies suggest that diminished levels of chloroquine accumulation is more important. The observation that verapamil and related drugs can reverse the chloroquine resistant phenotype has led to speculation that an ATP dependent transporter plays a role in drug efflux and chloroquine resistance, similar to the multidrug resistance (MDR) in cancer. A MDR-like transporter, designated PfMDR1, has been identified on the food vacuole membrane. However, no definitive correlations between PfMDR1 and chloroquine resistance could be demonstrated. An ancillary role for PfMDR1 in chloroquine resistance cannot be ruled out though.

A genetic cross and mapping studies between a chloroquine resistant clone and a chloroquine sensitive clone resulted in the identification of a 36 kb region on chromosome 7 associated with chloroquine resistance. One of the 10 genes in this 36 kb region encodes a protein with 10 transmembrane domains and resembles a transporter protein similar to chloride channels. The gene has been designated as PfCRT (for *P. falciparum* chloroquine resistance transporter) and the protein is localized to the food vacuole membrane. Several mutations in the PfCRT gene show correlations with the chloroquine resistance phenotype and one mutation, a substitution of a threonine (T) for a lysine (K) at residue 76 (K76T) shows good correlation with chloroquine resistance. Presumably these mutations affect the accumulation of chloroquine in the food vacuole, but the exact mechanism of chloroquine resistance is not known. Furthermore, the observation that chloroquine resistance has arisen relatively few times and then subsequently spread has led to speculation that multiple genes are involved in the development of resistance.