28 *Plasmodium* spp.

A 26-year-old model went to see her doctor about 1 week after returning from a job in the Gambia. She complained of an abrupt onset of bouts of shivering and feeling cold, vomiting, rigors, and profuse sweating accompanied by a headache and nausea. On examination, she was noted to be pale with a temperature of 39.5°C and had tachycardia. She gave a history of having taken anti-malarial tablets before and during her stay in the Gambia but was admitted to hospital with a provisional diagnosis of malaria.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

The organism causing malaria is *Plasmodium*, a eukaryotic protozoan that infects the erythrocytes of humans. It has the characteristics of eukaryotes, with a nucleus, mitochondria, endoplasmic reticulum, and so forth. There are approximately 156 named species of *Plasmodium* which infect various vertebrates. Five species of *Plasmodium* are able to infect humans: *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae* and *P. knowlesi*. *P. falciparum* is the most **virulent** species of malaria but *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa. All of these species have similar life cycles in which the organisms undergo both sexual and asexual reproduction in the vector and host and alternate between intracellular and extracellular forms. The female *Anopheles* mosquito is the vector for malaria. The risk of malaria transmission is therefore restricted to those areas where mosquitoes can breed and where the parasite can develop within the mosquito – see Epidemiology below.

ENTRY AND SPREAD WITHIN THE BODY

The transmission stage of *Plasmodium* is the sporozoite, which is injected into the bloodstream of a human when the female *Anopheles* mosquito takes a blood meal (**Figure 28.1**). The detailed life cycle is shown in **Figure 28.2**. Following the mosquito bite, at least some of the sporozoites remain in the dermis for some time before crossing the endothelial cells and entering the bloodstream; some pass directly into draining lymph nodes. Only a few dozen sporozoites are transmitted during feeding but there is rapid translocation into the liver to begin the first stage of disease.

Liver Stage (Pre-Erythrocytic Stage)

The blood-borne sporozoites localize in the liver via the sinusoids, where through interactions between their surface circumsporozoite protein (CSP – most abundant protein on the sporozoite surface) and highly sulfated heparan sulfate proteoglycans (HSPGs), they cross the sinusoidal cellular barrier through liver sinusoidal endothelial cells or Kupffer cells (KC). The sporozoites actively enter the hepatocytes (invade – rather than are taken up passively by **endocytosis**), and may traverse several hepatocytes before they switch to a replicative form. There, within a parasitophorous vacuole (to avoid lysosomal degradation), they increase in number and develop into schizonts. This asexual stage takes up to 2 weeks. Rupture of the liver cells releases the schizonts into the bloodstream as merozoites (with about 10–40 000 being released from the liver). *P. vivax* and *P. ovale* also produce a resting stage within the liver cell called hypnozoites. In this case, some sporozoites once in the liver do not develop immediately into schizonts, but remain at an uninucleate stage, in a quiescent form named hypnozoite, before resuming

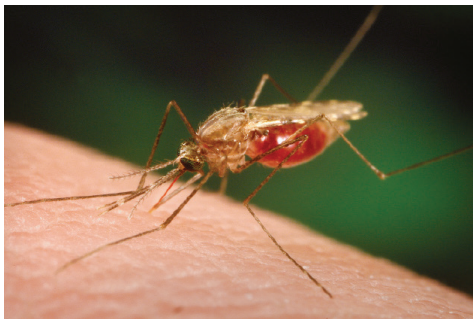

Figure 28.1 *Anopheles funestus* mosquito taking a blood meal from its human host. This mosquito species, together with *Anopheles gambiae*, is one of the two most important malaria vectors in Africa. Note the blood passing through the proboscis. *From the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #7192. Additional photographic credit is given to James Gathany, Dr Frank Collins and the University of Notre Dame. The image was taken in 2005 by James Gathany.*

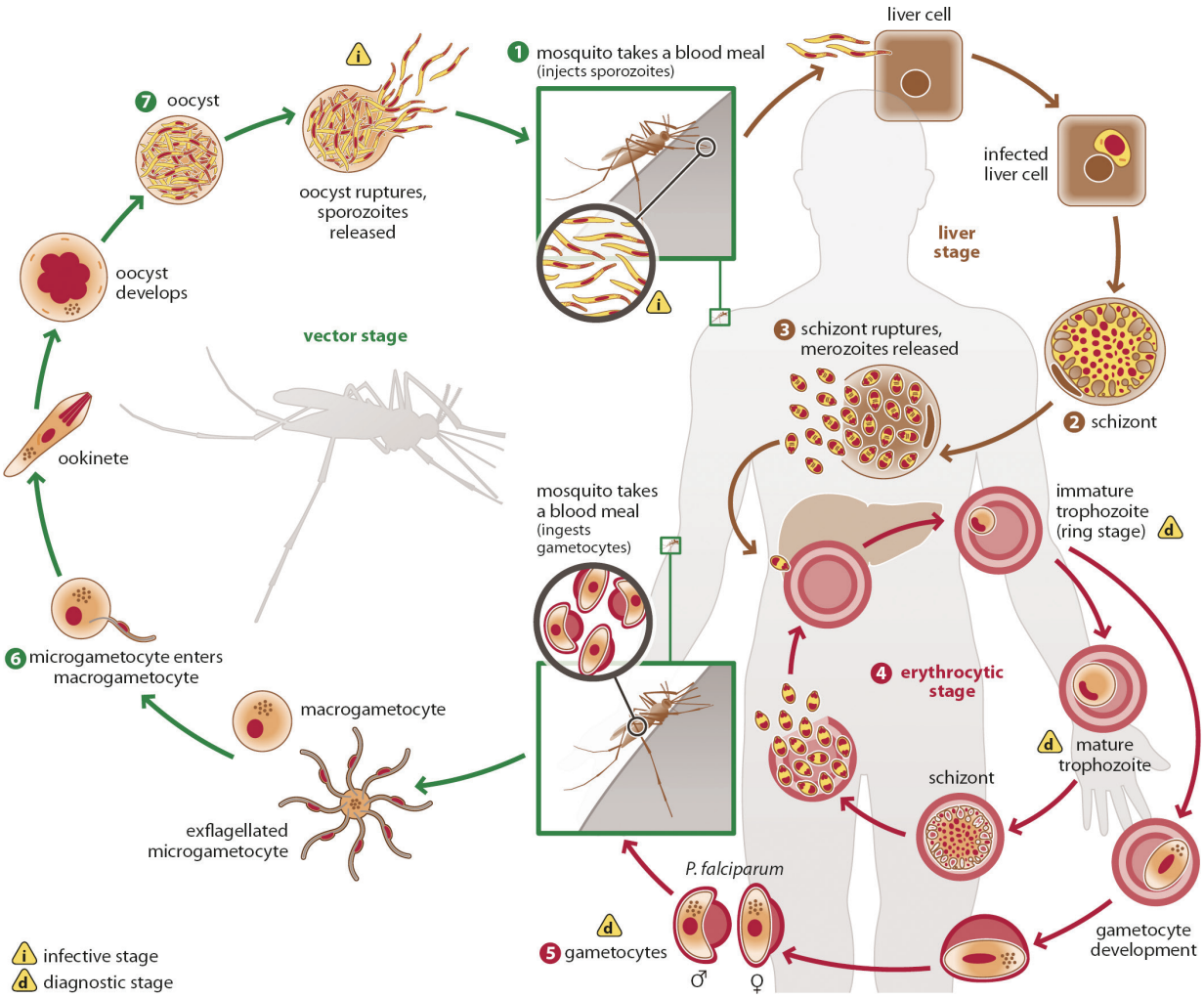

Figure 28.2 The lifecycle of *Plasmodium*. (1) The mosquito injects saliva containing sporozoites as it takes a blood meal and the parasite localizes in the liver (liver stage), where it undergoes a stage of development to produce a schizont which contains developing merozoites, in the infected liver cell (2). *P. vivax* and *P. ovale* also produce a resting stage within the liver cell called hypnozoites, which can persist in the liver and result in relapses months or even years later. The dead liver cell breaks open and the shizont ruptures producing small vacuoles (merosomes) which release their merozoites into the bloodstream. These invade erythrocytes (erythrocytic stage) and undergo developmental stages as trophozoites, which mature and produce schizonts, at which stage, the erythrocyte bursts rupturing the shizonts to release further merozoites (4). Further cycles of asexual development within uninfected erythrocytes occur, releasing more merozoites to infect further erythrocytes. Differentiation of the immature trophozoite into male and female gametocytes occurs in some erythrocytes (5) and these are ingested when a mosquito takes a blood meal. The male (microgametocyte – exflagellated) fertilizes the female macrogametocyte (6) to form a zygote within the intestine of the mosquito (vector stage) and this becomes an ookinete that invades the intestinal wall where it develops into an oocyte (7). The oocyte matures into sporozoites, which are released and migrate to the salivary gland of the mosquito. Here, they will be transmitted to a new human host when the mosquito takes a blood meal and the cycle starts again (1). *From the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #3405. Additional photographic credit is given to Alexander J da Silva, PhD and Melanie Moser. The image was created in 2002.*

hepatic development induced by as yet unknown factors that cause relapses weeks, months or even years after the primary infection. The sporozoite has small vacuoles (micronemes) that release substances onto their surface in a co-ordinated way to ensure successful migration to and invasion of hepatocytes and production of merozoites.

Erythrocyte Stage

Merozoites (the smallest extracellular parasite form) invade and destroy erythrocytes giving rise to symptoms (see Section 3). Invasion is a multistep process that includes firstly binding of the merozoite, reorientation, discharge of secretory organelles (called rhoptries and micronemes), formation of an electron-dense "tight junction" between the merozoite apical end and the erythrocyte membrane. This leads to actinomyosin-powered entry into the erythrocyte with formation of a membrane-bound parasitophorous vacuole and resealing of the erythrocyte cell membrane. Within this vacuole, the parasite feeds on hemoglobin and multiplies. Invasion takes less than 30 seconds. Entry of the merozoites into erythrocytes is achieved through attachment of a number of surface molecules (merozoite surface proteins (MSPs) that

are anchored by glycosylphosphatidylinositol (GPI), and a number of erythrocyte binding ligands (EBL). At least four have been identified for *P. falciparum* merozoites. It is generally accepted that glycophorins A, B, C, and D are important "receptors" on erythrocytes and are known for *P. falciparum* Glycophorin A on erythrocytes. Another important structure on erythrocytes is band 3 protein that binds MSP1 complex on *P. falciparum* merozoites. *P. vivax* has a specific reticular binding protein to enable it to attach and invade **reticulocytes** but not mature erythrocytes. In addition, *P. vivax* has surface molecules (**Duffy binding proteins** – DBP-1) that bind to Duffy blood group antigens on the erythrocytes. Although initially thought that DBP-1 was essential for binding of *P. vivax* to erythrocytes, some cases have been seen where infection occurs in individuals that genetically lack DBP-1. The search is on for other receptors for *P. vivax* merozoites on reticulocytes.

Within the erythrocyte, the merozoites undergo further development as a trophozoite (seen as a "ring" stage – see **Figure 28.2**) and then undergo asexual reproduction to produce schizonts, at which stage, the erythrocyte bursts releasing merosomes containing 16–32 daughter merozoites into the bloodstream.

Each asexual cycle takes 44–48 hours and is followed by cell rupture and re-invasion steps that induce periodic waves of fever in the patient (see **Figure 28.3** and Section 3). This erythrocytic cycle may continue for months or years. However, in some erythrocytes, the trophozoites differentiate into male and female gametocytes and a mosquito taking a blood meal will take up some of the gametocyte-containing erythrocytes, heralding the sexual developmental phase in the vector.

Vector Stage

Within the intestine of the insect, male (exflagellated microgametocytes) and female gametes (macrogametocytes) fuse to become a zygote. These become an ookinete, which then invades the intestinal wall where it develops into an oocyte. The oocyte develops into thousands of sporozoites, which then migrate to the mosquito's salivary gland.

PERSON-TO-PERSON SPREAD

The sporozoites are injected into an individual when an infected female *Anopheles* mosquito feeds and the whole cycle starts again.

NON-MOSQUITO SPREAD

Malaria can also be transmitted through blood transfusion, hypodermic needle sharing or accidents, and from mother to fetus.

EPIDEMIOLOGY

Malaria transmission occurs in five WHO regions. Globally, an estimated 3.4 billion people in 92 countries are at risk of being infected with malaria and developing disease (see map – the atlas project website), and 1.1 billion are at high risk (>1 in 1000 chance of getting malaria in a year). There was a marked reduction in global malaria case incidence and mortality rates between 2000 and 2019. The malaria case incidence rate (cases per 1000 population at risk) fell from 80 in 2000 to 57 in 2019. Total malaria cases declined from 238 million in 2000 to 227 million in 2019. The mortality incidence rate (deaths per 100 000 population at risk) was reduced from 25 in 2000 to 10 in 2019. The total number of deaths fell from 736 000 in 2000 to 409 000 in 2019. Children under 5 years of age are the most vulnerable group affected by malaria and in 2019 they accounted for 67% (274 000) of all malaria deaths worldwide. Africa continues to carry a disproportionately high share of the global malaria burden. In 2019, the region had 94%

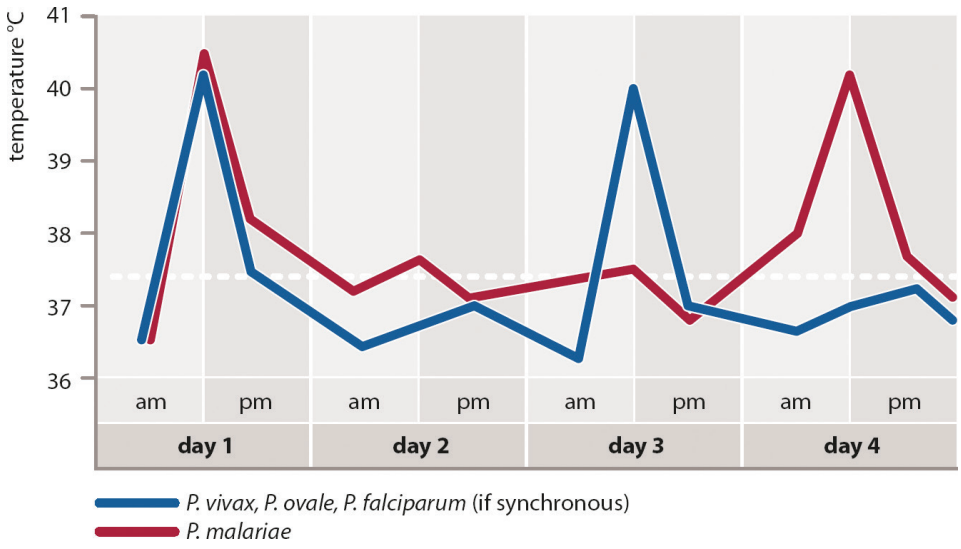

Figure 28.3 Cyclical fever coincident with the release of merozoites. *Adapted from Goering R, Dockrell H, Zuckerman M et al. (2008) Mim's Medical Microbiology, 4th edition, Figure 27.11. With permission from Elsevier.*

of all malaria cases and deaths. Six countries accounted for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%) and Niger (4%). In 2020, there was a slight increase in cases worldwide from 227 (in 2019) to 241 million cases. The estimated number of malaria deaths stood at 627000 in 2020. The WHO African Region carries a disproportionately high share of the global malaria burden. In 2020, the WHO African region was home to 95% of the malaria cases and 96% of malaria deaths.

With increasing international travel, prior to the SARS-2 (COVID-19) pandemic there continued to be a rise in the number of cases of malaria in travelers returning to non-malarious areas from countries where malaria is endemic. In Europe, in 2018, 8349 malaria cases were reported, 8347 (> 99%) of which were confirmed. Among 7338 cases with known importation status, 99.8% were travel related. As in previous years, the overall rate of confirmed malaria cases was higher among men than women (1.6 cases and 0.7 cases per 100000 population, respectively; male-to-female ratio 1.9:1). However, the travel-related cases were reduced during the SARS-CoV-2 pandemic due to limited travel.

Pregnancy is associated with a higher risk of malaria. An estimated 10000 pregnant women and 200000 of their infants died annually in sub-Saharan Africa as a result of malaria infection during pregnancy. HIV-infected pregnant women are at increased risk.

HUMAN GENETIC FACTORS THAT DECREASE THE INFECTION RATES OF PLASMODIUM

As already mentioned, the absence of DBPs in most West Africans prevents most infections by *P. vivax*, since it uses the Duffy blood group antigen-1 as a means of attachment. Sickle cell trait (heterozygous for HbS with HbA) gives an increasing amount of immunologic protection against malaria for young children during their first 10 years of life (a 29% reduction in malaria incidence). A number of mechanisms have been proposed to explain malaria resistance, including sickling of the infected red blood cells, increased splenic phagocytosis, premature hemolysis and parasite death, impaired hemoglobin digestion, weakened cytoadherence, acquired host immunity, translocation of HbS-specific parasite-growth inhibiting microRNAs, and induction of heme-oxygenase-1. Glucose-6-phosphate dehydrogenase (G6PD) deficiency confers resistance to malaria. Although the mechanism is unclear, it has been found that when the parasites are at the ring stage, erythrocytes deficient in the enzyme were phagocytosed 2–3 times more intensely than normal ring-stage erythrocytes. There was, however, no difference when the parasites were at the more mature trophozoite stage.

The Impact of the SARS-CoV-2 (COVID-19) Pandemic on Malaria

During the pandemic, a recent modeling analysis by the WHO predicted a >20% rise in malaria morbidity and >50% mortality in sub-Saharan Africa as a result of 75% reduction in routine malaria control measures. These might significantly increase the longer the pandemic goes on. Other indirect effects of the pandemic, particularly those that affect people's lives and well-being, such as increased malnutrition, poverty, and social instability, may further influence malaria burden. Current guidelines by WHO at the time of writing have included the continuation of all routine malaria control measures while adhering to Covid-19 local personal and physical distancing guidelines established by the authorities.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

The understanding of the immune response to *Plasmodium* is far from complete. Some immunity does develop to the parasite with continuous infection (especially to blood-stage parasites) and children who survive early attacks become resistant to severe disease by about 5 years of age. Levels of parasites fall progressively until adulthood, when they are low or absent most of the time. This immunity, however, appears to be lost after spending a year away from exposure, presumably due to lack of repeated antigenic stimulation needed for its maintenance. **IgG** antibodies to blood-stage parasites are transferred across the placenta and limit parasitemia and severe disease in neonates. Thus, although immune responses do develop to different stages of the lifecycle in infected individuals, they are weak and cannot eradicate the parasite.

PRINCIPAL MECHANISMS THAT ARE THOUGHT TO BE RESPONSIBLE FOR IMMUNITY AT DIFFERENT STAGES OF THE LIFECYCLE OF PLASMODIUM

Liver Stage

- Initial entry of the sporozoites into the lymph nodes and bloodstream on the way to the liver: antibodies.
- Evidence for antibodies to sporozoites and CSP to block binding to hepatocytes.
- Activation of complement by the antibodies activates complement fixation, phagocytosis, and lysis by cytotoxic NK and NKT cells through their Fc receptors. It also recognizes parasite neoantigens at the surface of infected hepatocytes and kills through an antibody-dependent cell-mediated mechanism by KC and NK cells.

- Since the liver stage of malaria infection is clinically silent, it has long been thought that parasites inside hepatocytes grow undetected by the innate immune system. However, a number of pattern recognition receptors (PRR) inside hepatocytes are stimulated leading to production of IFN-α. DNA of the parasite probably acts as a (pathogen) microbe-associated molecular pattern (MAMP) in the hepatocyte cytosol. This results in recruitment of macrophages, neutrophils, and lymphocytes.
- Cytotoxic CD8+ T cells that produce interferon-γ are mainly involved in killing of intrahepatic parasites. NK, NKT, and γδ T cells also kill intrahepatic parasites through secretion of type I interferons and IFN-γ.

This stage is "silent" without any clinical symptoms.

Erythrocyte Stage

- Merozoites in the bloodstream are targeted by antibodies to their erythrocyte attachment ligands, for example GPI anchoring MSPs (see Section 1) that can both opsonize and prevent entry.
- GPI is also a major MAMP, recognized by the PRR, Toll-like receptor 2 (TLR 2) on macrophages that induces pro-inflammatory cytokines such as IL-1 and TNFα.
- At the intra-erythrocyte stage, antibodies can mediate cellular killing, block adhesion of infected RBCs to endothelium, and neutralize parasite toxins to prevent the induction of excessive inflammation. Also, complement can lyse infected RBC.
- CD8+ T cells play little role at this stage but CD4+ T cells contribute to activation of macrophages.
- NK cells and γd T cells are involved at this stage. IFN-γ, perforins and granzymes produced by NK cells can also kill *P. falciparum* infected RBCs.

Male and female gametocytes in the bloodstream are also thought to be targeted by antibodies.

Recent studies have indicated a possible role for polymorphisms in the *Fc receptor gamma* (*FcR*γ) genes since they have been associated with either susceptibility or resistance to malaria. *FcR*γ are present on many immune cells and have several functions particularly in relation to antibody-mediated immune mechanisms.

Some innate immunity is also induced in the mosquito to the gametes and sporozoites of *Plasmodium*.

Malaria Immune Escape Mechanisms

Plasmodium has a number of "escape mechanisms" that allow it to avoid the immune response.

The **liver stage**: a) In order to invade hepatocytes, the sporozoites have first to pass through KC and endothelial cells. Binding of CSP to KC surface proteins produces high levels of intracellular cAMP that prevents the formation of reactive oxygen species (ROS). b) Sporozoite contact with KC also down-regulates inflammatory Th-1 cytokines and up-regulates anti-inflammatory Th-2 cytokines. c) KC apoptosis may be induced and expression of major histocompatibility complex (MHC)-I reduced. d) Once inside the hepatocyte, the parasitophorous vacuole prevents lysosomal degradation. e) Host heme oxygenase-1 (HO-1) also enhances the development of intrahepatic parasites through modulating the host inflammatory response.

The **erythrocyte stage**: a) The intracellular localization in the RBC escapes direct interaction with the immune cells. b) Lack of MHC-I molecule expression on the surface RBCs also avoids recognition by CD8+ T cells. c) Expression of variable antigenic surface proteins on infected RBCs helps the parasite to evade host immune responses. d) The phagocytic functions of macrophages are also hindered by *P. falciparum* malaria pigment or hemozoin. Hemozoin (released from ruptured erythrocytes) inside the macrophages reduces phagocytosis of infected RBC as well as reducing the production of radical oxygen intermediates.

PATHOGENESIS

There are three major pathologic mechanisms that account for the pathology and clinical signs and symptoms in patients with malaria (see below). These are as follows:

- The response of the **mononuclear phagocytic system** to the parasite leads to release of **cytokines** from host cells. Cytokines, such as IL-1 and TNF, act as endogenous pyrogens.
- Cyclical fever is caused by erythrocyte rupture leading to toxins being released into the bloodstream such as hemozoin. Defective production of red blood cells probably induced by cytokines and toxins including hemozoin and, leads to **anemia**. Modification of the erythrocyte membrane makes it less deformable and the cells are removed by the spleen, leading to **splenomegaly**. Additionally, antibody-mediated **lysis** of the erythrocytes occurs as parasite antigens are expressed on the surface of the erythrocytes.
- Obstruction of capillaries is caused by parasitized red blood cells. This is also caused by modification of the erythrocyte membrane as the surface molecules bind to adhesion molecules on the endothelium of the capillaries. This mechanism is particularly important in the pathogenesis of severe *P. falciparum* malaria such as development of cerebral malaria.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

In uncomplicated malaria, the first symptoms are fever, headache, chills, vomiting, muscle pains and diarrhea that appear 10–15 days after a person is infected. The pre-erythrocytic stage in the liver is asymptomatic. If not treated promptly with effective medicines, malaria can cause severe

illness that is often fatal. Patients may also have splenomegaly due to the sequestering of infected erythrocytes and anemia as the result of removal of erythrocytes.

With time, the fevers take on a periodicity depending on the species of malarial parasite.

Cyclical temperature fluctuations coincide with the rupture of erythrocytes and the release of merozoites into the bloodstream which, during the erythrocytic stage, occurs every 48 hours for *P. falciparum*, *P. ovale*, and *P. vivax*, and every 72 hours for *P. malariae* (see **Figure 28.3**). The cyclic temperature fluctuations are due to the release of toxins and hemozoin during erythrocyte rupture and GPI on fragments into the circulation. Through PRRs and other receptors, this induces the release of pyrogenic cytokines including TNFα, IL-1β, and IL6 by macrophages that cause rapid elevation of core temperature by acting on the hypothalamus.

COMPLICATIONS

In the most severe form of malaria (*P. falciparum*), there can be cerebral complications – **thrombosis** may occur due to occlusion of the cerebral vessels caused by the increased stickiness of the erythrocytes (see above). Multi-organ damage and renal failure can also result from the infection. The latter is uncommon in children with severe malaria who present with prostration, respiratory distress, severe anemia, and/or cerebral malaria.

Blackwater fever can also occur due to intravascular **hemolysis** leading to **hemoglobinuria** and kidney failure. *P. falciparum* also causes pulmonary **edema** and *P. malariae* causes **nephrotic syndrome**. In patients with splenomegaly, the spleen may rupture due to its large size.

The pattern of severe malaria is not fully understood, although genetic factors, age, and intensity of transmission determine susceptibility to severe anemia and cerebral malaria. Other infectious diseases can interact with malaria and modify susceptibility and/or severity of either disease. HIV infection with malaria increases the risk of both uncomplicated and severe malaria, and *Plasmodium* causes a transient increase in viral load, which might promote transmission of the virus.

Plasmodium and Epstein-Barr virus are concurrent risk factors for **Burkitt's lymphoma** in Africa.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

Clinical diagnosis of malaria can be quite difficult because of the overlap of symptoms with other infectious diseases.

There are three main diagnostic tests for malaria:

1. ***Microscopic diagnosis.***
   The gold standard for diagnosis is by a blood film. Thick or thin films are prepared from finger-prick blood. These are stained with Giemsa or Fields stain and viewed under the microscope. The degree of parasitemia is noted and the morphologic appearance of the trophozoite (or gametocyte) can be used to identify the species of malaria (see diagnostic stages in **Figure 28.2**).
   P. falciparum: ring forms, often resembling stereo headphones, may be seen at the edge of the cell. The red cell is of normal size (**Figure 28.4**).
   P. vivax: irregular large thick ring. The red cells are enlarged.
   P. ovale: regular dense ring. The red cell is oval.
   P. malariae: dense thick ring or band form. The red cell is normal in size.
   Blood films may also be stained with acridine orange to identify the species.
2. ***The rapid detection test (RDT).***
   Malaria can be diagnosed serologically by the detection of antibodies or antigen, the latter being used for rapid identification of acute cases. A number of commercial kits are available for rapid diagnosis of *P. falciparum*. Although many are highly sensitive, some are not as sensitive as a blood film. They detect *P. falciparum*, histidine-rich protein (HRP), for example, ParaSight F® kits, or lactate dehydrogenase (LDH), for example Kat-Quick® kits.
3. ***Molecular diagnosis (PCR).***
   The detection of parasite nucleic acids using polymerase chain reaction (PCR), although slightly more sensitive than smear microscopy, takes longer and is often not available quickly enough to be of value in establishing the diagnosis of malaria infection. PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT.

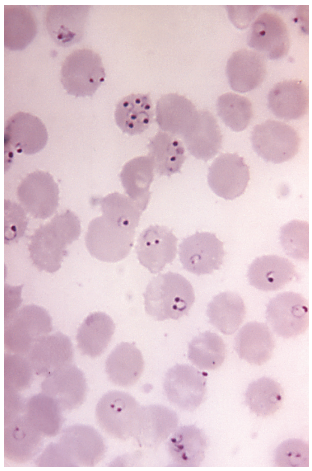

Figure 28.4 Blood film showing the presence of *P. falciparum* rings in human erythrocytes (×1125). Note that some red blood cells contain multiple parasites, which is more common with *P. falciparum* than other *Plasmodium* spp. *From the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #4884. Additional photographic credit is given to Dr Mae Martin who took the photo in 1971.*

DIFFERENTIAL DIAGNOSIS

- Meningitis or encephalitis: Lower respiratory tract infection: COVID-19;
- Influenza and other viral infections such as Epstein-Barr virus or cytomegalovirus;
- Gastroenteritis: Urinary tract infection: Lymphoma: Sepsis: Viral hepatitis: HIV seroconversion: Legionellosis: Leptospirosis.

Malaria may present with similar symptoms to other travel-related infections, including:

- Viral hemorrhagic fevers such as Lassa fever, Crimean-Congo hemorrhagic fever, Marburg, and Ebola – patients suspected of having a viral hemorrhagic fever require strict isolation.
- Enteric fevers such as typhoid or paratyphoid.
- Arboviruses such as Dengue, West Nile virus, and Japanese encephalitis.
- Rickettsial infection such as scrub typhus and relapsing fever.
- Trypanosomiasis.
- Rabies.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

Early diagnosis and prompt treatment are the basic elements of malaria control and this is crucial to prevent the development of complications and the majority of deaths from malaria. The two main approaches to malaria control are:

- drug treatment of the patients with infection, and
- prevention and control of the vector.

MANAGEMENT

Antimalarial treatment policies vary between countries depending on epidemiology of the disease, transmission, patterns of drug resistance, and political and economic contexts.

Historically, chloroquine has been the first-line drug for treating malaria and is still used for *P. ovale*, *P. vivax*, and *P. malariae* except in Indonesia, Sabah, and Papua New Guinea where there is high-level chloroquine resistance. *P. falciparum* became increasingly resistant to chloroquine and sulfadoxine-pyrimethamine combinations of drugs were used and are still used. However, there is now significant resistance to these drugs. Currently, the first-line drugs against *P. falciparum* and adopted by the WHO, are the artemisinin-based combination therapies (ACTs). Artemisinin, first isolated and developed in China in the 1980s from the plant *Artemisia annua*, has several derivatives including artesunate, artemether, and dihydro-artemisinin. ACTs work by combining a derivative of artemisinin, a fast-acting antimalarial endoperoxide, with a longer-lasting partner drug that continues to reduce the parasite numbers after the artemisinin has dropped below a therapeutic level. ACTs not only act on the asexual blood stages to alleviate symptoms but also on the gametes, therefore reducing the spread of the disease. From April 2019, the WHO recommended **artesunate** as the first-line treatment of **severe** malaria.

Companion drugs for the artemisinin derivatives include lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine, and chlorproguanil/dapsone.

One drug that targets the pre-erythrocytic phase of the disease is primaquine and this is still used to kill the hypnozoites of *P. vivax* and *P. ovale* in the liver. The FDA-approved tafenoquine, an anti-plasmodial 8-aminoquinoline derivative is indicated for the radical cure (prevention of relapse) of *P. vivax* malaria in patients aged 16 years or older who are receiving appropriate antimalarial therapy for acute *P. vivax*.

Sequencing of the parasite genome has aided and will continue to aid in the discovery of new drugs to treat malaria.

PREVENTION AND VECTOR CONTROL

Personal prevention measures include the use of a) indoor residual spraying (IRS) of walls in dwelling places using organochlorines, pyrethroides, organophosphates, carbamates or neonicotinoids and b) window screens, repellents (such as DEET) and wearing of light-colored clothes, long pants, and long-sleeved shirts. Insecticide-treated bed nets (ITNs) have been shown to reduce malaria illness, severe disease, and death due to malaria in endemic regions. In several African settings, ITNs have been shown to reduce the death of children under 5 years from all causes by about 20%. Due to the resistance of mosquitos to DDT, only two insecticide classes are approved for net treatment – pyrroles and pyrethroids. These have been shown to pose very low health risks to humans and other mammals but are toxic to insects and kill them. Resistance is, however, already developing against pyrethroides. Nets, to be effective, have to be retreated every 6–12 months and now the WHO recommends that long-lasting insecticidal nets (LLINs) be distributed to and used by all people ("universal coverage") in malarious areas, not only by the most vulnerable groups who are pregnant women and children under 5 years. These nets have been shown to be effective for up to 3 years.

Infection is also prevented by controlling the breeding cycle of the vector, which should significantly reduce the number of cases and rate of parasite infection. Control of the larval stage includes:

- Oils applied to the water surface, suffocating the larvae and pupae. Most currently used oils are rapidly biodegraded.
- Toxins from the bacterium *Bacillus thuringiensis var. israelensis* (Bti) can be applied in the same way as chemical insecticides. They are highly specific, affecting only mosquitoes, black flies, and midges.

- Insect-growth regulators such as methroprene are specific to mosquitoes and can be applied in the same way as chemical insecticides.

These approaches are generally considered to be environmentally friendly methods of mosquito control.

PREVENTION FOR TRAVELERS TO ENDEMIC AREAS

With travel to malaria endemic areas now very common, a number of drugs are given to prevent infection with the parasite, as well as steps taken to avoid mosquito bites. The drugs currently used in prophylaxis vary depending on which area you are travelling to. They include: Atovaquone-proguanil, doxycycline, mefloquine, tafenoquine and primaquine.

The patient described in this case had been taking anti-malarial tablets as a preventive measure against getting malaria. Possibilities as to why she still contracted malaria are: a) the patient was taking homeopathic tablets; b) the patient did not take the drugs on a regular basis; or c) the particular malaria species with which the patient was infected was resistant to the prophylactic treatment.

Information as to the appropriate drugs for the area currently to be visited is available at several websites, for example https://www.cdc.gov/malaria/travelers/drugs.html.

VACCINES

Development of highly effective and durable vaccines to prevent infection with *Plasmodium falciparum* and *P. vivax* have been a key priority. Strategies for producing vaccines to *P. falciparum* have been focused on its life cycle. Vaccines include:

- *whole sporozoite antigens* – using whole attenuated sporozoites,
- *liver-stage vaccines* – using thrombospondin-related adhesion protein linked to a multi-epitope string (ME-TRAP) inserted into a chimpanzee adenovirus vector,
- *blood-stage vaccines* – using immunodominant and non-polymorphic merozoite antigens (e.g. *P. falciparum* reticulocyte-binding protein homolog 5),
- *transmission-blocking vaccines* (targeting the sexual stages to impact the parasite's life cycle in the mosquito vector aiming to prevent sporozoite development and onward transmission) - using ookinete surface protein Pfs25 and the gametocyte antigens Pfs48/45 and Pfs230.

The most extensively tested vaccine candidate for prevention of *P. falciparum* malaria is RTS,S/AS01 which has been under development for several years. This vaccine directs immune responses against the major circumsporozoite protein (PfCSP) covering the surface of the infecting sporozoite. The WHO is now recommending widespread use of the RTS,S/AS01 (RTS,S) malaria vaccine among children in sub-Saharan Africa and in other regions with moderate to high *P. falciparum* malaria transmission. It is recommended that this malaria vaccine be given in a schedule of four doses to children from 5 months of age for the reduction of malaria disease and burden.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- The organism causing malaria is *Plasmodium*, a protozoan with a complex life cycle.
- Five species of *Plasmodium* are able to infect humans: *P. ovale*; *P. vivax*, and *P. malariae*, *P. knowlesi*, and *P. falciparum* being the most virulent species of malaria.
- The female *Anopheles* mosquito is the vector for malaria.
- The sporozoite is transmitted from the mosquito into the blood during a blood meal and localizes in the liver. Schizonts are produced through asexual reproduction.
- Liver schizonts rupture and release merozoites into the bloodstream, which invade and destroy erythrocytes giving rise to symptoms.
- Within the erythrocyte, the merozoite undergoes another round of schizogony to produce trophozoites.
- Differentiation of the trophozoite into gametocytes occurs in some erythrocytes.
- When another mosquito feeds it takes in the gametocytes, where they fuse to form a zygote, which becomes an oocyte. The oocyte matures into sporozoites, which migrate to the salivary gland of the mosquito and are inoculated into another human when the mosquito feeds.
- The malaria case incidence rate, total malaria cases and deaths fell from between 2000 and 2019. In 2020, there was an estimated 241 million malaria cases. The estimated number of malaria deaths stood at 627000 in 2020. The WHO African Region carries a disproportionately high share of the global malaria burden. In 2020, the region was home to 95% of all malaria cases and 96% of all malaria deaths. Children under 5 are still the most vulnerable group.
- Asia, Latin America, the Middle East, and parts of Europe are also affected.
- With increasing international travel, there continues to be a rise in the number of cases of malaria in travelers returning to non-malarious areas from countries where malaria is endemic. However, since the start of the SARS-2 pandemic with reduced global mobility it is likely that travel-associated malaria has decreased.
- During the SAR-2 pandemic, a recent modeling analysis by the WHO predicted a >20% rise in malaria morbidity and >50% mortality in sub-Saharan Africa as a result of 75% reduction in routine malaria control measures.

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- Pregnancy significantly increases the risk of malaria.
- Absence of Duffy blood group antigen in West Africans, sickle cell trait, and glucose 6 phosphate dehydrogenase (G6PD) deficiency are examples of genetic factors leading to reduced infection rates for malaria.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- The parasite uses a number of "escape mechanisms" at both the liver stage and erythrocyte stages to avoid the immune response. These include different life cycle forms and direct immunosuppression by the parasite.
- Some immunity does develop to the parasite with continuous infection but this is weak and does not eliminate the parasite.
- Immune mechanisms that exist are different against the different stages of the life cycle. In the pre-erythrocyte phase: antibodies (and complement) against the sporozoites, phagocytosis, NK and cytotoxic T cells against the hepatocytes. Erythrocyte stage: antibodies against the merozoites and gametes. NK and $\gamma\delta$ T cells. CD8+ T cells play little role but CD4+T cells contribute to macrophage activation.
- Pathogenesis occurs due to: (a) cytokine release leading to spikes of fever at regular intervals, (b) destruction of erythrocytes leading to anemia, and (c) obstruction of capillaries due to binding of erythrocytes through parasite encoded surface molecules to endothelium.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- In uncomplicated malaria, the first symptoms are fever, headache, chills, vomiting, muscle pains, and diarrhea that appear 10–15 days after a person is infected.
- Patients can develop splenomegaly and anemia.
- Cyclical temperature fluctuations coincide with the rupture of erythrocytes and the release of merozoites into the bloodstream which, during the erythrocytic stage, occurs every 48 hours for *P. falciparum*, *P. ovale*, and *P. vivax*, and every 72 hours for *P. malariae*. This gives rise to fevers of differing periodicity.
- The cyclic temperature fluctuations are due to the release of toxins and hemozoin during erythrocyte rupture and GPI on fragments into the circulation. Through PRRs and other receptors, this induces the release of pyrogenic cytokines including TNFα, IL-1β, IL6 by macrophages that cause rapid elevation of core temperature by acting on the hypothalamus.
- Complications include cerebral malaria where thrombosis may occur (with *P. falciparum*, severe anemia and blackwater fever).
- Co-infection with HIV increases the risk of both uncomplicated and severe malaria, and *Plasmodium* causes a transient increase in viral load, which might promote transmission of the virus.
- *Plasmodium* and Epstein-Barr virus are concurrent risk factors for Burkitt's lymphoma in Africa.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- There are three diagnostic tests.
- Microscopic diagnosis: the gold standard is by a blood film; the morphologic appearance of the trophozoite or gametocyte can be used to identify the species.
- The rapid detection test (RDT): malaria can be diagnosed serologically using the detection of antibodies or antigens; there are kits available.
- Molecular diagnosis: PCR used to detect parasite nucleic acids; useful to confirm species after microscopic or RDT diagnosis.
- Clinical diagnosis of malaria can be quite difficult because of the overlap of symptoms with many other infectious diseases, e.g. meningitis, enteric fevers, rickettsial infections, rabies, etc.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- The two main approaches to malaria control are drug treatment of the patients with infection and prevention and control of the vector.
- Most drug treatments target the erythrocytic stage of the infection.
- Chloroquine has been the first-line drug for treating malaria and is still in use for *P. ovale*, *P. vivax*, and *P. malariae*. *P. falciparum* has become increasingly resistant to chloroquine.
- Drugs currently used against *P. falciparum* and adopted by WHO are the artemisinin-based combination therapies (ACTs). Companion drugs for the Artemisinin derivatives include lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine, and chlorproguanil/dapsone.
- Primaquine targets the pre-erythrocytic stage of the disease and is used to kill the hypnozoites of *P. ovale* and *P. vivax* in liver cells.
- ACTs act not only on the asexual blood stages to alleviate symptoms but also on the gametes, therefore reducing the spread of the disease.
- Sequencing of the parasite genome has aided and will continue to aid in the discovery of new drugs to treat malaria.
- Personal prevention measures include the use of IRS for spraying internal surfaces and window screens, repellents (such as DEET), and wearing of light-colored clothes, long pants, and long-sleeved shirts.
- Insecticide-treated bed nets (ITNs) reduce malaria illness, severe disease, and death due to malaria in endemic regions. In several African settings, ITNs have been shown to reduce the death of children under 5 years from all causes by about 20%. Due to the resistance of mosquitos to DDT, only two insecticide classes are now approved for net treatment – pyrroles and pyrethroids.
- Nets have to be retreated every 6–12 months and now the WHO recommends that long-lasting insecticidal nets (LLINs) be distributed to and used by all people ("universal coverage") in malarious areas.

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- Mosquito larval control is also used. This is achieved by using oils applied to the water surface, toxins from *Bacillus thuringiensis*, and insect growth regulators such as methoprene.
- Prophylactic drugs for international travelers depend on drug resistance in the endemic areas to be visited and include atovaquone-proguanil, doxycycline, mefloquine, tafenoquine, and primaquine.
- There are a number of strategies for producing vaccines to *P. falciparum* including liver-stage vaccines, blood-stage vaccines, and transmission-blocking vaccines. The WHO have recently recommended the widespread use of a vaccine (RTS,S) against the major circumsporozoite protein (PfCSP) covering the surface of the infecting sporozoite. The vaccine should be used to treat children in sub-Saharan Africa and in other regions with moderate to high *P. falciparum* malaria transmission.

FURTHER READING

Goering R, Dockrell HM, Zuckerman M, Chiodini PL, (eds). Mims' Medical Microbiology and Immunology, 6th edition. Academic Press/Elsevier, Cambridge, 2018.

Male D, Peebles S, Male V. Immunology, 9th edition. Elsevier, Philadelphia, 2020.

Murphy K, Weaver C. Janeway's Immunobiology, 9th edition. Garland Science, New York/London, 2016.

REFERENCES

Amiah MA, Ouattara A, Okou DT, et al. Polymorphisms in Fc Gamma Receptors and Susceptibility to Malaria in an Endemic Population. Front Immunol, 11: 561142, 2020.

Chu CS, White NJ. The Prevention and Treatment of Plasmodium vivax Malaria. PLoS Med, 18: e1003561, 2021.

Cowman AF, Tonkin CJ, Tham W-H, Duraisingh M. The Molecular Basis of Erythrocyte Invasion by Malaria Parasites. Cell Host Microbe, 22: 232–245, 2017.

Draper SJ, Sack BK, King CR, et al. Cell Malaria Vaccines: Recent Advances and New Horizons. Host Microbe, 24: 43–56, 2018.

Gowda DC, Wu X. Parasite Recognition and Signaling Mechanisms in Innate Immune Responses to Malaria. Front Immunol, 9: 3006, 2018.

Jaskiewicz E, Jodłowska M, Kaczmarek R, Zerka A. Erythrocyte Glycophorins as Receptors for Plasmodium merozoites. Parasit Vectors, 12: 317, 2019.

Kurtovic L, Reiling L, Opi DH, Beeson JG. Recent Clinical Trials Inform the Future of Malaria Vaccines. Commun Med, 1: 26, 2021.

Liu Q, Jing W, Kang L, et al. Trends of the Global, Regional and National Incidence of Malaria in 204 Countries from 1990 to 2019 and Implications for Malaria Prevention. J Travel Med, 28: taab046, 2021.

Milner, DA, Jr. Malaria Pathogenesis. Cold Spring Harb Perspect Med, 8: a025569, 2018.

O'Leary, K. A Malaria Vaccine at Last. Nat Med, 27: 2057, 2021.

Talapko J, Škrlec I, Alebic' T, et al. Malaria: The Past and the Present. Microorganisms, 7: 179, 2019.

Thriemer K, Ley B, von Seidlein L. Towards the Elimination of Plasmodium vivax Malaria: Implementing the Radical Cure. PLoS Med, 18: e1003494, 2021.

Tse EG, Korsik M, Todd MH. The Past, Present and Future of Anti-Malarial Medicine. Malar J, 18: 93, 2019.

WEBSITES

BMC, Part of Springer Nature, Malaria Journal: http://www.malariajournal.com/

Centers for Disease Control and Prevention, Parasites – Malaria, 2022: https://www.cdc.gov/parasites/malaria

European Centre for Disease Prevention and Control, Malaria, Annual Epidemiological Report for 2018, 2018: https://www.ecdc.europa.eu/sites/default/files/documents/malaria-annual-epidemiological-report-2018.pdf

Map – The Malaria Atlas Project: https://malariaatlas.org/

National Institute for Health and Care Excellence, Malaria, treatment: https://bnf.nice.org.uk/treatment-summary/malaria-treatment.html

World Health Organization, Malaria, 2022: https://www.who.int/news-room/fact-sheets/detail/malaria

Students can test their knowledge of this case study by visiting the Instructor and Student Resources: [www.routledge.com/cw/lydyard] where several multiple choice questions can be found.