MALARIA

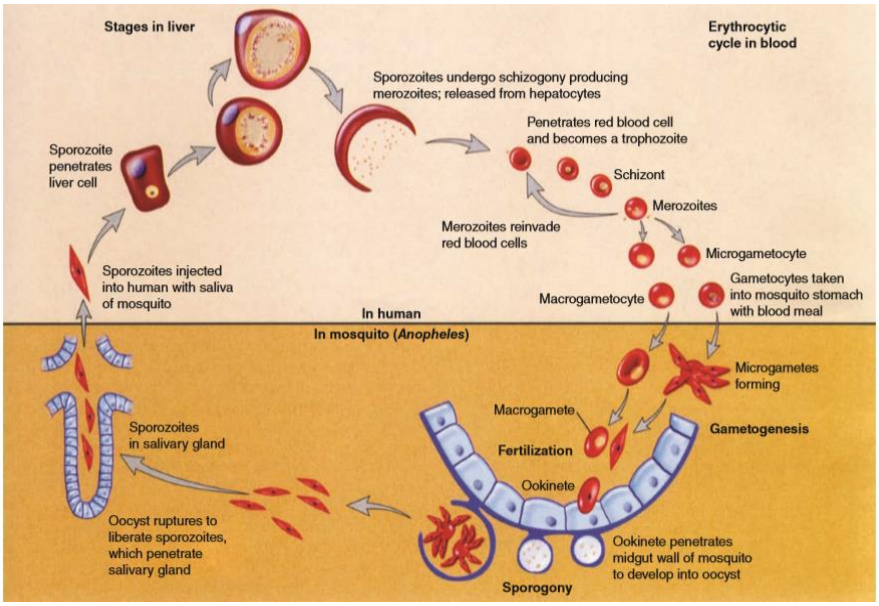
Apicomplexa are obligate intracellular protozoan parasites.
Why are they named so? Name derived from the complex of organelles located at the apical end of parasite life cycle stages that are involved in penetrating cells. These organelles include the rhoptries, micronemes, and associated microtubular complexes located in this region of the parasite.
Diseases caused by Apicomplexans:

- Malaria and toxoplasmosis, are common diseases in humans; together, they affect more than one-third of the world's population and kill or deform perhaps a million neonates and children each year.
- Cryptosporidium, Cyclospora, and Isospora are Apicomplexa that can cause diarrhea.
- Babesia, a relative of malaria, and transmitted by ticks, is also a member of this group.

PLASMODIUM SPECIES

The plasmodia are Apicomplexa in which the sexual and asexual cycles of reproduction are completed in different host species. This statement is justified by their lifecycle (Figure given below).
Sexual phase occurs within the gut of mosquitoes and results in the formation of a motile zygote, the ookinete. These arthropods subsequently transmit the parasite as sporozoites while feeding on a vertebrate host.
Asexual phase- within the vertebrate, the plasmodia reproduce asexually, first in the liver and then in erythrocytes; they eventually burst from the erythrocyte and invade other uninvolved RBCs. This event produces periodic fever and anemia in the host, a disease process known as malaria.
Five species of plasmodia are known to infect humans. They are: *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium knowlesi,* and *Plasmodium falciparum.*

LIFE CYCLE



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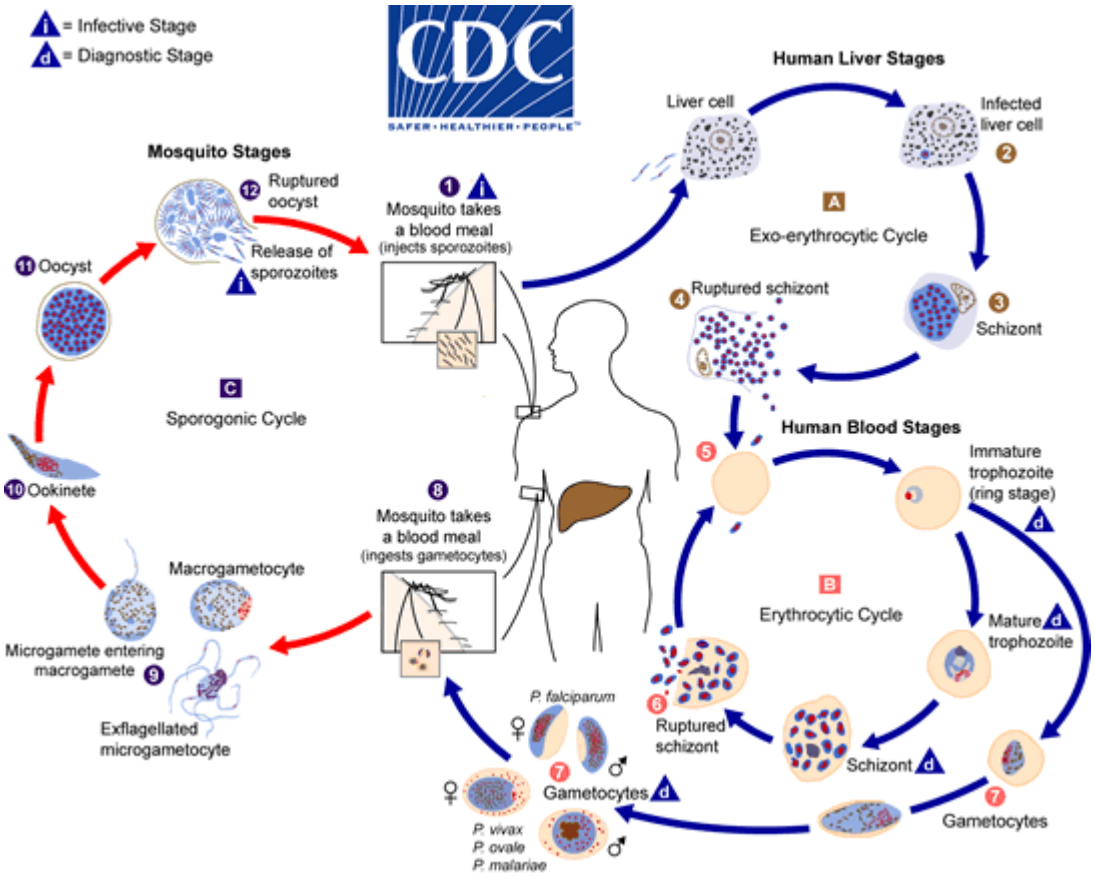

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶ . Sporozoites infect liver cells ❷ and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver (if untreated) and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❺. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿ which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶ into a new human host perpetuates the malaria life cycle.

MORPHOLOGY OF ERYTHROCYTIC PARASITES

- The parasitized erythrocyte in *P vivax* and *P ovale* infections is pale and enlarged and contains numerous Schüffner dots. All asexual stages (trophozoite, schizont,

merozoite) may be seen simultaneously. Cells infected by *P ovale* are elongated and frequently irregular or fimbriated in appearance.

- In *P malariae* infections, the RBCs are not enlarged and contain no granules. The trophozoites often present as "band" forms, and the merozoites are arranged in rosettes around a clump of central pigment.
- In *P falciparum* infections, the rings are very small and may contain two chromatin dots rather than one. There is often more than one parasite per cell, and parasites are frequently seen lying against the margin of the cell. Intracytoplasmic granules known as Maurer dots may be present, but are often cleft shaped and fewer in number than Schüffner dots. Schizonts and merozoites of *P falciparum* are not present in the peripheral blood as they are sequestered in postcapillary venules. Gametocytes are large and banana shaped.
- *Plasmodium knowlesi* shares many of the morphologic characteristics of *P malariae*, but can be distinguished from the latter by its fever cycle and diagnostically by using polymerase chain reaction (PCR). All the features are tabulated below:

TABLE 51–1 Differential Characteristics of *Plasmodium* Species

CHARACTERISTICS	P VIVAX	P OVALE	P MALARIAE	P FALCIPARUM	P KNOWLESI
Erythrocyte					
Enlarged, pale	+	+	–	–	–
Oval, fimbriated	–	+	–	–	–
Schüffner dots	+	+	–	–	–
Maurer dots	–	–	–	+	–
Parasite					
All asexual stages seen	+	+	+	–	+
Band forms	–	–	+	–	+
Double infections	–	–	–	+	–
Double chromatin dots	–	–	–	+	–
Banana-shaped gametocytes	–	–	–	+	–

PHYSIOLOGY

- Parasites vary in ability to attack subpopulations of erythrocytes
- RBC Duffy antigen and glycoprotein A are RBC receptors

Protective Effect of Sickle Cell Trait Against Malaria

Certain RBC genetic polymorphisms may also affect parasitism. The sickle cell gene is caused by a single amino acid mutation (valine instead of glutamate at the 6th position) in the beta chain of the hemoglobin gene. Inheritance of this mutated gene from both parents leads to sickle cell disease and people with this disease have shorter life expectancy. On the contrary, individuals who are carriers for the sickle cell disease (with one sickle gene and one normal hemoglobin gene, also known as sickle cell trait) have some protective advantage against malaria. As a result, the frequencies of sickle cell carriers are high in malaria-endemic areas.

Parasite growth appears to be retarded in RBCs heterozygous for hemoglobin S (SA) when they are exposed to conditions of reduced oxygen tension such as those which might be present in the visceral capillaries. These conditions cause the hemoglobin in infected cells to polymerize, rendering it unusable by the parasite. In essence, the parasite starves to death. Sickling may also render the erythrocyte more susceptible to phagocytosis or directly damage the parasite.

A similar protective effect may be exerted by **hemoglobins C, D, and E; thalassemias; and glucose-6-phosphate dehydrogenase (G6PD) or pyridoxal kinase deficiencies**, because these abnormalities have also been found more frequently in malarious areas. The protection in these conditions may be related to the increased susceptibility of such RBCs to oxidant stress. In thalassemia, the protection may also be related in part to the production of fetal hemoglobin, which retards maturation of *P falciparum*, as well as an increased binding of antibodies to modified parasitic antigens (neoantigens) presenting on the surface of the erythrocytes.

Changes introduced by malaria parasite: Once invasion has occurred; malaria parasites may induce several changes in the erythrocytic membrane. These include alteration of its lipid concentration, modification of its osmotic properties, and incorporation of parasitic neoantigens, rendering the RBCs susceptible to immunologic attack.

Plasmodium vivax and P ovale stimulate the production of caveolae–vesicle complexes, which are visualized as Schüffner dots in stained smears. In *P falciparum* infections, electron-dense elevated knobs or excrescences form on the RBC surface. These produce a strain-specific, high–molecular-weight adhesive protein (PfEMP1), which mediates binding to receptors on the endothelium of capillaries and postcapillary venules of the brain, placenta, and other organs, where they can produce obstruction and microinfarcts. Malarial parasites generate energy by the anaerobic metabolism of glucose. They appear to satisfy their protein requirements by the degradation of hemoglobin within their acidic food vacuoles, resulting in the formation of the malarial pigment (hemozoin) mentioned previously. It has been estimated that the average plasmodium destroys between 25% and 75% of the hemoglobin of its host erythrocyte. Unlike their vertebrate hosts, malarial parasites synthesize folates de novo. Thus, **antifolate antimicrobials such as pyrimethamine are effective antimalarial agents.**

EPIDEMIOLOGY

Distribution in tropical areas worldwide. *Plasmodium vivax* is the most widely distributed of the four species. *Plasmodium falciparum* is the dominant organism of the tropics. Malaria kills approx. 700,000 annually, mostly children.

PATHOGENESIS

Fever, anemia, circulatory changes, and **immunopathologic phenomena** characteristic of malaria are all the result of the erythrocytic cycle of the plasmodia. There are no clinical signs of infection associated with the liver phase of infection.

Fever- the hallmark of malaria, appears to be initiated by the process of RBC rupture that leads to the liberation of a new generation of merozoites. To date, all attempts to detect the factor(s) mediating the fever have been unsuccessful. Fever may arise due to: release of parasite-derived pyrogens the time of red cell rupture, or release of interleukin-1 (IL-1)

and/or tumor necrosis factor (TNF) from macrophages involved in the ingestion of parasitic or erythrocytic debris.
Early in malaria, RBCs appear to be infected with malarial parasites at several different stages of development, each inducing erythrocyte destruction at a different time. The resulting fever is irregular and hectic. Because temperatures higher than 40°C destroy mature parasites, a single population eventually emerges, parasite replication is synchronized, and fever occurs in distinct paroxysms at 24 hour (*P knowlesi*), 48 hour (*P falciparum*, *P vivax*, *P ovale*) or, in the case of *P malariae*, 72 hour intervals.

Anemia- Parasitized erythrocytes are phagocytosed by a stimulated reticuloendothelial system or are destroyed at the time of parasite-induced cell rupture, releasing toxic products. This not only results in destruction of infected cells, but noninfected ones as well, resulting in an anemia that may be disproportionate to the degree of parasitism.
Depression of marrow function, sequestration of erythrocytes within the enlarging spleen, and accelerated clearance of nonparasitized cells all appear to contribute to the anemia.
When hemolysis is massive, hemoglobinuria develops, resulting in the production of dark urine. This process in conjunction with malaria is known as **blackwater fever**.

Circulatory Changes- The high fever results in significant vasodilatation. In *P falciparum* malaria, vasodilatation leads to a decrease in the effective circulating blood volume and hypotension, which may be aggravated by other changes in the small vessels and capillaries. The intense parasitemias of *P falciparum* is capable of producing comas and the adhesion of infected RBCs to the endothelium of visceral capillaries can impair the microcirculation and precipitate tissue hypoxia, lactic acidosis, and hypoglycemia. Although all deep tissues are involved, the brain is the most intensely affected resulting in what has been described as **cerebral malaria**.

Cytokines: Elevated levels of IL-1 and TNF are consistently found in patients with malaria, essential part of the host's immune response to malaria. TNF levels increase with parasite density, and high concentrations appear harmful. TNF has been shown to cause upregulation of endothelial adhesion molecules; high concentrations might precipitate cerebral malaria by increasing the sequestration of *P falciparum*-parasitized erythrocytes in the cerebral vascular endothelium. Alternatively, excessive TNF levels might precipitate cerebral malaria by directly inducing hypoglycemia and lactic acidosis.

- Thrombocytopenia and nephritis common.

IMMUNITY

Once infected, the host quickly mounts a stage-, species-, and strain-specific immunologic response that typically limits parasite multiplication and moderates the clinical manifestations of disease, without eliminating the infection.

- A prolonged recovery period marked by recurrent exacerbations in both symptoms and number of erythrocytic parasites follows.
- The exact mechanisms involved in this recovery are uncertain. In simian and probably in human malaria, recovery is known to require the presence of both T and B

lymphocytes. It is probable that the T lymphocytes act partially through their helper effect on antibody production.

- B lymphocytes begin production of stage- and strain-specific antiplasmodial antibodies within the first 2 weeks of parasitemia. With the achievement of high levels of antibodies, the number of circulating parasites decreases.
- The infrequency with which malaria occurs in young infants has been attributed to the transplacental passage of such antibodies. It is uncertain whether they are directly lethal, act as opsonizing agents, or block merozoite invasion of RBCs.
- Antibody responses are also detectable against sporozoites and, because of this, much attention has been given to develop a vaccine against this parasite stage. Because sporozoites clear so quickly from the peripheral circulation, however, they may escape immune detection and all it would take is one to initiate hepatic schizogony resulting in blood stage infection.
- In *P falciparum* malaria, chronic infection is maintained through the insertion of highly polymorphic variant antigens that are inserted into the infected erythrocyte membrane. With *P falciparum*, the disease typically does not exceed 1 year, but with *P malariae* the erythrocytic infection can be extremely persistent, lasting in one case up to 53 years.
- In a closely related simian malaria, splenectomy results in rapid cure, suggesting that suppressor T lymphocytes in the spleen may play a protective role. In infection with *P vivax* and *P ovale*, latent hepatic infection may result in the discharge of fresh merozoites into the bloodstream after the disappearance of erythrocytic forms. This phenomenon, known as relapse, can maintain infection for 3 to 5 years or longer.

MANIFESTATIONS

The incubation period between the bite of the mosquito and the onset of disease is approximately 2 weeks. In the United States, the interval between entry into the country and onset of disease exceeds 1 month in 25% of P falciparum infections and 6 months in a similar proportion of P vivax cases.

The **clinical manifestations** of malaria vary with the species of plasmodia but typically include **chills, fever, splenomegaly**, and **anemia**.

The hallmark of disease is the malarial paroxysm. This manifestation begins with a **cold stage**, which persists for 20 to 60 minutes. During this time, the patient experiences continuous rigors and feels cold. With the consequent increase in body temperature, the rigors cease and vasodilatation commences, ushering in a hot stage. The temperature continues to rise for 3 to 8 hours, reaching a maximum of 40°C to 41.7°C before it begins to fall. The **wet stage** consists of a decrease in fever and profuse sweating. It leaves the patient exhausted but otherwise well until the onset of the next paroxysm. Typical paroxysms first appear in the second or third week of fever, when parasite replication within erythrocytes becomes synchronized. In *falciparum* malaria, synchronization may never take place, and the fever may remain hectic and unpredictable. The first attack is often severe and may persist for weeks in the untreated patient. Eventually the paroxysms become less regular, less frequent, and less severe. Symptoms finally cease with the disappearance of the parasites from the blood. In falciparum malaria, capillary blockage can lead to several serious complications. When the central nervous system is involved (cerebral malaria), the patient may develop

delirium, convulsions, paralysis, coma, and rapid death. Acute pulmonary insufficiency frequently accompanies cerebral malaria, killing about 80% of those involved. When splanchnic capillaries are involved, the patient may experience vomiting, abdominal pain, and diarrhea with or without bloody stools. Jaundice and acute renal failure are also common in severe illness. These pernicious syndromes generally appear when the intensity of parasitemia exceeds 100 000 organisms per cubic millimeter of blood. Most deaths occur within 3 days.

DIAGNOSIS

- Malarial parasites can be demonstrated in **stained smears of the peripheral blood** in virtually all symptomatic patients. Typically, capillary or venous blood is used to prepare both thin and thick smears, which are stained with Wright or Giemsa stain and examined for the presence of erythrocytic parasites. Thick smears, in which erythrocytes are lysed with water before staining, concentrate the parasites and allow detection of very mild parasitemia.
- To improve the standard thin and thick smear method- procedure involves acridine orange staining of centrifuged parasites in quantitative buffy coat (QBC) tubes. Although it is expensive, this requires a fluorescence microscope and permits less reliable parasite speciation; its rapidity and ease of use make it attractive to laboratories that are only occasionally called on to identify patients with malaria.
- Simple, **specific card antigen** detection procedures are now available.
- The most widely used test, **ParaSight F**, detects a protein (HRP2) excreted by *P falciparum* within minutes. The test can be performed under field conditions and has a sensitivity of more than 95%.
- A second rapid test, **OptiMAL**, detects parasite lactate dehydrogenase, and, unlike ParaSight F, can distinguish between *P falciparum* and *P vivax*.
- Numerous **PCR** assays for the laboratory diagnosis of malaria.

TREATMENT

The complete treatment of malaria requires the destruction of erythrocytic schizonts, hepatic schizonts, and erythrocytic gametocytes. The first terminates the clinical attack, the second prevents relapse, and the third renders the patient noninfectious to Anopheles and thus breaks the cycle of transmission. Unfortunately, no single drug accomplishes all three goals.

Termination of Acute Attack-Several agents can destroy asexual erythrocytic parasites. **Chloroquine, a 4-aminoquinoline-** has been the most commonly used. It acts by inhibiting the degradation of hemoglobin, thereby limiting the availability of amino acids necessary for growth. It has been suggested that the weak basic nature of chloroquine also acts to raise the pH of the food vacuoles of the parasite, inhibiting their acid proteases and effectiveness.

Other schizonticidal agents include **quinine/quinidine, antifolate–sulfonamide combinations, mefloquine, halofantrine,** and the **artemisinins**.

The **artemisinins** are also unique in their capacity to reduce transmission by preventing gametocyte development.

Plasmodium falciparum has now become variably resistant to all drug groups, including the artemisinin compounds. There is a growing consensus that the most effective way to slow the

further development of drug-resistant strains of *P falciparum* is to use one of the artemisinins in combination with quinine/quinidine, antifolate–sulfonamide compounds, mefloquine, or halofantrine.

Radical Cure- In *P vivax* and *P ovale* infections, hepatic schizonts persist and must be destroyed to prevent reseeding of circulating erythrocytes with consequent relapse. **Primaquine**, an **8-aminoquinoline**, is used for this purpose.

PREVENTION

- Personal protection-Mosquito protection with screens and repellents
- Chemoprophylaxis choice must consider resistance in area
- General- Reduce human reservoir contact with mosquitoes and eradicate mosquitoes

Attempts at complete eradication have failed

Vaccines: The establishment of a continuous *in vitro* culture system, successful propagation of malaria in laboratory raised mosquitos, development of the hybridoma technique allowed the preparation of monoclonal antibodies with which antigens responsible for the induction of protective immunity could be identified and recombinant DNA procedures enabled scientists to clone and sequence the genes encoding such antigens, permitting the amino acid structure to be determined and peptide sequences suitable for vaccine development to be identified.

In 2012, a phase III clinical trial consisting of a protein fragment from the outer surface of *P falciparum*, fused with a hepatitis B virus protein, and combined with an immune adjuvant (subunit vaccine) reduced episodes of both clinical and severe malaria in children aged 5 to 17 months by approximately 50%. This vaccine targets the preerythrocytic stage of the disease. Studies are continuing, with development of new adjuvants that may be even more potent. Other attenuated sporozoite vaccines are currently in clinical trial.

References

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