MALARIA

‘Roll Back Malaria, Protect Women & Children’
Despite all differences in biological detail and clinical manifestations, every parasite's existence is based on the same simple basic rule:

A PARASITE CAN BE CONSIDERED TO BE THE DEVICE OF A NUCLEIC ACID WHICH ALLOWS IT TO EXPLOIT THE GENE PRODUCTS OF OTHER NUCLEIC ACIDS - THE HOST ORGANISMS

John Maynard Smith
Today:

- The history of malaria
- The biology of malaria
- Host-parasite interaction
- Prevention and therapy
About 4700 years ago, the Chinese emperor Huang-Ti ordered the compilation of a medical textbook that contained all diseases known at the time. In this book, malaria is described in great detail - the earliest written report of this disease.
Tests show King Tut died from malaria, study says

CHICAGO (Reuters) - King Tutankhamen, the teen-aged pharaoh whose Egyptian tomb yielded dazzling treasures, limped around on tender bones and a club foot and probably died from malaria, researchers said on Tuesday.

There has been speculation about the fate of the boy king, who died sometime around 1324 BC probably at age 19, since the 1922 discovery of his intact tomb in Egypt's Valley of Kings.

Hawass et al., Journal of the American Medical Association 303, 2010, 638
Today, malaria is considered a typical „tropical“ disease. As little as 200 years ago, this was quite different. And today it is again difficult to predict if global warming might cause a renewed expansion of malaria into the Northern hemisphere.
A pious myth relates that in the year 452, the the ardent prayers of pope Leo I prevented the conquest of Rome by the huns of king Attila. A more biological consideration might suggest that the experienced warrior king Attila was much more impressed by the information that Rome was in the grip of a devastating epidemic of which we can assume today that it was malaria.
In Europe, malaria was a much feared disease throughout most of European history. An example: in the coastal counties of Southern England, infant mortality due to malaria in the sixteenth and seventeenth centuries was at about the same level as it is nowadays in Nigeria.

Comparison of infant mortality between coastal and inland districts of Essex County, England from 1551 - 1750
Until fairly recently, malaria was found in many places . . .

. . . in Washington DC

. . . in the old Rome

. . . in modern Italy
1879: Edwin Klebs and Corrado Tommasi-Crudeli describe for
the first (and last . . .) time the *Bacillus malariae* as the
causative agent of malaria

(What is a bit embarrassing with this story is that Edwin Klebs was at that time
Professor of Pathology at the University of Bern . . .)

(However, he has also contributed more
lasting achievements to microbiology, and
the bacterium *Klebsiella* is named in
his honour)
In November 1880, the French military doctor Charles Louis Alphonse Laveran published observations made with blood from malaria patients in Algeria:

„Note sur un nouveau parasite trouvé dans le sang de plusieurs malades atteints des fièvres palustres“

Bull. de l’Académie de Méd. 9, 1880, 1235

„Monsieur Moustiques“
The Mosquito Connection

Giovanni Maria Lancisi

„De noxiis paludum effluviis eorumque remediis“

1717

Ronald Ross

„On some peculiar pigmented cells found in two mosquitoes fed on malarial blood“

Sir Ronald Ross
poet, writer, painter,
military doctor

While serving in India, he discovered the transmission cycle of Plasmodium, and he developed a first mathematical model of malaria epidemiology

Nobel Prize 1902
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The phylogeny of the apicomplexa (Chromalveolata)
Plasmodium belongs to the order of the Apikomplexa

Many species of Plasmodium are parasites of various vertebrates (e.g. P. gallinaceum in birds, P. berghei or P. yoelii in the mouse, or P. knowlesi in the monkey)

P. falciparum is restricted to humans and chimpanzees. It may represent a new species that originated only about 5000 - 20'000 years ago
The apikoplast

- a characteristic organelle of the Apicomplexa (Plasmodium spp., Eimeria spp., Theileria spp., Toxoplasma gondii, ...).
- contains a circular genome with many genes that correspond to those of plant or algal plastids
- may have originated via endosymbiosis
- fulfills many specific functions (e.g. the syntheses of fatty acids, heme and isoprenoids). Contains enzymatic functions that are absent in other eukaryotes).
- is essential for cell proliferation
- might be an interesting target for the development of novel chemotherapeutic agents
Current model of the origin of the apicomplexans from photosynthetic algae

Moore et al., Nature 451, 2008, 959
The genome of *Plasmodium falciparum*

- haploid
- 14 chromosomes
- ca. 30 Mb
- extremely high AT-content (ca. 80 %)
- many genes contain numerous introns
- mRNAs often have very long 5′-UTRs

Genome databases: http://PlasmoDB.org
http://EuPathDB.org

Completely sequenced genomes:
- *P. falciparum* Human
- *P. vivax* Human
- *P. knowlesi* Human and monkey
- *P. yoelii* Rodents
- *P. chabaudi* Rodents
- *P. berghei* Rodents
Malaria Fact Sheet

- 2.5 billion people at risk
- 300 - 500 million cases every year, with increasing tendency
- ca. 1 million deaths per year
- every 40 seconds a child dies from malaria
- various factors lead to the prediction that the number of disease cases will double over the next twenty years
- 90 % of all malaria cases occur in Africa
- in the endemic areas of Asia or South America, an average person is stung by a malaria vector about 5 times a year. In certain regions of Africa this happens over 1000 times!
The various human malarias I

**Malaria quartana:** - *Plasmodium malariae*
- Fever attacks with a periodicity of 72 h
- good prognosis except when kidney complications arise
- risk of relapse up to 30 years after infection

**Malaria tertiana:** - *Plasmodium vivax* or *P. ovale*
- Fever attacks with a periodicity of 48 h
- rarely fatal
- frequent relapses up to two years after infection

**Malaria tropica:** - *Plasmodium falciparum*
- irregular fever attacks
- life threatening, particularly for small children between 6 months and five year
- no risk of relapses
## The various human malarias II

<table>
<thead>
<tr>
<th>organism</th>
<th>P. falciparum</th>
<th>P. vivax</th>
<th>P. ovale</th>
<th>P. malariae</th>
</tr>
</thead>
<tbody>
<tr>
<td>duration of liver stage (days)</td>
<td>5-6</td>
<td>8</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>formation of hypnozoites</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>type of erythrocyte infected by parasite</td>
<td>preferentially young ECs, but all ages can be infected</td>
<td>reticulocytes</td>
<td>reticulocytes</td>
<td>old ECs</td>
</tr>
<tr>
<td>maximal duration of infection (years)</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

- ECs: Erythrocytes
- NO: No
- YES: Yes

*(ts 03/07)*
About 400 species of Anopheles are known. Sixty six of them are able to transmit Plasmodium. The most efficient vector is *A. gambiae*. It is long lived, has a short generation cycle (about 10 days), prefers humans to all other potential blood donors, adapts well to changes in the environment, and reaches high population densities.

Transmission of malaria can happen wherever the temperature is between 16 and 33 °C, the air is moderately humid, and the altitude is less than 2000 m above sea level.
Malaria pathogenesis

- High fever (> 40 °C) alternating with shivers
- Anaemia and hypoferremia
- Disruption of liver functions
- Lactic acidosis
- Hypoglycemia
- Disruption of placental functions
- Cerebral malaria (predominantly in infants) leads to convulsions, coma and death
- Opportunistic bacterial infections
- „Black water fever“ in patients with G6PD deficiency
- Edema of the lung - respiratory problems
- Kidney failure
Today:

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Malaria infection I: The life cycle

- Proliferation in erythrocytes (schizonts)
- Infected erythrocytes burst and release infectious merozoites
- Gametocyte
Infectious sporozoites get injected into the skin together with saliva during Anopheles bite.
The sinusoids of the liver form a network of blood vessels where blood from the nutrient rich portal vein and the oxygen-rich hepatic artery mix, and that is drained by the central vein. Hepatocytes are organized in thin layers (the cords) with excellent access to the sinusoids. The Kupffer cells are macrophages that eliminate the bacteria that might have entered the blood
Malaria infection III: Liver invasion

Sporozoites leave the bloodstream in the liver and invade hepatocytes

Prudêncio et al., Nature Reviews Microbiology, 4, 2006, 849
Sporozoites may migrate through several hepatocytes before they decide to stay and proliferate. What are they looking for?

Prudêncio et al., Nature Reviews Microbiology, 4, 2006, 849
Malaria infection V: escape from the hepatocytes

Infectious merozoites are released from the hepatocytes in the form of vesicles filled with merozoites (merosomes)

Sturm et al., Science 313, 2006, 1287

Prudêncio et al.,
Nature Reviews Microbiology,
4, 2006, 849
The complex migrations of Plasmodium from skin to blood
A single point mutation in the beta-chain of hemoglobin produces a protein with strongly changed oxygen-binding behaviour. This S-hemoglobin is highly insoluble in its oxygen-free form and precipitates as crystals.

\[ \text{H-A: } \text{NH}_2\text{-Val-His-Leu-Thr-Pro-Glu-Glu-Ser-} \]
\[ \text{H-S: } \text{NH}_2\text{-Val-His-Leu-Thr-Pro-Val-Glu-Lys-} \]

**Sickle cell anaemia provides partial protection from malaria**

Sickling erythrocytes

Frequency of sickle cell anaemia
Normal erythrocytes infected with plasmodia adhere to the capillaries - this prevents the parasites from being eliminated in the kidney too rapidly.

If sickle-cell erythrocytes adhere: the oxygen-poor environment causes S-hemoglobin to crystallize, which leads to a deformation of the cell and to mechanical stress on the cell membrane.

The erythrocyte membrane gets permeable and K⁺-ions leak out.

The parasite in the damaged erythrocyte dies very quickly under these circumstances.
Glucose-6-phosphate dehydrogenase deficiency protects from severe malaria

In erythrocytes (that do not contain mitochondria), defence against oxidative damage is dependent on G6PH

G6PD

Pentose phosphate pathway

Cappellini, Lancet 3712, 2008, 64
Glucose-6-phosphate dehydrogenase deficiency protects from severe malaria

The frequency distribution of G6PD deficiency (green) closely overlaps with the distribution of malaria (red). Genetic studies have demonstrated that this selection co-evolved with P. falciparum malaria over the last 10'000 years.

www.ch.ic.ac.uk
The adhesion of infected erythrocytes to the endothelium of capillaries is essential for parasite survival, but it is also an important cause of malaria pathology.

ADHESION: important for the parasite and an important cause of pathology
Infected erythrocytes present various parasite-coded proteins on their surface. The genome of P. falciparum codes for about 50 var genes (PfEMP1) and about 200 rif genes (Rifins). Additional proteins are also involved in cell adhesion. These specify the type of surface structure of the endothelial cells to which the parasites adhere.

(Nature 400, 1999, 506)

(Nature 439, 2006, 926)
Various PfEMP proteins adhere to different molecules of the host’s endothelial cells

„Panning“ of infected erythrocytes with mammalin cells expressing different surface proteins (e.g. ICAM-1, CD36, CSA or complement receptor) or other cell surface structures such as chondroitin sulfate, a polysaccharide

Isolation of P. falciparum clones that express PfEMPs with different specificities
Parasite lines that specifically adhere to chondroitin-sulfate cause placental malaria in women who are pregnant for the first time. This causes high maternal, fetal and infant morbidity (100'000 - 200'000 cases per year), maternal anemia and low birthweight.

Chondroitin-sulfate, a sulfated glycosaminoglycan consists of alternating residues of N-acetyl-galactosamine and glucuronic acid. It is linked to serines of cell surface proteins in the placenta.

Though cell adhesion proteins of Plasmodium are usually very variable, the CSA-binding protein contains some conserved domains which allow the women to eventually generate protective antibodies.
The parasite’s dilemma:

- to be able to survive in the host, the parasite must continuously express adhesion molecules at the surface of the infected erythrocytes.

- sadly (for the parasite), these are recognized by the immune system of the host, and the altered erythrocytes are eliminated.

- to prevent this from happening simultaneously to all infected erythrocytes, Plasmodium expresses only one of its 50 PfEMP genes at any given time. How can these gene be regulated so selectively?

- The phenomenon is known as “allelic exclusion” and is known from other systems as well, such as the expression of immunoglobulin genes, olfactory receptors, variable surface proteins of trypanosomes.

The mechanism is still poorly understood.
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Anti-malarial strategies

- Fight the mosquito vectors (drainage of swamps and ponds, larvivorous fish, insecticides)
- Prevent infection by mosquito nets (with or without insecticide treatment)
- Drugs (for prophylaxis and for therapy)
- Vaccination: pipe dreams for the distant future !!
Vector control

- Drain or avoid the generation of unnecessary waterholes (e.g. during road construction)

- Use of larvivorous fish in larger bodies of water such as rice fields

- Spray living quarters with insecticides. Since 2006, DDT is again admitted for this use. Its prohibition 30 years ago turned out to be a disaster for malaria vector control. (WHO press release, Sept. 15. 2006)
Mosquito nets

A relatively cheap and highly cost-effective measure

Nets can be locally produced and treated with insecticides
Chinin - an alkaloid from the bark of the Cinchona tree

China bark („Jesuit‘s bark“) was exported from South America to Europe already around 1630 and rapidly became the standard medication for fever, a symptom that was frequently, but by no means always, caused by malaria.

Its active component, quinine, was isolated for the first time by Pierre Pelletier and Joseph Caventou in 1820

Quinine kills the intraerythrocytic stages of Plasmodium
Sir Robert Talbors miracle cure for "the ague" (malaria)
Chloroquine

1924 first synthesized by Bayer Leverkusen

Mechanism of action:

Hem is detoxified by polymerization into insoluble polymers within the infected erythrocytes (hemozoin)

Chloroquine binds to heme and blocks polymerization
ANTI-FOLATES: Mechanism of action I

Dihydrofolate reductase / Thymidylate synthase

dUMP → dTMP → DNA-synthesis

Methylene-tetrahydrofolate
Glycin
Serin

NADPH+ H+

Dihydrofolate

Pyrimethamin
Chlorproguanil
Proguanil

Tetrahydrofolate

Antifolates: Mechanism of action I

dUMP → dTMP → DNA-synthesis

Methylene-tetrahydrofolate
Glycin
Serin

NADPH+ H+

Dihydrofolate

Pyrimethamin
Chlorproguanil
Proguanil

Tetrahydrofolate
Anti-Folates: Mechanism of action II

GTP

Dihydroneopterin-aldolase

Pyrophosphokinase

Dihydropteroate synthase

Dihydrofolate synthase

Dihydrofolate reductase

Serin hydroxymethyl transferase

Thymidylate synthase

TMP

SULFONAMIDE
(e.g. Sulfadoxin)

ANTIFOLATE
(e.g. Pyrimethamin)
Example of a prophylactic anti-folate

Fansidar®

Combined action anti-folate:
S inhibits dihydropteroate-synthase
P inhibits DHFR/TS

http://www.malariafreefuture.org
2001 WHO and GlaxoSmithKline agreed to develop a cost-effective combination anti-folate (LAPDAP; Chlorproguanil und Dapson) and to provide this to endemic countries at cost.

Chlorproguanil is a “prodrug“

The sulfonamide Dapson inhibits dihydropteroate-synthase.
Artemisinine derivatives

They are derived from the traditional Chinese herb medication "qinghaosu", that is produced from the plant Artemisia annua.

Problem: an extremely short half life in the body

Artemether

Artesunate

Dihydroartemisinine (active metabolite)
Artemisia annua
Novartis concluded an agreement with WHO to increase the production of this new combination drug. For 2005, about 30 million doses were envisaged, but not enough Artemisia annua could be harvested by that time. Currently, Novartis and WHO jointly develop a formulation of the drug that allows application as suppositories, an important route of application for infants and persons with severe disease who are no longer able to swallow tablets.

The medication is produced in China. Artemisinin is a substance known in traditional medicine. Lumefantrin was developed in the seventies in the Academy of Military Medical Sciences in Beijing.
Artemisinic acid, a precursor of artemisinin can also be produced by plant cell cultures or in engineered S. cerevisiae.
The problem of drug resistance

Chloroquin-resistance originated twice independently but almost simultaneously. The resistance then rapidly spread across the entire world.

The development of chloroquine resistance was a very complex, multi-step genetic process, but it occurred nevertheless!
### A selection of current antimalarials

<table>
<thead>
<tr>
<th>Antimalarial</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td></td>
</tr>
<tr>
<td>Primaquine (Primaquine®)</td>
<td>intercalates in DNA specifically against P. vivax</td>
</tr>
<tr>
<td>Pyrimethamine / sulfadoxin (Fansidar®)</td>
<td>Anti-folates</td>
</tr>
<tr>
<td>Doxycyclin (e.g. Vibramycin®)</td>
<td>inhibits bacterial protein synthesis (apikoplast, mitochondria?)</td>
</tr>
<tr>
<td>Atovaquone / Proguanil (Malarone®)</td>
<td>A inhibits mitochondrial electron transport. P inhibits dihydrofolate reductase</td>
</tr>
<tr>
<td>Mefloquin (Lariam®)</td>
<td>forms toxic complexes with heme also active against hypnozoites</td>
</tr>
<tr>
<td>Arthemeter Lumfantrine (Coartem®)</td>
<td>A: fast actin, short half-life also reduces gametocytogenesis C: blocks haem detoxification</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>fatally cardiotoxic (Bouchau et al., Malaria Journal 8, 2009, 289)</td>
</tr>
</tbody>
</table>
Drug resistance appears to be an inevitable problem with any chemotherapy against infectious diseases, and once they occur they disseminate rapidly across the world.
The rapid development of drug resistance:

- market introduction of mefloquin (1984)

![Graph showing the percentage of drug-sensitive infections from 1976 to 1992 for mefloquin, quinine, Sulfadoxin / Pyrimethamin, and Chloroquine.](image)

- mefloquin
- quinine
- Sulfadoxin / Pyrimethamin
- Chloroquine
Further information

Prophylaxis / Drugs:
http://www.tropenreisemed.ch/merkblaetter_malaria.html
http://www.safetravel.ch/

General information about malaria:
www.who.int/topics/malaria/en
www.theglobalfund.org/en/about/malaria
www.mmv.org
www.rollbackmalaria.org
www.gatesfoundation.org/topics/pages/malaria.aspx

Malaria Genome-Database:
www.plasmoDB.org/