**Exoerythrocytic Cycle**

- Schizonts take 5-7 days to develop
- Formation of >10,000 merozoites
- Upon rupture of merozoite, released merozoites will invade RBC's

The final step involves the release of merozoites (green) into the bloodstream.

The signal(s) that trigger the release remain unknown.

Plasmodium merozoites are released by the formation of merozoite-filled vesicles (merosomes), which bud off from the infected hepatocytes into the sinusoidal lumen.

---

**The Disease**

- **Cold Stage**
  - Intense cold
  - Shivering
  - Lasts 15-60 min

- **Hot Stage**
  - Intense heat
  - Dry burning skin
  - Throbbing headache
  - Lasts 2-6 hours

- **Sweating Stage**
  - Declining temperature
  - Exhausted, weak
  - Lasts 2-4 hours

Should I stay or should I go? Antigenic Variation!!!
- **Malaria tertiana**: 48h between fevers (P. vivax and ovale)
- **Malaria quartana**: 72h between fevers (P. malariae)
- **Malaria tropica**: irregular high fever (P. falciparum)

**Malaria Paroxysm**

- Paroxysms are associated with the synchrony of merozoite release - rupture of RBCs - release of toxins
- Rapid climb in body temp.
- Mild delirium
- Between paroxysms patient feels well with normal temps
- P. falciparum may not exhibit classic paroxysms
  - Continuous fever
  - Less synchronous
- Concurrent infections with more than 1 species is common

**Disease Severity**

<table>
<thead>
<tr>
<th></th>
<th>Pv</th>
<th>Po</th>
<th>Pm</th>
<th>Pf</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average</strong></td>
<td>20,000</td>
<td>9,000</td>
<td>6,000</td>
<td>50,000-500,000</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>50,000</td>
<td>30,000</td>
<td>20,000</td>
<td>2,500,000</td>
</tr>
<tr>
<td><strong>Paroxysm</strong></td>
<td>moderate to severe</td>
<td>mild to moderate</td>
<td>severe</td>
<td></td>
</tr>
<tr>
<td><strong>Severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>3-8 w</td>
<td>2-3 w</td>
<td>3-24 w</td>
<td>2-3 w</td>
</tr>
<tr>
<td><strong>Infection</strong></td>
<td>5-8 y</td>
<td>12-20 m</td>
<td>&gt;20 y</td>
<td>6-17 m</td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td>renal</td>
<td>cerebral</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Why is falciparum malaria so severe?

1. High parasitemia
   - Large number of merozoites per schizont (30,000 merozoites)
   - Severe anemia

2. Will infect any stage of RBC
   - No preference

3. Sequestration
   - Only ring and early trophozoites are found in circulating blood
   - Immune evasion - avoids clearance by the spleen

3. Cytoadherence
   - Cerebral malaria

Pathogenesis of falciparum Malaria

- Main problem - Cytoadherence
  - Induction of cytokines (rupture of RBCs) leads to an increase in the level of endothelial adhesion molecules
  - Vascular occlusions
  - Infected RBCs will adhere to endothelial cells as well as to each other (rosetting, clumping)

- Rosetting was found in 50% of field isolates - correlated well with severity of disease

Result of Cytoadherence

- cytoadherence
- cerebral ischemia (Cerebral Malaria ~10% of hospital cases)
- hypoxia, metabolic effects
- coma
- death

Cerebral Malaria accounts for 80% of deaths
**Pathogenesis of cerebral malaria**

- Cerebral malaria is characterized by multiple brain hemorrhages
- Excessive serum and tissue levels of TNFα and INFγ (two important cytokines) are associated with severe malaria
- Some researchers believe this inflammation is the main cause for pathology (an overshooting immune response against a chronic pathogen that cannot be cleared can cause severe disease)

**Knobs and cytoadherence**

- Proteins synthesized by the parasite are transported to the RBC membrane
- KAHRP, PfEMP2 remain at the subsurface of RBC membrane
- Possibly reorganize submembrane cytoskeleton to form knobs
- PfEMP1 transmembrane protein - exposed on the RBC surface
  - One player that mediates adherence to endothelial cells
  - Cytoplasmic domain (acidic) interacts with KAHRP (Highly basic)

**What mediates cytoadherence?**

- Proteins synthesized by the parasite are transported to the RBC membrane
- KAHRP, PfEMP2 remain at the subsurface of RBC membrane
- Possibly reorganize submembrane cytoskeleton to form knobs
- PfEMP1 transmembrane protein - exposed on the RBC surface
  - One player that mediates adherence to endothelial cells
  - Cytoplasmic domain (acidic) interacts with KAHRP (Highly basic)
Knobs and cytoadherence

- Knobs
  - Electron dense protuberance found on RBC membrane of infected cells
  - Composed of parasite derived proteins that are exported to RBC surface

  - F: ring stage
    - Protein is in parasite

  - G,H: young trophozoites
    - Protein is found in RBC cytoplasm

  - I: mature trophozoites
    - Protein is nearly completely localized to the RBC membrane surface

Plasmodium Cytoadherence

- PfEMP-1 Structure
  - Cloned and sequenced
  - Family of ~ 60 var genes (variant)
  - Conserved intracellular ATS
    - Acidic terminal segment - binds KAHRP
  - Transmembrane domain - tm
  - Conserved head structure
    - Cysteine-rich interdomain region
    - Duffy binding like domain
  - Variable extracellular domain
    - 2-7 copies of DBL

![Diagram of PfEMP-1 Structure]
Location within the nucleus: role in control of expression.

All var genes, regardless of chromosomal location or transcription status, appear to physically reside at the nuclear periphery usually in telomeric clusters.

Antigenic Variation!!

Antigenic switching is accompanied by changes in binding phenotype

Possible Host Cell Receptors
- CD36
- Ig super-family
  - VCAM-1
  - ICAM-1
  - PECAM-1
- E-selectin
- Thrombospondin
- Chondroitin sulfate A
- Hyaluronic acid
- Rosetting Receptors
  - CR-1
  - Glycosaminoglycan
  - Blood group A

Very big topic - look for review on antigenic variation

Summary
- Only *P. falciparum* infections result in cytoadherence
  - Knob production
- Parasite modifies host RBC via exported proteins
  - Tubovesicular network
- PEMP-1 may be the main mediator of cytoadherence
- PEMP-1 undergoes antigenic variation
  - Family of var genes
  - Recombination mediated at telomeric or subtelomeric ends during meiosis
Pathogenesis of malaria

- Parasitemia
- Anemia
- Cerebral malaria

Immunity to Malaria

- High endemic areas
  - People do develop immunity - very slow to develop, short-lived
  - NOT sterilizing immunity - does not protect against infection
  - Premunition - immunity that is dependent upon parasite being present - known about this for a long time
  - Mostly resistance to superinfection
  - Tolerant or asymptomatic carriers - parasitemias are present

Innate Resistance

- Duffy blood group antigens
  - Fy/Fy - no merozoite binding
- Sickle cell anemia
  - Mutation is hemoglobin (single aa sub.)
  - Homozygous individual suffer greatly from disease - life expectancy ~30 years
  - Heterozygous individual are protected
  - Presence of sickle cell trait confers about 85-90% protection
  - Selective pressure has led to maintenance of a disease trait
  - 1 in 10 African Americans are heterozygous
- Thalassemia
Malaria in the US?

First reported outbreak of Malaria with extended transmission in US since 1986

Cases presented between July 22-August 26, 2003
Patient 1 and 2 attended the same party of July 4

Think about sporulation in mosquito (~10 days)
Think about EE cycle (8 days) and E cycle (11 days)

All patients lived within 10 miles of Palm Beach International Airport

Malaria Treatments

- Quinine related drugs
  - Quinine
  - Chloroquine
  - Halofantrine

- Antifolates
  - Sulphadoxine
  - Pyrimethamine
  - Proguanil

- Other drugs
  - Artemisinin
  - Clindamycin
  - Atovaquone

- Why chloroquine?
  - Synthesized in 1934
  - First used in WWII
  - Most widely used drug
  - Safe, fairly cheap to make
Cinchona the source of quinine

- Peruvian Indians seem to be the first to know about the potential benefits of quinine as they chewed bark while working in cold streams and mine for the Spanish...bark from the "fever tree".
- In the early 1600s the bark was used to treat the fever of the Countess of Chinchon and became well known as Jesuit's powder or Peruvian bark.

- Initial preparations were often quite variable in the amount of active ingredient resulting in varying effects. Very bitter taste – mixed with sweetened water (1st tonic water?)
- Not until 1820 was quinine isolated – was also used in tonic water - gin and tonics were used for prophylaxis of malaria
- Quinine was used as a muscle relaxant – halt shivering.

A little history on cinchona

- High demand had brought the Cinchona tree almost to extinction in the wild – outlawed exporting cinchona seeds etc.
- Charles Ledger a trader in Peru sent out Manuel Incra Macrami to locate a stand of special tree they had found earlier.
- After three years Manuel came back with 15 kg of seeds which they sold for 100 guilders to the Dutch, as the British were not interested.
- Cincnosa ledgeriana formed the basis of a very profitable Dutch quinine monopoly which lasted through World War II. In the 1930s, the Dutch had plantations in Java that were producing 22 million pounds of cinchona bark, or 97% of the world's quinine production!!!
Chloroquine - an alternative

- Quinine was the drug of choice for Malaria from 17th Century to 1940s!
- Chloroquine an artificial quinine analog was a very potent drug which was very cheap to make and had no really serious side effects
- Acts within 24-48 hrs
- None of the drugs developed since come close to chloroquine

Hemoglobin degradation

- Proposed chloroquine mode of action - still debated
  - Weak base, uncharged @ pH7
  - Rapid diffusion through plasma and lysosomal membranes
  - Inside lysosome - charged molecule is trapped
  - Accumulation via conc. Gradient
  - Chloroquine binds FP - interferes with pigment formation
    - Inhibits heme polymerase
    - HIGHLY TOXIC TO PARASITE
    - May also interfere with nucleic acid biosynthesis

Drug resistance

- CQ was WIDELY used after 1940s – for any type of fever; even added to salt!
- Ability of a pathogen to survive and/or multiply despite the administration and absorption of the drug given in doses equal to or higher than doses normally given.
- Important factors associated with resistance:
  - Longer half-life
  - Single mutation for resistance
  - Poor compliance - use chloroquine to treat any fever - takes 2-3 doses in stead of 10
  - Host immunity
  - Large numbers of people using the drug
Chloroquine resistance

- Characterized using the following:
  - Sensitive (S): asexual parasite count reduces to 25% of the pre-treatment level in 48 hours after starting the treatment and complete clearance after 7 days, without subsequent recrudescence - Complete Recovery.
  - R: Delayed Recrudescence: asexual parasitemia reduces to < 25% of pre-treatment level in 48 hours, but reappears between 2-4 weeks.
  - R: Early Recrudescence: asexual parasitemia reduces to < 25% of pre-treatment level in 48 hours, but reappears earlier.
  - RII Resistance: Marked reduction in asexual parasitemia (decrease >25% but <75%) in 48 hours, without complete clearance in 7 days.
  - RIII Resistance: Minimal reduction in asexual parasitemia, (decrease <25%) or an increase in parasitemia after 48 hours.

Spread of chloroquine resistance

- Used since the early 1950s
- Effective and safe treatment
- Widespread use of chloroquine
- First cases were seen in the late 1950s at two different foci
  - Columbia
  - Cambodia-Thailand border
- Interesting transfer to the African continent - spread very quickly at that point
- Now additional resistance
  - Mefloquine
  - Antifolates
Mechanisms of drug resistance

- Changes in target enzyme
  - Mutations in target gene
  - Decreased affinity to drug
- Overexpression of target
  - Amplification
- Decreased activation of drug
- Changes in accessibility
  - less import, or more export of drug

Resistance to chloroquine

- Drug resistant parasites show lower chloroquine concentrations in the food vacuole - decreased accumulation
- This seems to be due to an increased pH of this acidic organelle (chloroquine is a weak base)
**Resistance to chloroquine**

- In vitro data suggests that a series of point mutations in the newly identified membrane transporter PfCRT are responsible for resistance.
- This putative transporter localizes to the membrane of the food vacuole.
- Large field studies have found strong association of these mutations with chloroquine resistance (different mechanism in *P. vivax*).

**Malaria antifolates**

- While *Plasmodium* parasites cannot synthesize purines de novo, they do synthesize pyrimidines de novo.
- Folates - important cofactors in this process (also for amino acid biosynthesis) and are made de novo as well - *Plasmodium* cannot utilize preformed folates.
- Pyrimethamine and sulfa drugs (Fansidar) act synergistically as they target the same pathway at two different points.
- Humans take up folate as a vitamin in the food.

**Malaria antifolates**

- Pyrimethamine and proguanil are dihydrofolate analogs inhibiting parasite DHFR but not human DFHR.
- Leads to depletion of folate pool and subsequently reduces the pool of thymidine for nucleic acid synthesis.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>Major Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadoxine + pyrimethamine</td>
<td>Dihydropterote synthase</td>
<td>A437G, K540E, A581G</td>
</tr>
<tr>
<td>Sulfadoxine + pyrimethamine</td>
<td>Dihydrofolate reductase</td>
<td>S108N, N51I, C59R, I164L</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Chloroquine resistance transporter</td>
<td>K76T</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Multidrug resistance gene</td>
<td>D86Y</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Multidrug resistance gene</td>
<td>Increased copy number</td>
</tr>
</tbody>
</table>

**Prevention of drug resistance**

- Drug selection based on type of case
- Avoid drugs with longer-half lives if possible
- Do not use anti-malarials for non-malarial indications
  - Use of chloroquine to treat rheumatoid arthritis is endemic area
- Ensure patient compliance with treatment
- Monitoring for resistance and early treatment
- Clear policies for use of new anti-malarials
- Use drugs in combination to inhibit emergence of resistance
  - Drugs that target two different biological pathways

**Artemisinin - Newest Treatment**

- An ancient herbal remedy with good potency! (Qinghao)
  - Earliest document use of herbal remedy dating back to 168 BC.
- Extract from the wormwood plant
- Great attention since early 1990s as a “new” treatment for malaria – especially for drug resistant forms
- Can be used for cases that exhibit multi-drug resistance
- Extremely fast acting - within 12 hr
- Inhibits the trophozoite stage
Artemisinin – Supply and Demand

- Cultivation and extraction: takes up to 9 months
- Artemisinin and its derivatives can cure malaria within 3 days
- Drug stability issues - light sensitive
- 2001: WHO recommended Artemisinin-based combination therapy (ACT)
- Backing from company Novartis to supply at cost
- 2004: projected 10 Million doses needed
- 2005: projected 60 Million doses needed

Chemical synthesis
- Jay Keasling - Scientist of the Year (2006)

HUGE SHORTAGE
Supply and demand drove the cost up

Atovaquone

- HIGHLY Lipophilic compound - essentially insoluble in water
- Resembles Ubiquinone
- Alone - fast recrudescence in patients decreased parasite susceptibility following treatment (High relapse rate!)

Atovaquone

- Interferes with parasite mitochondrial electron transport - collapses the membrane potential
- Also effective against Toxoplasma and Pneumocystis
- Plasmodium parasites developed resistance to atovaquone quickly
- Mutations in cyt b1 complex
- Combined therapy: Malarone
  - Atovaquone + proguanil
  - Travellers use this a prophylaxis

Atovaquone

- Ubiquinone
- Atovaquone
Prophylaxis - Malarone

Atovaquone/Proguanil

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