The Kinetoplastids

*Leishmania spp.*

*Trypanosoma spp.*

Mitochondrion

- a membrane-enclosed organelle found in most eukaryotic cells
- function to generate most of the cell's supply of ATP (*Krebs cycle/tricarboxylic acid-citric acid cycle occurs here*)
  - **glycolysis** – in cytoplasm: glucose $\rightarrow$ pyruvate & 2 ATP
  - **TCA & oxidative phosphorylation** – in mitochondria: pyruvate $\rightarrow$ $\sim$28 more ATP
- several copies of a single, circular chromosome
  - likely derived from an α-proteobacteria by endosymbiosis
  - genes for of the respiratory chain
  - genes for ribosomal RNAs & 22 tRNAs
  - other genes are nuclearly encoded & proteins imported
The Kinetoplastid Kinetoplast

- early eukaryotic lineage & one of earliest to have mitochondria
- single mitochondrion
- mitochondrial DNA is known as kinetoplast DNA
- network of catenated minicircles & maxicircles
  - ~10,000 minicircles
  - 50 maxicircles (rRNA & structural genes)

The Kinetoplastid & RNA Editing

Huge mass of mitochondrial DNA (kDNA)
- catenated minicircles & maxicircles
- maxicircle genes
  - have frameshifts
  - lack initiation codons
- post-transcriptional RNA modification = non-encoded U residues inserted into mRNA
- U deletions occur at a lower frequency
  - after transcription → overcomes coding frameshifts

Guide RNAs (gRNAs) small RNAs transcribed from maxicircles & minicircles → gRNAs form duplexes with pre-edited mRNA to provide editing information for insertions & deletions

The enzymes that carry out RNA editing are unique to trypanosomes, so are an excellent future target for drugs.
An edited gene showing U insertions (blue) & deletions (red)

A gRNA-mRNA duplex showing information for insertions & deletions

Leishmania spp.

Cutaneous: papules develop into ulcers, healing with scars.

Mucocutaneous: mouth, nose, throat & skin lesions cause disfigurement.

Visceral: “kala azar” fever & enlarged spleen & liver. Fatal if not treated.
Kill or Cure:
Kala Azar
Season 1 Episode 5
Transmitted by the bite of a sand fly, Kala Azar or visceral leishmaniasis is a deadly disease that attacks the internal organs. An estimated 1.5 million people worldwide are currently infected; 200 million people are at risk. Hopes are high in India that a new drug, paramomycin, can deliver an affordable and efficacious cure. The drug goes into production, signaling the possible elimination of this deadly disease in South Asia.

http://www.rockhopper.tv/programmes/22/

Terms from the documentary-Kala Azar
Bihar: state in eastern India
~ 58% of population is below age 25
has the lowest GDP/person in India
per capita income of $436/year 2011

Spleen: an organ that participates in filtration of RBCs and assists in developing immune response
Antimony: element (Sb) used in electronics, flame-proofing, paints, rubber, ceramics, enamels & drugs to treat Leishmania infection.
Paromomycin: antibiotic; inhibits protein synthesis by binding to 16S ribosomal RNA.
Pneumonia, dysentery (extreme diarrhea) = secondary infections of person with Leishmania infection.
Genus *Leishmania*

• genetically diverse vector-borne parasites
• transmitted from host to host by sandfly bite
• zoonotic *(infects a variety of vertebrates in tropics & subtropics)*
• all possess a kinetoplast
• all live within macrophages or other phagocytic cells

• several million humans suffer from leishmaniasis & >350 million people live within endemic area

• Leishmaniasis occurs in 88 countries: southern Europe, Africa, Asia, South Asia & South & Central America

• Clinical conditions caused by *Leishmania* vary greatly due to *Leishmania* species *(thermo-tolerance)*
  - host immune status *(immune intact or compromised)*

  can present as cutaneous lesions that resolve over time or as a systematic disease of the reticuloendothelial system.
  • >90 percent of the cases of cutaneous leishmaniasis occur: Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, & Syria (in the Old World) & in Brazil and Peru (in the New World)
  • Over 90 percent of the cases of visceral leishmaniasis occur: India, Bangladesh, Nepal, Sudan, & Brazil

*Cutaneous*: involving the skin, i.e. cutaneous or mucocutaneous disease
*Reticuloendothelial system*: part of immune system, consists of phagocytic cells located in connective tissue, primarily monocytes & macrophages. These cells accumulate in lymph nodes & spleen.
**Morphological Forms: *Leishmania***

- **Promastigote** (insect = sandfly)
  
  Metacyclic promastigotes from sandfly infect macrophages

- **Amastigote** (mammal, intracellular, no flagellum)
  
  Sandfly feeds on infected macrophages in blood

**Insect** → Infectious form; must release from insect midgut

**Mammal** → in macrophages

**Leishmania Life Cycle**

**Infection of insect vector:**
- Sandfly feeds on blood from an infected mammal
- Uptake of macrophages containing amastigotes during meal
- Sandflies become maximally filled with blood
- Cannot regurgitate excess blood
  
  *Parasite prevents contraction of stomach muscle*
  
  Enhances sandfly infection

**Proliferation & differentiation in insect vector:**
- Parasite must develop into infectious stage for mammalian host
- 1-2 weeks needed for infectious forms to develop
- Complex developmental changes in sandfly gut
- Transition from **procyclic** to **metacyclic** promastigotes
Leishmania Life Cycle

Proliferation & differentiation in insect vector:

amastogotes from blood meal become procyclic promastigotes
non-infectious, replicative stage
attached to gut wall via sugar-lectin interactions
insect galectins
parasite lipophosphoglycan (LPG)
must be retained in the gut
complete replication cycle
produce infectious metacyclic promastigotes

Galectin: a beta-galactoside-binding family of lectins that contains 1 or more carbohydrate-recognition domains. These function in immunity, homeostasis and embryogenesis.
Beta-galactoside: galactose attached to a non-carbohydrate moiety via a glycosidic bond.
Lipophosphoglycan: (LPG) GPI-anchored polysaccharide, plays many roles in Leishmania infection (protection from complement, macrophage adhesion & altered macrophage signaling).

Leishmania Life Cycle

Proliferation & differentiation in insect vector:

~1 week → flagellated metacyclic promastigote stage
infectious form, transmitted by sandfly bite
Metacyclic promastigotes attached to gut
must release from insect midgut
altered parasite surface sugars no longer bind galectin
Leishmania substance blocks digestive tract of sandfly
insect regurgitates parasites during feeding
metacyclic promastigotes injected into host

Insect salivary proteins aid Leishmania infection of mammal host:
interfere with IL-10 production
inhibit TNF-alpha release
stimulates prostaglandin E2 production
upregulate macrophage IL-6 synthesis (Th2 response favored)
altered host responses have role in establishing parasite in host
Infection of mammalian host:
  metacyclic promastigotes deposited at bite site adhere
  induce antibodies & become opsonized
  complement component C3 coats parasite surface
  engulfed by macrophages
  differentiates into amastigotes
  (intracellular phase of life cycle)
  amastigotes live in macrophage phagosomes
  inhibit lysosome fusion (evade digestion)
  inhibition by host cell interaction with LPG
  mutant parasites with altered LPG are digested

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Virulence Factors & Pathogenesis

• Temperature transition:
  sandfly (ambient temperature) → mammalian host (37°C)
  induction of heat shock genes
  induction of developmentally regulated genes
• Parasite down-regulates IL-12
  delays onset of cell mediated immune response
• Parasite interferes with macrophage antigen presentation
  (cysteine protease inhibitors)

Protective Immune Mechanisms

Cutaneous forms typically induce well-defined $T_{H1}$ responses
T-cell mediated immunity is critical to control & eliminate
antibodies → no role in immunity to cutaneous leishmaniasis
(probably aid the parasite in gaining entry to macrophage)
Distinct *Leishmania* Infections

differences between *Leishmania* spp.

• Some species only cause *cutaneous* lesions
  remain at bite site throughout infection
  amastigotes grow inside host cells at site of injection
  sandflies contact infected host cells at raised margin of
  cutaneous lesions

• Some species cause *visceral* or *mucocutaneous* lesions
  disperse to other sites
  amastigotes carried by phagocytes to mucocutaneous
  junctions or reticuloendothelial tissues
  infected macrophages in blood taken up by vector

Cutaneous Leishmaniasis:

*Leishmania (L) major*
(Yakimov & Schockov 1915)

*Leishmania (L) tropica*
(Wright 1903)

*Leishmania (L) mexicana*
(Biagi 1953)
Several million people suffer from cutaneous leishmaniasis each year. Rodents are a primary reservoir for human infection caused by Leishmania major & dogs are a reservoir for other species.

Oriental sore: traditional name for cutaneous Leishmania, which is endemic in Middle East, India & Africa

Leishmanization: uninfected individuals deliberately inoculated in areas other than face with scrapings containing organisms from active lesions. This controls the region of the body where scars develop & is a rudimentary immunization.

Cutaneous Leishmaniasis - Clinical Disease

Small red papules at site of bite wound
- 2-8 weeks after injection of metacyclic promastigotes.
- Lesion changes over time
  - Painless nodule (1 cm in diameter)
  - Area ulcerates due
  - Becomes depressed then heals through scarring

Organisms are found only in living tissue at raised margin of lesion

As ulcer heals (weeks to months) immunity is permanent & is effective against other Leishmania species that cause only cutaneous lesions (viable parasites remain at this site).
Occasionally *Leishmania tropicalis* visceralizes (Saudi Arabia & other parts of Middle East among coalition force forces in region during Gulf War & troops in Afghanistan).

outward signs & symptoms are unpredictable
  - months to manifest
  - splenomegaly
  - daily fever spikes
  - leucopenia & malaise
  - fatality in untreated individuals is high

*Splenomegaly*: an enlargement of the spleen, which usually lies in the left upper quadrant of the human abdomen.

*Leukopenia*: a decrease in the number of circulating white blood cells in the blood. Since the function of these leukocytes is to combat infection, decreased numbers can place patients at risk for infection.

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*Old Leishmania major lesion*  
*Healing lesion due to Leishmania*

*Chichlero’s ulcer due to Leishmania spp.*  
*Skin sample showing Leishmania amastigotes (arrows) in dendritic cells & macrophages.*
Treatment - Cutaneous *Leishmania*

Treatment:
sodium stibogluconate is drug of choice
cure rates of 85-95% are typical
antimony containing drug
serious side effects
   *rash, headache, arthralgias & myalgias,*
   *pancreatitis & hematologic suppression*)
ketoconazole (*antifungal drug*) is alternative with fewer side effects (*but not as effective*).

**Arthralgia**: joint pain; it is a symptom of injury, infection or illnesses.
**Myalgia**: muscle pain; a symptom of many diseases.
**Pancreatitis**: inflammation of the pancreas.
**Hematologic Suppression**: decreased numbers of blood cells.

Cutaneous *Leishmania* - Prevention & Control

- eradication of sandfly breeding sites
- *pyrethroid* impregnated collars on domestic dogs
- sleeping under insecticide impregnated bed nets

- *transmission from person to person rarely occurs* -- *infection from reservoir hosts (rodents & dogs)* therefore treating all infected individuals has little effect

- sand flies usually bite in early morning & late evening – avoiding outdoor activities at these times reduces the chance of being bitten & infected

- vaccine not currently available

*Pyrethroids*: natural organic compounds purified from chrysanthemums that have potent insecticidal activity.
Multiple cutaneous lesions due to *L. panamanensis*

**Cutaneous Leishmania:** infection progresses at the site of the bite only

Mucocutaneous Leishmaniasis:

*Leishmania (V) braziliensis*  
(Vianna 1911)
Leishmania braziliensis
zoonotic infection
typically causes cutaneous lesions
2-3% of infections metastasize to mucocutaneous junctions
(oral cavity, urogenital area & anal area)
eroses soft tissues
lesions in nasal passage: necrotizing inflammation
lesions of oral cavity
destruction of soft palate & nasal septum
invasion of larynx

Mucocutaneous Leishmaniasis
 lesions do not heal spontaneously
secondary infections are common
patient may die from secondary infection that spreads to lungs

Treatment: very toxic drugs!
sodium stibogluconate
liposomal amphotericin-B
pentamidine
additional drugs are needed!
Cutaneous lesion on lower lip due to L. braziliensis.

Espundia: this lesion resulted in the erosion of the soft palate (most lesions do not advance this far before medical intervention).

Visceral Leishmaniasis

*Leishmania (L) donovani*
(Ross 1903)

*Leishmania (L) infantum*
(Cunha & Chagas 1937)

*Leishmania (L) infantum chagasi*
(Cunha & Chagas 1937)
parasites infect macrophages routinely invade reticuloendothelial tissues throughout body cause an often fatal disease → visceral leishmaniasis hepatosplenomegaly & high fever especially prevalent in children hundreds of millions of people are at risk worldwide

Visceral Leishmaniasis

• reservoirs: humans are primary source, sometimes dogs
• bite of infected sandfly →
• promastigote taken up by dendritic cells or macrophages
• parasites transform into amastigotes
• disseminate to new areas of body & infect macrophages spleen, liver & bone marrow are most seriously affected organs
• infected person/animal bitten by uninfected sandfly ingests macrophages containing amastigotes duration of lifecycle within the fly is ~10 days

• *L. donovani* can be transmitted by organ transplantation (rare)
• congenital infection: fetus acquires from infected mother
Liver biopsy with *L. donovani* amastigotes

Bone marrow aspirate with *L. donovani* amastigotes

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**Visceral Leishmaniasis - Clinical disease**

*Kala azar*: means “black fever” in Hindi characterizes appearance of patient’s skin (in light-skinned patients skin darkens)

3-6 month incubation period

- intermittent high fever
- individual weak but does not feel ill
- generalized lymphadenopathy
- hepatomegaly

Disease is progressive

Individuals have compromised immunity

People die with visceral leishmaniasis rather than from it, due to concurrent disease (TB, pneumonia, dysentery).
**Visceral Leishmaniasis**

- The ability of some Leishmania to visceralize may relate to the ability of some parasites to tolerate higher (internal) body temperatures.
- Immunity is dependent upon $T_{H1}$ responses.
- Parasites try to bias response to $T_{H2}$.
- Antibodies play no role in protection.

**Treatment:**
- Sodium stibogluconate (**Pentostam**)
- 30 day treatment since drug resistance exists.

Sodium stibogluconate is exceedingly phlebotoxic; after a few doses it is difficult to find a vein in which to inject the drug. The drug can be given intramuscularly but is exceedingly painful when given by this route. Sodium stibogluconate can cause pancreatitis, cardiac conduction disturbances, a reduced appetite, metallic taste in mouth, nausea, vomiting, diarrhea, headache, tiredness, joint pains, muscle aches, dizziness & anaphylaxis.