### History of Leishmaniasis

- **W. Leishman & C. Donovan**
  - One of the first accounts of parasites associated with visceral disease
- **Reports dating back as far as 7 BC!**
  - Description of conspicuous lesions (OW)
  - 5th century Spanish missionary records (NW)

- 350 million at risk
- 12 million infected
- 1.5-2 million clinical cases/year

### Leishmania sp.

- **Intracellular parasite**
- **Primarily reside in macrophage**
- **Promastigotes**
  - 15-20 μm in length
  - Flagellated, motile
  - Quickly attach to and invade macrophages
- **Amastigotes**
  - 2-5 μm in length
  - Non-flagellated
  - Reside in phagolysosome
**Leishmania sp. Life Cycle**

**Sandfly Stages**
1. Sandfly takes a blood meal (injects promastigote stage into the skin)
2. Promastigotes are ingested (macrophages)
3. Promastigotes transform into amastigote stage in midgut
4. Amastigotes multiply in cells (including macrophages) of various tissues
5. Sandfly exits its host

**Human Stages**
1. Amastigotes transform into promastigotes inside macrophages
2. Promastigotes are ingested by macrophages
3. Human macrophages are killed
4. Amastigotes multiply in cells
5. Ingested or transmitted by sandfly

**Phlebotomine vectors**

- **Sandfly vectors**
- **Old World**
  - *Phlebotomus*
  - Desert, semi-arid
- **New World**
  - *Lutzomyia*
  - Forest dwelling
- **Animal reservoirs**
  - Wild and domestic canines
  - Rodents
  - Small mammals
Habitat of *Leishmania* promastigotes

Abundant Surface Molecules

- **LPG** - lipophosphoglycan - major molecule
- **PPG** - proteophosphoglycan
- **gp63** - highly glycosylated protein with protease activity
- **GIPL** - glycosylinositol phospholipids

LPG is a multi-functional surface molecule

- Attachment to insect midgut
- Resistance to complement when promastigote is injected into tissue
- Attachment to macrophage receptors for invasion
- Resistance of parasites to oxidative attack inside macrophage
- Modulation of macrophage signaling cascades
Clinical Spectrum of Leishmaniasis

- **Cutaneous Leishmaniasis (CL)**
  - most common form, relatively benign self-healing skin lesions
  - (aka, localized or simple CL)
- **Diffuse Cutaneous Leishmaniasis (DCL)**
  - rare cutaneous infection with non-ulcerating
  - nodules resembling leprosy
- **Leishmaniasis Recidiva**
  - rare hypersensitive dermal response
- **Mucocutaneous Leishmaniasis (MCL)**
  - simple skin lesions that metastasize, especially to nose and mouth region
- **Visceral Leishmaniasis (VL)**
  - generalized infection of the reticuloendothelial system, high mortality

Some Species Infecting Humans

<table>
<thead>
<tr>
<th>New World Cutaneous, Mucocutaneous, and Diffuse Leishmaniasis</th>
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<th>Visceral Leishmaniasis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexicana Complex</strong></td>
<td><strong>L. tropica</strong></td>
<td><strong>L. donovani</strong></td>
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<tr>
<td><em>L. mexicana</em></td>
<td><em>L. major</em></td>
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<td><em>L. amazonensis</em></td>
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<tr>
<td><strong>Braziliensis Complex</strong></td>
<td><strong>L. aethiopica</strong></td>
<td><strong>L. infantum</strong>*</td>
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<tr>
<td><em>L. braziliensis</em></td>
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<tr>
<td><em>L. panamensis</em></td>
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<td><em>L. guyanensis</em></td>
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</table>

*Both dermatrophic and viscerotrophic strains exist.

**L. chagasi (Americas) may be the same as L. infantum (Mediterranean)**
Disease distribution

Cutaneous Leishmaniasis

- Most common form
- Usually one or more sores or nodules on skin
- Sores can change in size or appearance over time
- Described as looking like a volcano with a raised edge and central crater
- Usually painless sores unless secondarily infected
- May be accompanied by swollen lymph nodes
- Can be self-healing, but could take months to years

Oriental sore
Baghdad boil

Incubation period:
2 weeks to several months
Cutaneous Leishmaniasis

cutaneous lesions are usually self-limiting
DoD Preventative Measures

- Suppress animal reservoirs
  - Dogs, rats, gerbils and other rodents
- Suppress the Sandfly vector
  - Critical to preventing disease for stationary troop
- Prevent sandfly bites
  - Personal protective measures
    - Important at night
    - Sleeves rolled down
    - Insect repellent w/ DEET
    - Permethrin treated uniforms
    - Permethrin treated bed nets

Visceral Leishmaniasis

- 3 possibly related species
  - *L. donovani* (Asia, Africa)
    - India (kala azar)
  - *L. infantum* (Mediterranean, Europe)
  - *L. chagasi* (New World)
- reticuloendothelial system affected
  - spleen, liver, bone marrow, lymph nodes
- onset is generally insidious
- progressive disease
  - 75-95% mortality if untreated
  - death generally within 2 years
Visceral Leishmaniasis

- incubation period
  - generally 2-6 months
  - can range 10 days to years
- fever, malaise, weakness
- wasting despite good appetite
- spleno- and hepatomegaly, enlarged lymph nodes
- depressed hematopoiesis
  - severe anemia
  - leucopenia
  - hemorrhages in mucosa

Mucocutaneous Leishmaniasis

- Rare form of the disease
- Occurs with species found in Central and South America
- Very rarely associated with L. tropica found in the Middle East
- Mucosal involvement when a cutaneous lesion is near nose or mouth - more likely if a skin lesion is left untreated
- May occur months or years after an original skin lesion
- Difficult to confirm - low parasite numbers in lesion
- Lesions can be VERY disfiguring
**Diffuse cutaneous leishmaniasis**

- scaly, not ulcerated, nodules
- chronic and painless
- numerous parasites in lesions
- seldom heal despite treatment

- Post kala azar
  - due to inadequate treatment
  - nodular lesions
  - easily cured with treatment (in contrast to DCL)

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**Leishmanial/HIV co-infection**

Now emerging as a serious problem
HIV increases risk of visceral leishmaniasis by 100-1000x

![World map showing distribution of Leishmanial/HIV co-infection in 1998](image)
Key to persistance

Diagnosis - CL, DL, MCL

- suspected because of:
  - geographical presence of parasite
  - history of sandfly bite
  - + skin lesion:
    - chronic, painless, ‘clean’ ulcer
    - nasopharyngeal lesions
    - nodular lesions
  - demonstration of parasite
  - delayed hypersensitivity skin test
  - serology

- amastigotes (scrapings, biopsy, aspirates)
- in vitro culture (promastigotes)
- inoculate into hamsters
Skin scraping

Diagnosis of VL

• suspected because of:
  • geographical presence of parasite
  • history of sandfly bite
  • prolonged fever, splenomegaly, hepatomegaly, anemia, etc.

• amastigotes in bone marrow aspirates
• in vitro culture of aspirates
• serological tests
  • direct agglutination
  • ELISA dipstick (39 kDa Ag)
• Molecular - Real time PCR
Treatment for kinetoplastid diseases

- **Leishmaniasis**
  - **Pentavalent antimonial compounds** (1947, 1950)
    10-30 day treatment
  - **Pentamidine** (for failed cases) (1940)
  - **Amphotericine** (1959)
    Drug interacts with plasma membrane ergosterol (also in fungi)
    Discriminates between ergosterol and cholesterol
    New formulation w/ liposomes readily taken up by macrophages!
  - **Allopurinol** (experimental in humans, used for dogs)
    Inhibits hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) - feedback inhibition of purine biosynthesis

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**Treatments for Leishmaniasis**

<table>
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<th>Drug</th>
<th>1985</th>
<th>2005</th>
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Different approaches to intracellular survival

- Trypanosoma cruzi -- induce phagocytosis and escape into the cytoplasm
- Toxoplasma -- active invasion, parasitophorous vacuole is never part of the endocytic pathway
- Mycobacterium tuberculosis -- induce phagocytosis and block lysosomal maturation
- Leishmania ...

Leishmania parasitophorous vacuole

- Internalized via conventional phagocytosis
- Vacuole contains markers of a mature phagolysosome
- No delay to phagolysosome maturation
- Parasites replicate in a lysosome-like compartment!
- Mature parasitophorous vacuole continuously receives contents from secretory and endocytic pathways
**Leishmania phagosome**

- **A**
  - Image of a phagosome with a label "PL".

- **B**
  - Diagram showing nutrient acquisition mechanisms.

**Nutrient Acquisition**

- **Figure 2**
  - Diagram illustrating the process of nutrient acquisition in Leishmania phagosomes.