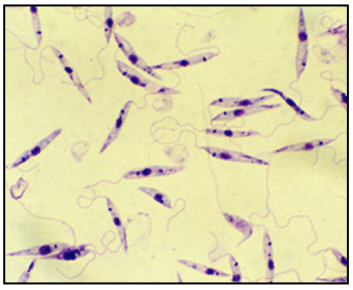
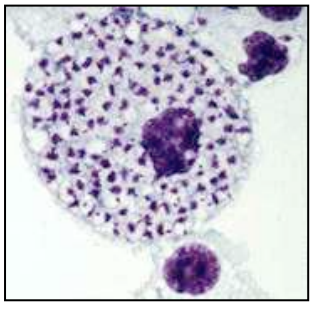
History of Leishmaniasis

- 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**

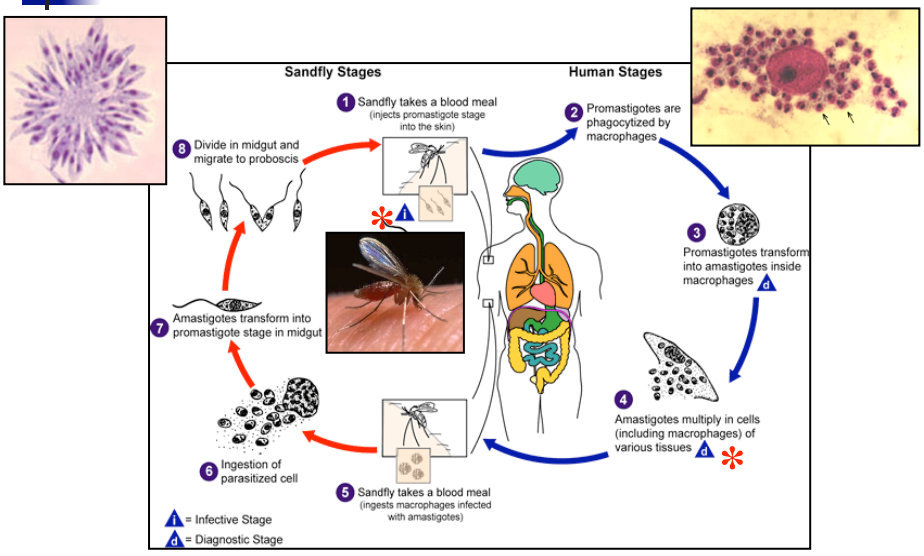
Leishmania sp.





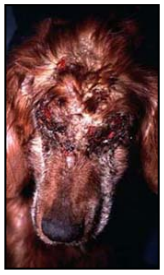
- Intracellular parasite
- Primarily reside in macrophage
- Promastigotes
  - 15-20 μm in length
  - Flagellated, motile
  - Quickly attach to and invade macrophages
- Amastigotes
  - 2-5 μm in length
  - Non-flagellated
  - Reside in phagolysosome

Leishmania sp. Life Cycle

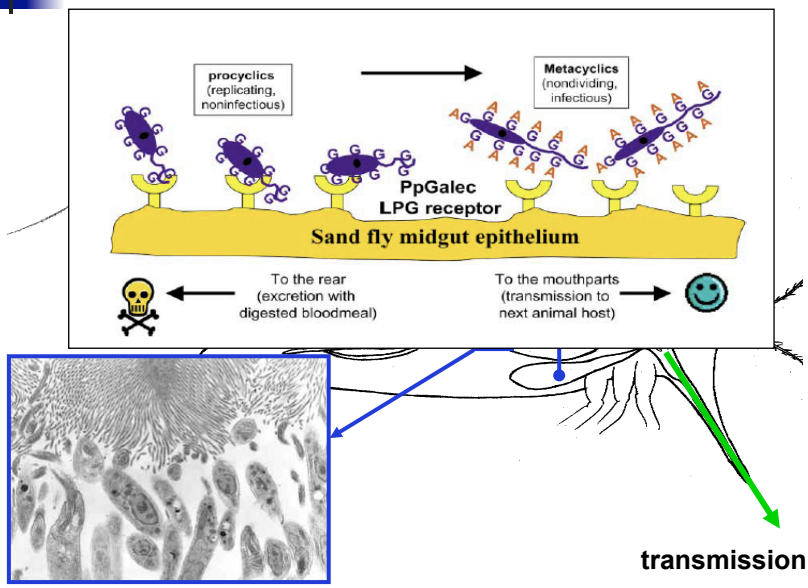


Phlebotomine vectors

- Sandfly vectors
- Old World
  - *Phelbotomus*
  - Desert, semi-arid
- New World
  - *Lutzomyia*
  - Forest dwelling
- Animal reservoirs
  - Wild and domestic canines
  - Rodents
  - Small mammals

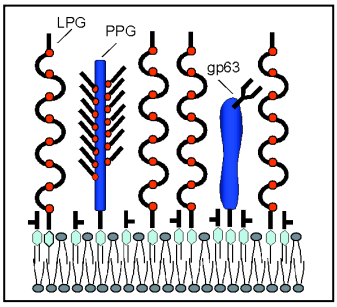


Habitat of *Leishmania* promastigotes



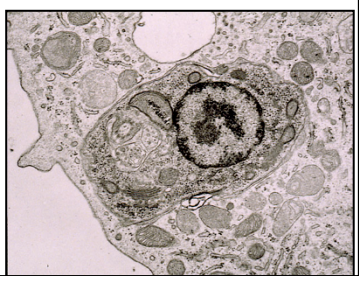
Abundant Surface Molecules

LPG - lipophosphoglycan - major molecule
PPG - proteophoshoglycan
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

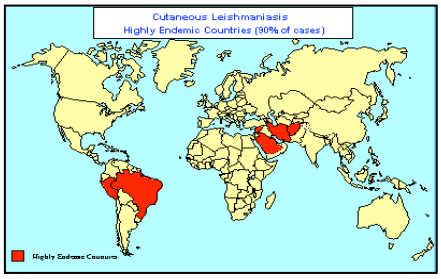
- **Cutaneous Leishmaniasis (CL)**
  - most common form, relatively benign self-healing skin lesions
  - (aka, localized or simple CL)
  - **Diffuse Cutaneous Leishmaniasis (DCL)**
    - rare cutaneous infection with non-ulcerating
    - nodules resembling leprosy
  - **Leishmaniasis Recivida**
    - 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

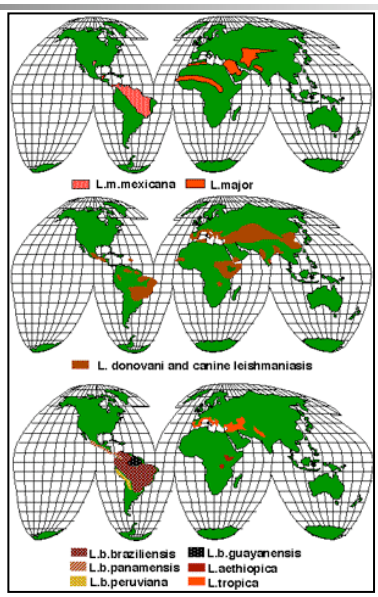
Some Species Infecting Humans

New World Cutaneous, Mucocutaneous, and Diffuse Leishmaniasis	Old World Cutaneous, Recidivans, and Diffuse Leishmaniasis	Visceral Leishmaniasis
Mexicana Complex	*L. tropica*	*L. donovani*
L. mexicana		
L. amazonensis	*L. major*	*L. infantum**
Braziliensis Complex	*L. aethiopica*	*L. chagasi* **
L. braziliensis		
L. panamensis	*L. infantum**	
L. guyanensis		

*Both dermotrophic and viscerotrophic strains exist.
***L. chagasi* (Americas) may be the same as *L. infantum* (Mediteranean)

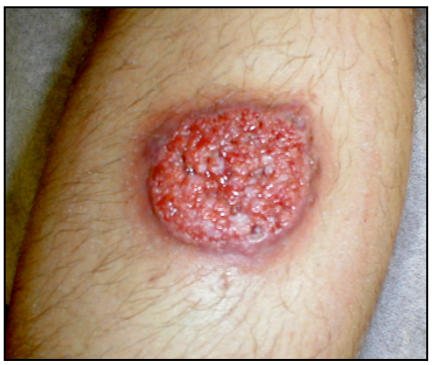
Disease distribution





Cutaneous Leishmanisis

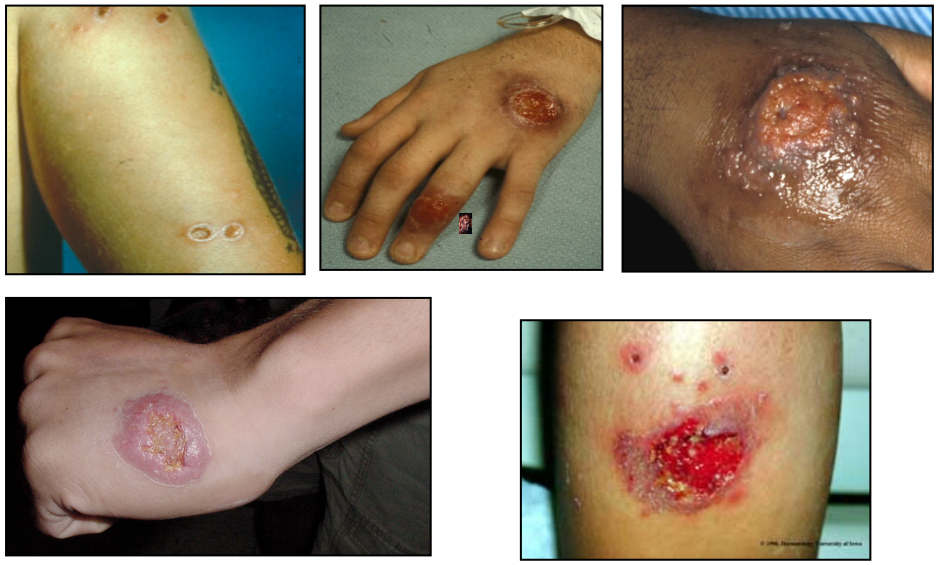
- Most common form
- Usually one or more sores or nodules on skin
- Sores can change in size or appearance over time
- Described as looking like a volcano with a raised edge and central crater
- Usually painless sores unless secondarily infected
- May be accompanied by swollen lymph nodes
- Can be self-healing,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



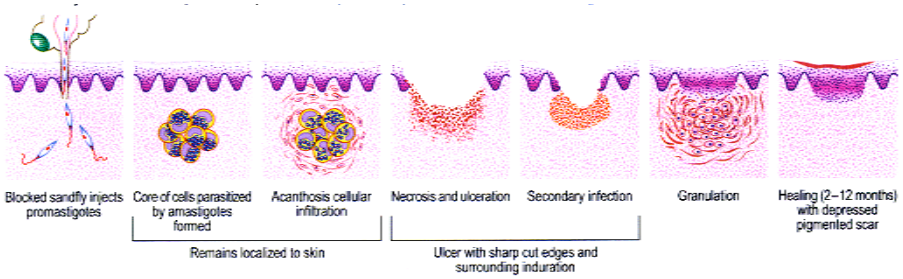
Oriental sore
Baghdad boil

Incubation period:
2 weeks to several months

Cutaneous Leishmaniasis

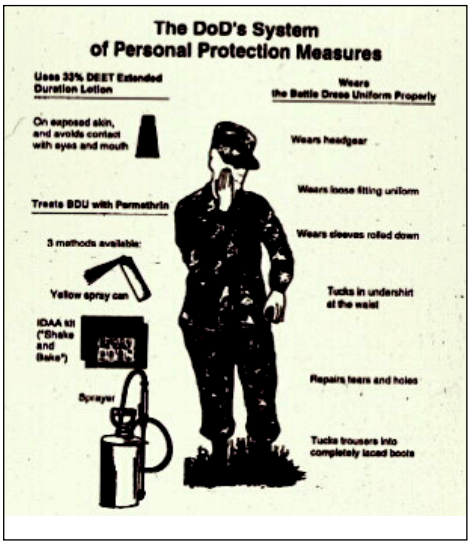


cutaneous lesions are usually self-limiting



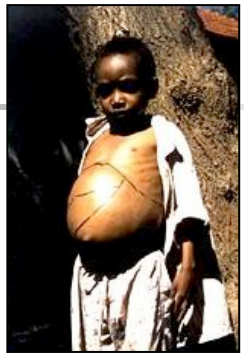
DoD Preventative Measures

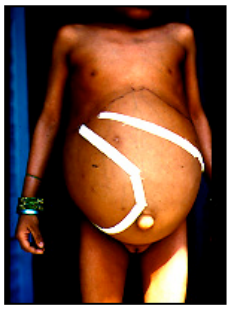
- Suppress animal reservoirs
  - Dogs, rats, gerbils and other rodents
- Suppress the Sandfly vector
  - Critical to preventing disease for stationary troop
- Prevent sandfly bites
  - Personal protective measures
    - Important at night
    - Sleeves rolled down
    - Insect repellent w/ DEET
    -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



Visceral Leishmanisis

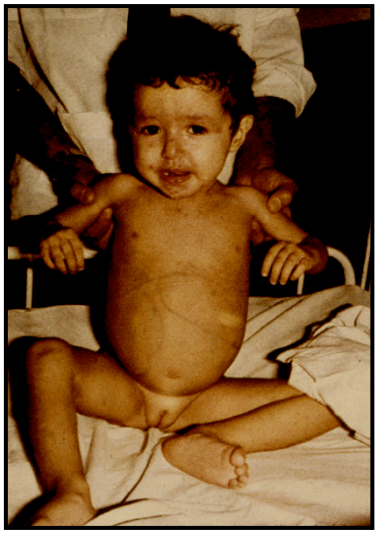
- **3 possibly related species**
  - ***L. donovani* (Asia, Africa)**
    - **India (kala azar)**
  - ***L. infantum* (Mediterranean, Europe)**
  - ***L. chagasi* (New World)**
- **reticuloendothelial system affected**
  - **spleen, liver, bone marrow, lymph nodes**
- **onset is generally insidious**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





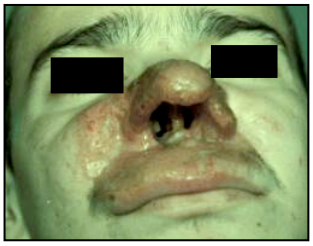
Visceral Leishmaniasis

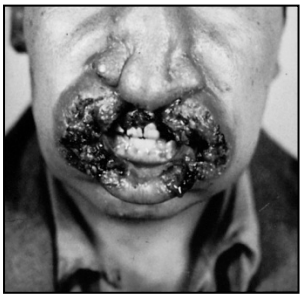
- **incubation period**
  - **generally 2-6 months**
  - **can range 10 days to years**
- **fever, malaise, weakness**
- **wasting despite good appetite**
- **spleno- and hepatomegaly, enlarged lymph nodes**
- **depressed hematopoiesis**
  - **severe anemia**
  - **leucopenia**
  - **hemorrhages in mucosa**



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



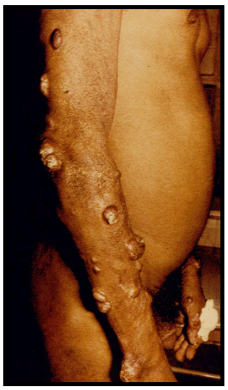


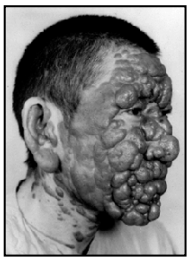
Diffuse cutaneous leishmaniasis

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

- Post kala azar

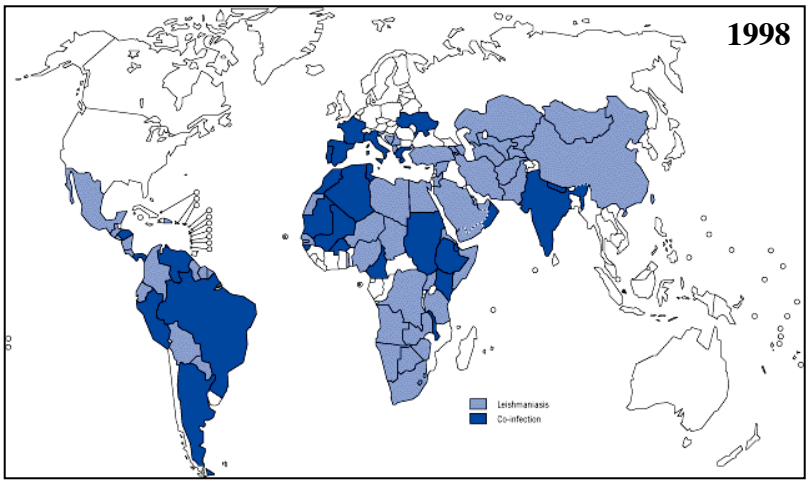
- due to inadequate treatment
- nodular lesions
- easily cured with treatment (in contrast to DCL)





Leishmania/HIV co-infection

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



Key to persistance

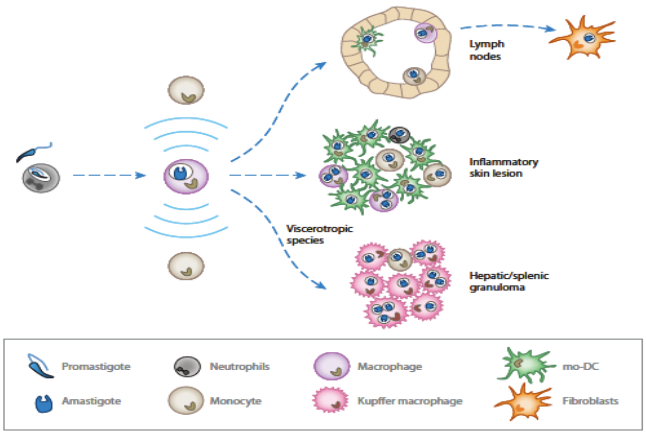


Figure 1

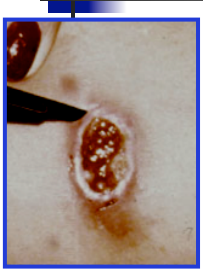
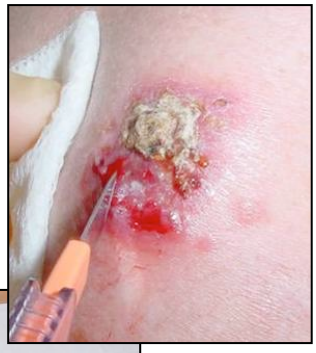
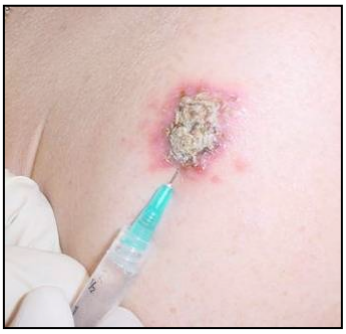
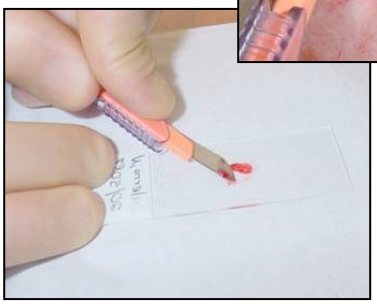
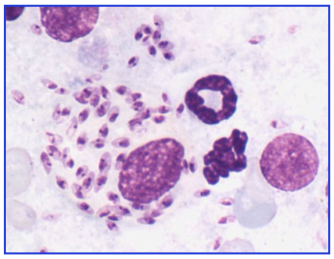
Multiple host niches occupied by *Leishmania* parasites. Metacyclic promastigotes injected into the skin by the sandfly are phagocytosed primarily by neutrophils and resident dendritic cells. Promastigotes released from apoptotic neutrophils are subsequently phagocytosed by macrophages and monocytes recruited to the site of infection (*blue lines*). Dendritic cells derived from recruited monocytes (mo-DC) can be major host cells in inflammatory lesions that develop at the site of the bite or in draining lymph nodes (27, 70). Other host cells, such as neutrophils and eosinophils, can also harbor parasites. Following resolution of clinical disease, parasites may persist as a quiescent population in nonprofessional phagocytic cells such as fibroblasts (12). Viscerotropic species (*Leishmania donovani*, *L. infantum/chagasi*) can disseminate from dermal tissues to the liver, spleen, and bone marrow to cause visceral leishmaniasis. The major host cells infected in the liver are resident Kupffer macrophages (5, 71).

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

Treatments for Leishmaniasis

Table 1. Drugs in use and on trial in 1985 and 2005

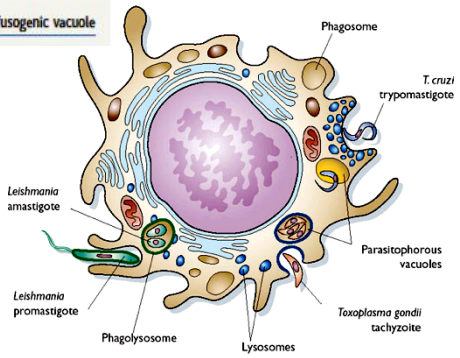
Visceral leishmaniasis	**1985**	**2005**
First-line drugs	Sodium stibogluconate (Pentostam); meglumine antimoniate (Glucantime)	Sodium stibogluconate (Pentostam, generic sodium stibogluconate); meglumine antimoniate (Glucantime)
	Amphotericin B (Fungizone)	Amphotericin B (Fungizone)
		Liposomal amphotericin B (AmBisome)
	Pentamidine	Pentamidine
Clinical trials	Allopurinol (Phase II)	Miltefosine (oral, Phase IV, registered in India)
		Paromomycin (Phase III)
		Sitamaquine (oral, Phase II)
		Other amphotericin B formulations
Drugs in preclinical development	–	–
Cutaneous leishmaniasis	**1985**	**2005**
First-line drugs	Sodium stibogluconate (Pentostam); meglumine antimoniate (Glucantime)	Sodium stibogluconate (Pentostam); meglumine antimoniate (Glucantime)
	Amphotericin B (Fungizone)	Amphotericin B (Fungizone)
	Pentamidine	Pentamidine
		Paromomycin (topical formulations)
Clinical trials	Paromomycin (topical formulation, Phase II)	Miltefosine (oral, Phase III)
	Allopurinol riboside (Phase II)	Paromomycin (other topicals, Phase II)
		Imiquimod (topical immunomodulator, Phase II)
Drugs in preclinical development	–	–

Different approaches to intracellular survival

Table 1. Summary of entry mechanisms and survival niches of intracellular parasites. Abbreviations: BR(2), bradykinin receptor 2; C1, complement receptor 1; C3, complement receptor 3; TGF-β, transforming growth factor-β.

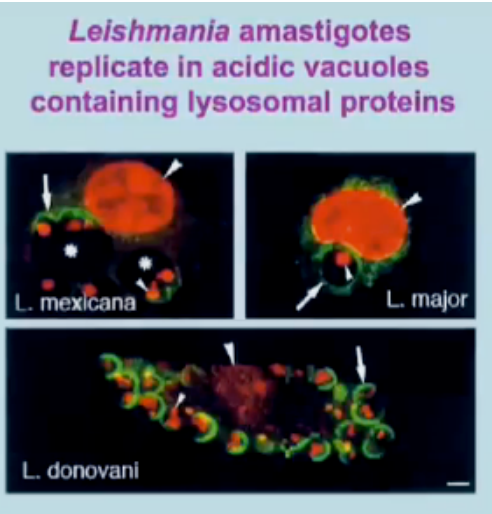
Property	Intracellular parasite		
	Leishmania	*T. cruzi*	*Toxoplasma*
Cell types	Primarily macrophages	Variety	All nucleated
Receptors	C1, C3, Scavenging	G protein, BR(2), TGF-β	GAGs, sialic acid
Entry mechanism	Phagocytosis	Calcium-induced lysosome fusion	Direct penetration
Host actin	Yes	No	No
Parasite actin	No	No	Yes
Niche	Lysosomal	Cytosolic	Nonfusogenic vacuole

- Trypansoma 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 ...

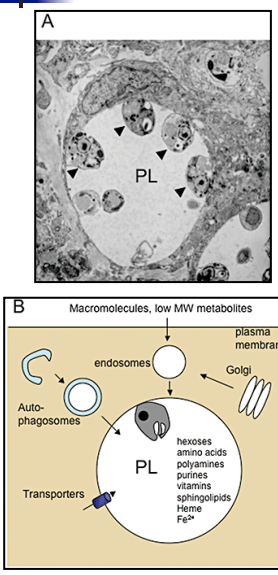


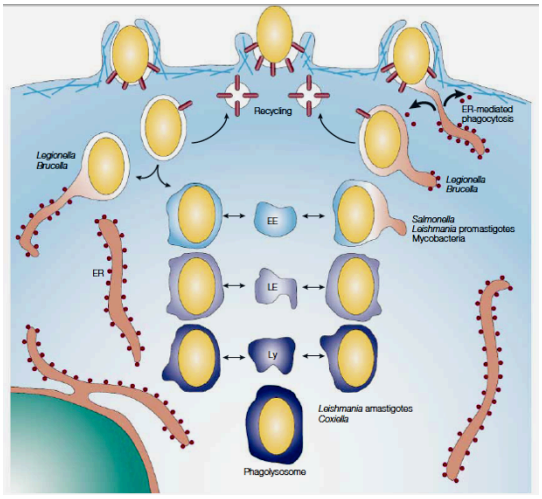
Leishmania parasitophorous vacuole

- 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 continuosly receives contents from secretory and endocytic pathways



Leishmania phagosome





Nutrient Acquisition

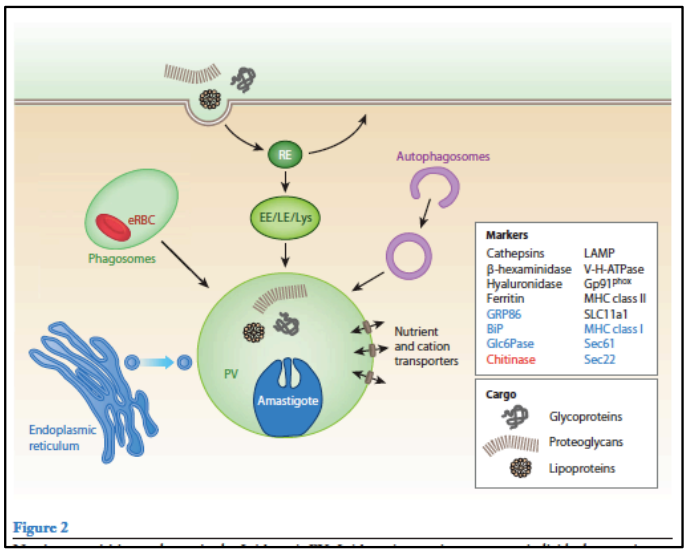


Figure 2