Genus : Leishmania

Subgenus

Leishmania Viannia

Species parasite to man

Lesimania

- Leishmania donovani complex
  - L.D. causing - Kala-azar (VL, AVL)
  - L. infantum - infantil kala azar / ZVL
  - L. chagasi - ZVL
  - L. archibaldi - VL
- L. maxicana complex
  - L. mexicana - CL
    chiclero's ulcer
  - L. amazonensis
  - L. venezuelensis
  - L. pifonoi
- L. tropica complex
  - L. tropica - CL
  - L. killicki - LCL
- L. major complex
  - L. major - LCL(ulcerative type)
- L. aethiopica complex - CL / DCL

Viannia

- L. Braziliensis complex
  - L. braziliensis – invde skin/ oro-nasal mucosa (espundia) DCL/ VL
  - L. peruviana - skin/ oro-nasal mucosa
- L. guyanensis complex
  - L. guyanensis – CL
  - L. panamensis – LCL
  - L. shawi - CL
- L. lainsoni complex - CL
- L. naiffi complex - CL

Clinical classification

- Visceral leishmaniasis(Kala-azar)(VL)
- Cutaneous leishmaniasis(CL)
- Muco-Cutaneous leishmaniasis(MCL)

Leishmania Parasites and Diseases

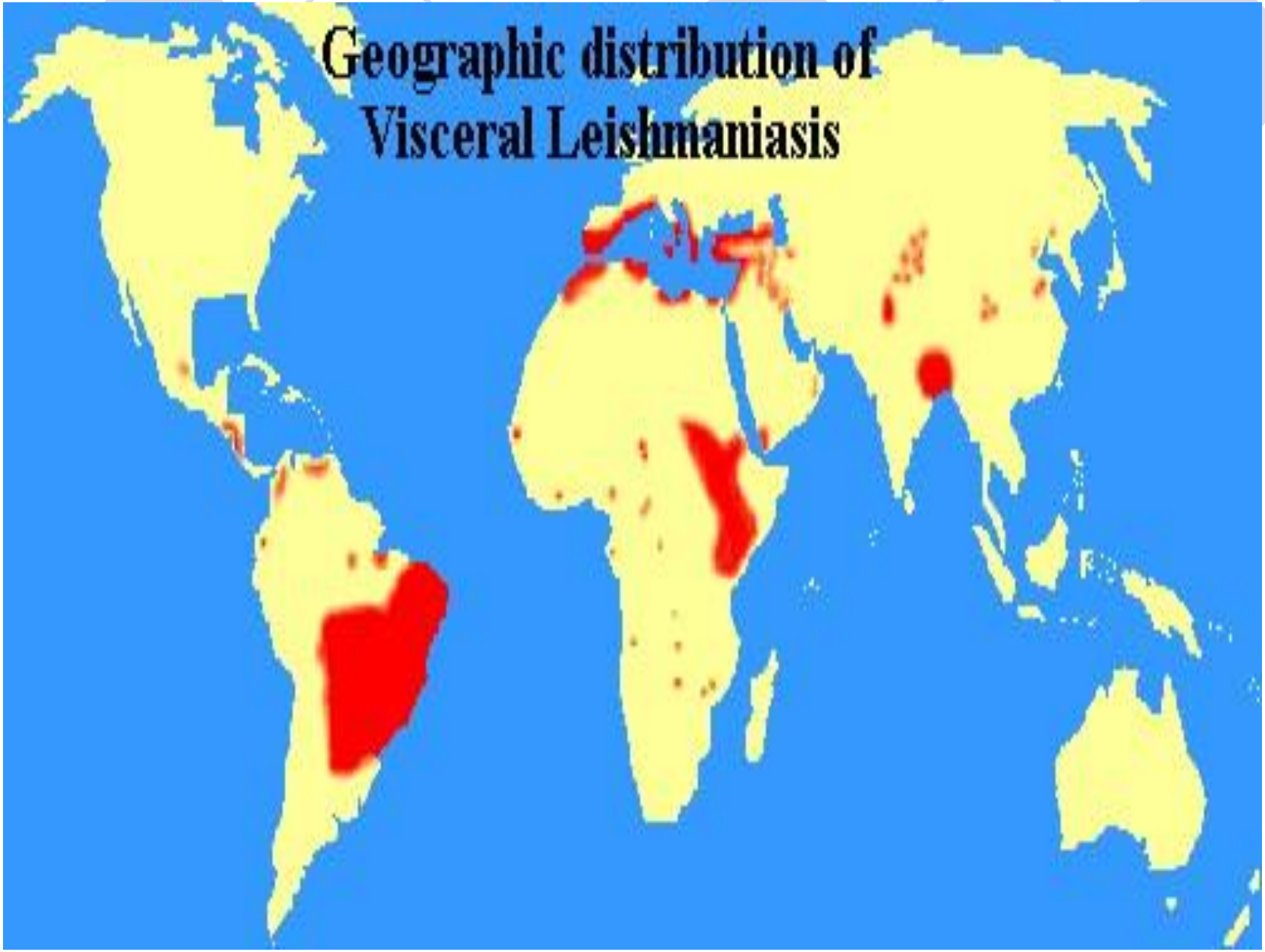
SPECIES	**Disease**
*Leishmania tropica**	Cutaneous leishmaniasis
*Leishmania major**	
Leishmania aethiopica	
Leishmania mexicana	
Leishmania braziliensis	Mucocutaneous leishmaniasis
*Leishmania donovani**	Visceral leishmaniasis
*Leishmania infantum**	
Leishmania chagasi	

- Leishmania donovani

  - Visceral leishmaniasis / Kala-azar

Geographic distribution

- More commonly seen in tropical countries than temperate
- India, Bangladesh, China, Middle east, East Africa & South and central America
- In India
  - Assam, Bengal(West), Bihar, Orissa, Tamilnadu & parts of Uttar Pradesh


Geographic distribution of
Visceral Leishmaniasis

History

- Leishman- London May 1903
- Donovan- Madras July 1903
  - Reported parasite in splenic smears of patients of Kala azar.

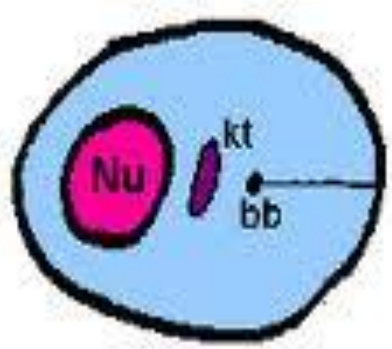
Habitat

- A parasite of R-E system
- Intracellular- in Macrophage, liver & splenic cells.

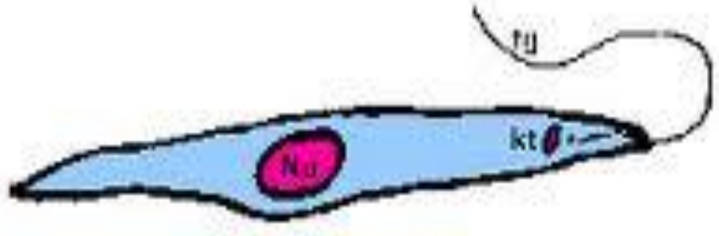
Morphology

- Two forms

- Amastigote - Aflgaellar form
  - Inside R-E cells of definite host-man

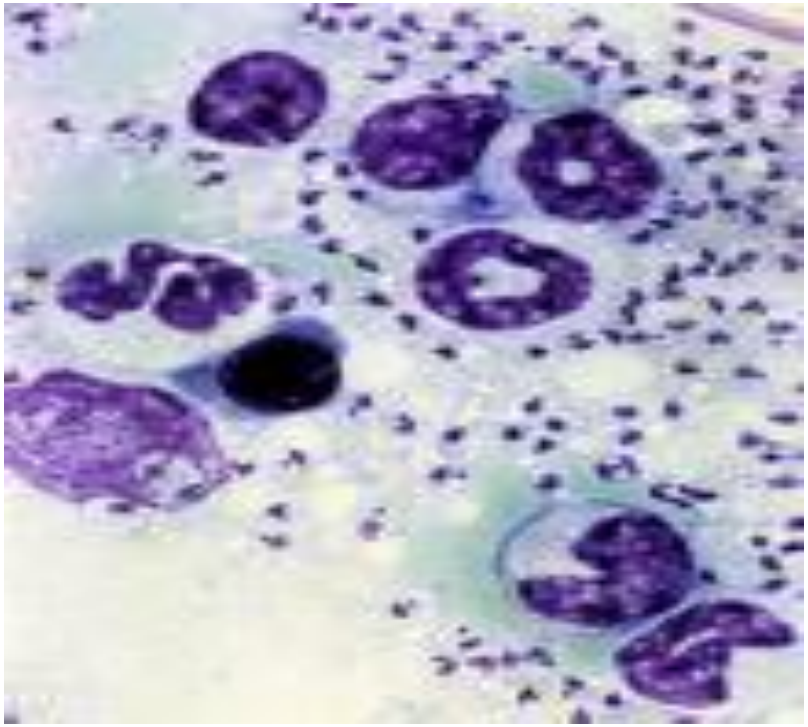


- Promastigote – Flagellar stage
  - Gut of sand-fly (insect)
  - Artificial culture



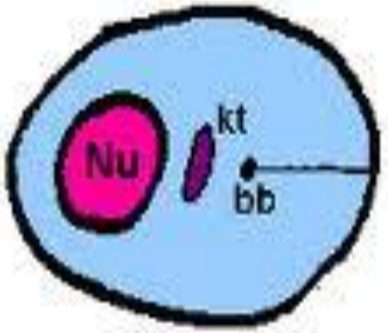
Sand fly - Phlebotomus

Amastigote & Promastigote



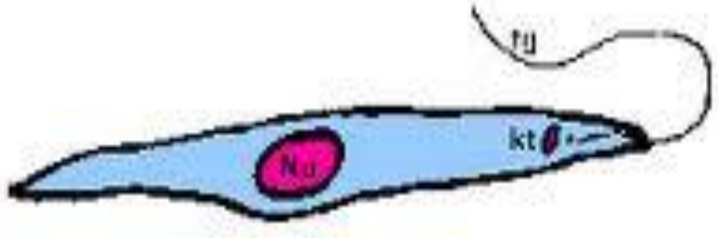
Amastigote form

- Size – 2 to 4 micron
- Shape – round or oval body
- Cell membrane – delicate
- Nucleus-
  - 1 micron in diameter, round to oval
  - Middle of cell or along side of cell wall
- Kinetoplast
  - At right angle to nucleus
  - Either rod shaped (Para basal body) or
  - tiny dot like body (blepharoplast)
- Axoneme
  - Delicate filament extending from the kinetoplast to the margin of the body
- Vacuole
  - A clear unstained space lying along side the axoneme.



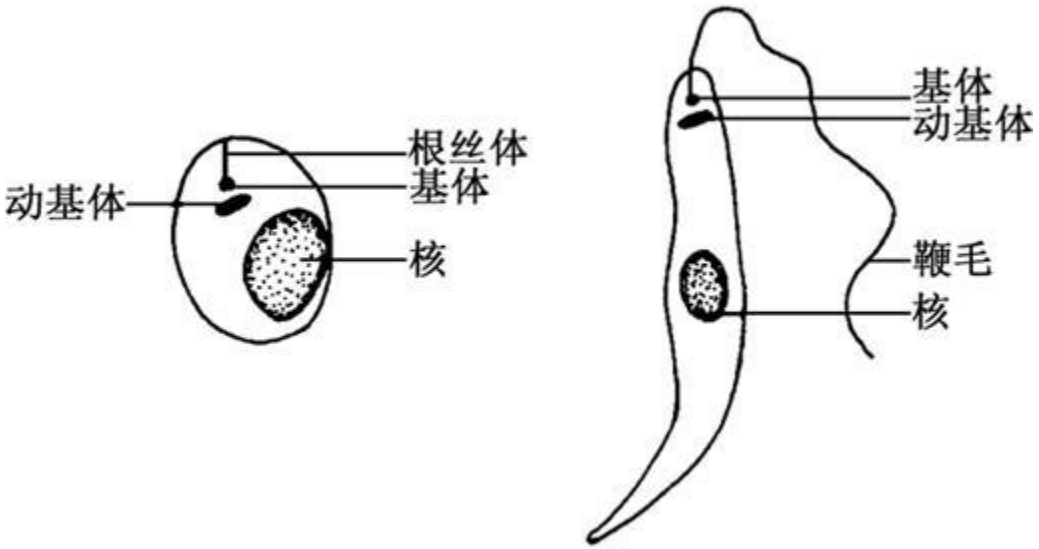
Promastigote stage

- Size – 15 to 20 u X 2 to 3 u
- Shape – long slender spindle shaped bodies
- Nucleus- centrally
- Kinetoplast
  - Lies transversely near anterior end.



- Axoneme
  - Delicate filament extending from the kinetoplast to the margin of the body
- Eosinophilic Vacuole
  - Light staining area lying in front of the kinetoplast.
- Flagellum
  - Same length as the body or even longer, projecting from front.
  - Does not curve around the body, so no undulating membrane.

Amastigote & Promastigote

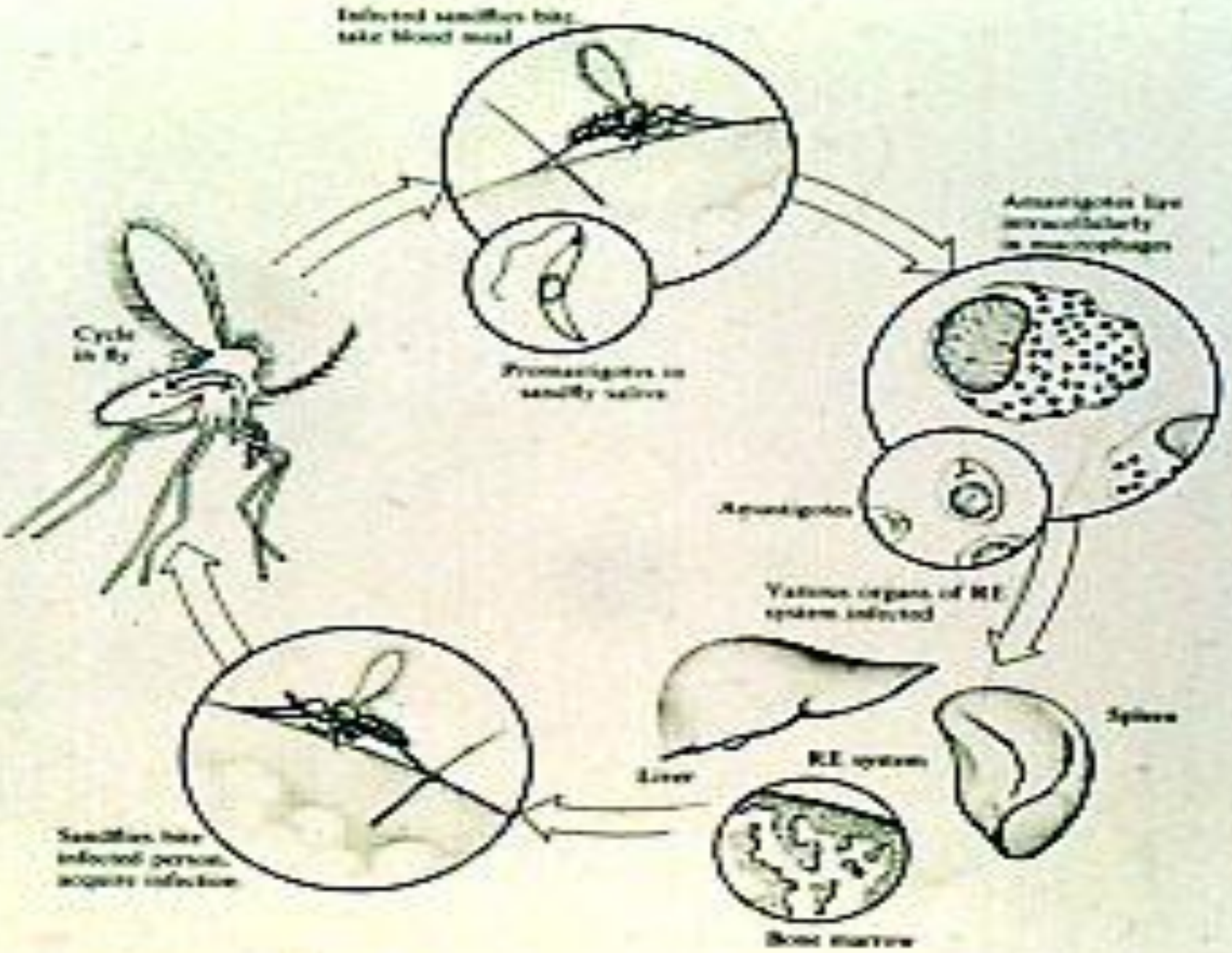


Life cycle

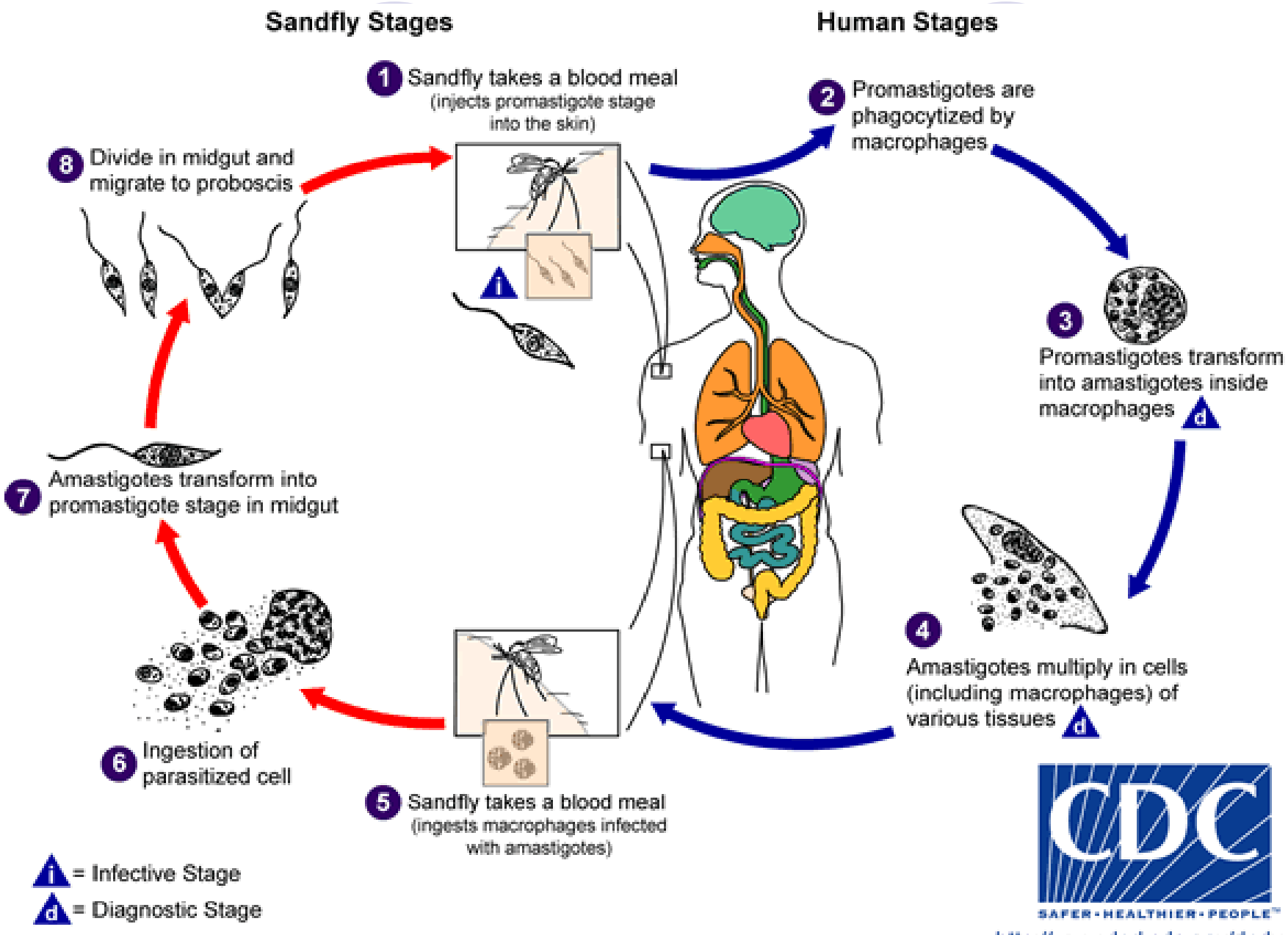
- Passes life cycle in two hosts.
  - Definite – Man & other vertebrate host
  - Intermediate-Sand fly

- Infective form- Promastigotes present in buccal mucosa
  - Bite of infected sand fly- enter tissues
  - Taken up by macrophage, lose flagella and transform into amastigotes
  - Multiply by binary fission to reach enormous numbers
  - Macrophage distends & ultimately ruptures
  - Liberated amastigotes enter other macrophages
  - Few are present free in peripheral blood.

Leishmania donovani

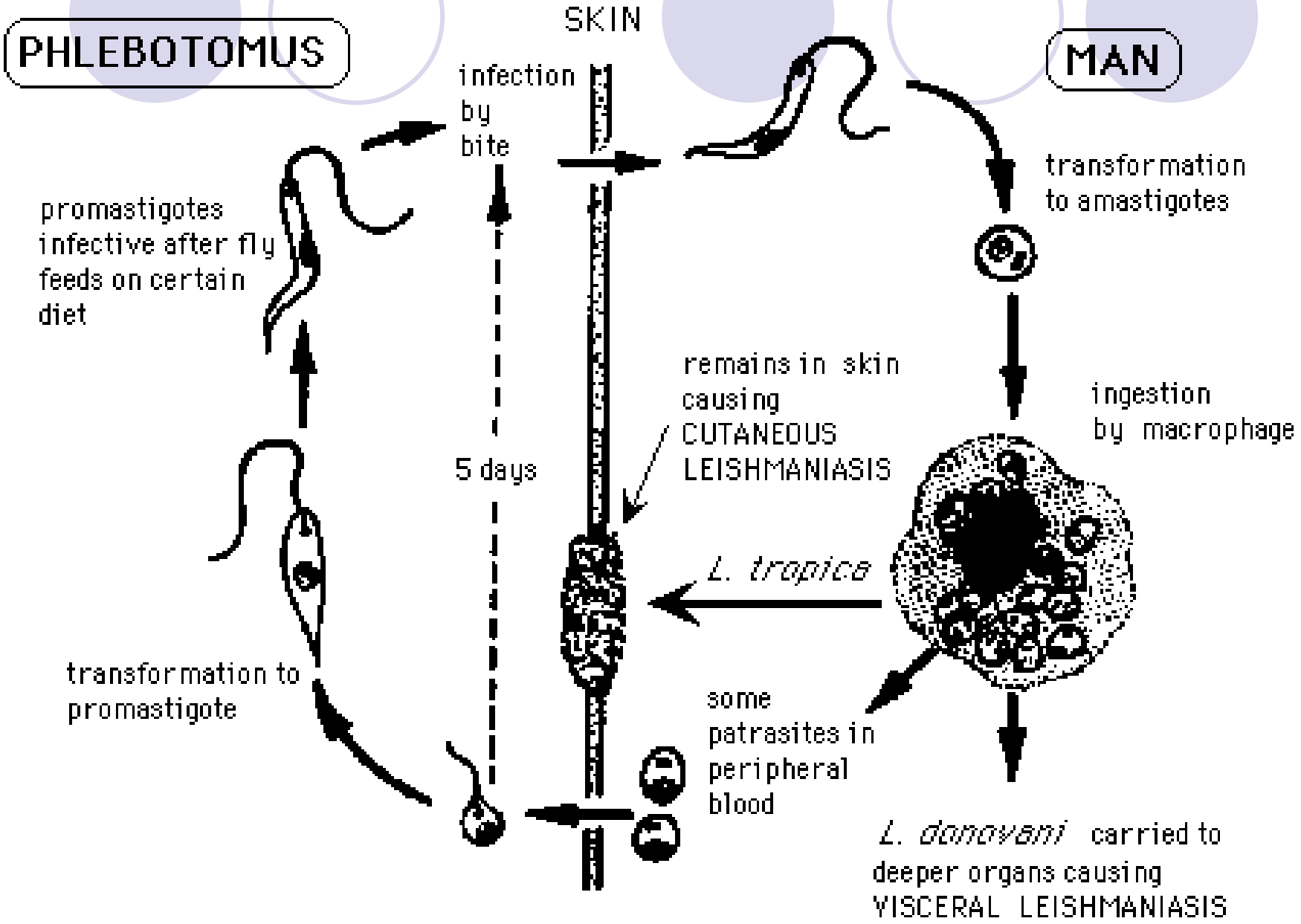


- When female sand fly bites infected person, amastigotes present in peripheral blood are taken up
- Reach midgut, change morphology
- Converted into promastigotes
- Multiply by binary fission
- Migrate to pharynx and buccal cavity in large numbers
- Mature promastigote block passages.
- Hence in order to take another blood meal sand fly has to liberate large number or parasites in person
- Process takes 6 to 10 days – Extrinsic incubation period


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal
(injects promastigote stage
into the skin)
2 Promastigotes are
phagocytized by
macrophages
3 Promastigotes transform
into amastigotes inside
macrophages
d
4 Amastigotes multiply in cells
(including macrophages) of
various tissues
d
5 Sandfly takes a blood meal
(ingests macrophages infected
with amastigotes)
6 Ingestion of
parasitized cell
7 Amastigotes transform into
promastigote stage in midgut
8 Divide in midgut and
migrate to proboscis
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx

Life cycle of *Leishmania donovani* & *L. tropica*

(After Smyth, 1994)



Pathogenicity & clinical features

Incubation period : 3 to 6 months

Disease produced is called **kala-azar or visceral leishmaniasis**

Pyrexia

early symptom, it may be continuous or remittent in type becoming intermittent at later stage

Splenic enlargement- one of the most striking feature of disease.

With progression it extends several inches below the costal margin filling up the entire abdomen.

Clinical features

- Liver is also enlarged but not as much as spleen
- Skin changes
  - Seen on face, hands, feet & abdomen
  - Dark pigmentation of skin-kala
  - Skin is dry, rough & harsh
- Lymphadenopathy is often seen in African & Chinese form of kala azar
- Infections in immunocompromised person
  - One of the important opportunistic infection occurring In patients of AIDS
- Anemia in kala azar
  - Due to hemolysis occurring due to hypersplenism
  - Autoimmune basis-

Laboratory diagnosis

- Direct evidence
  - Demonstration of organism in smear &/or culture from specimens like blood, splenic biopsy or bone marrow biopsy
- Indirect evidence
  - Demonstration of circulating antibody non-specific or specific by various serological tests

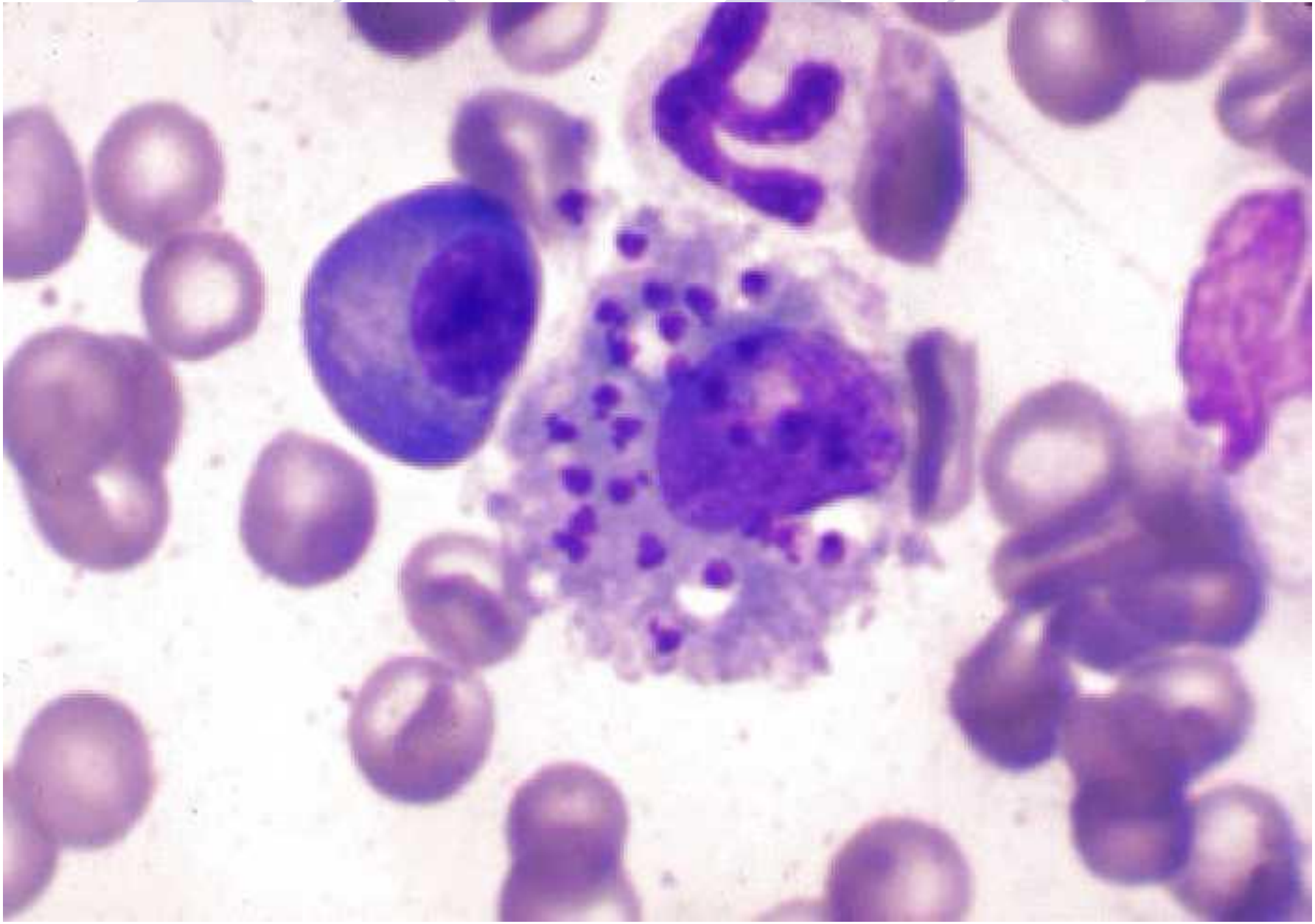
Cultivation

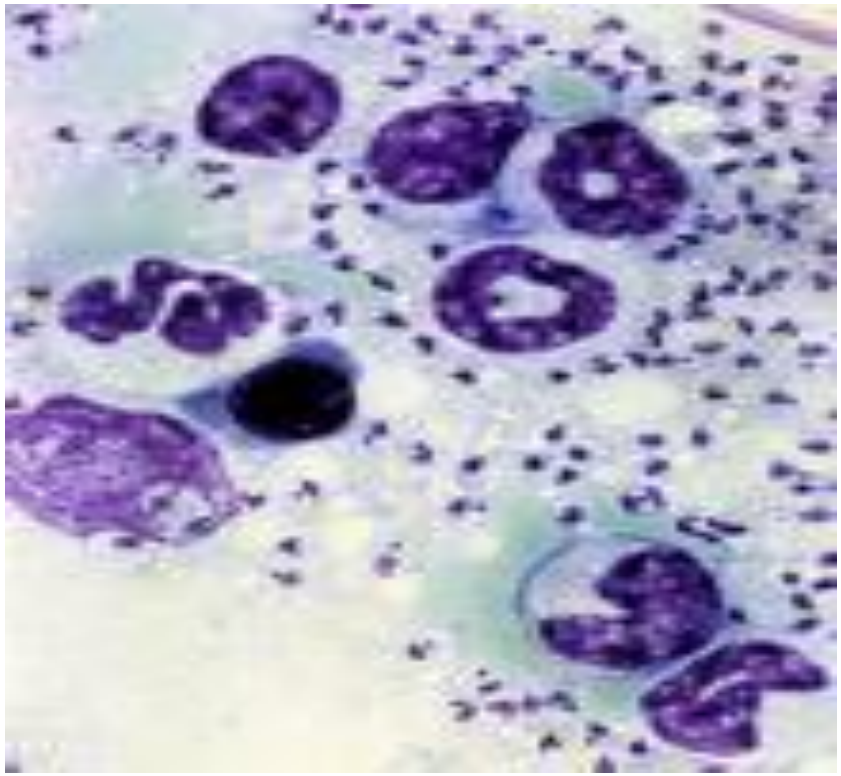
- NNN medium
  - 2 part of salt agar & 1 part of defibrinated rabbit's blood
  - Novy & Mac Neal prepared first, later modified by Nicolle
  - Material is inoculated into water of condensation and incubated at 24 c.
  - Presence of Ascorbic acid & hematin favors the growth of parasite
  - Amastigote form change morphology to promastigote

Demonstration of organism in smear

- Blood
  - By making a thick blood film
  - By producing straight leukocyte edge
  - By centrifuging citrated blood
  - Culture
    - 1 to 2 ml of blood is inoculated into water of condensation of NNN medium and incubated at room temp for 4 weeks with daily observation at weekly interval

- Splenic puncture
  - Important in those whose blood smear is negative
  - Only drawback is bleeding which might continue from puncture site
  - It is advisable to perform bleeding time & prothrombin time before doing it
- Bone marrow biopsy
  - A certain method of diagnosis particularly in early cases when spleen is not enough enlarged
  - Compared to splenic puncture it s safer method as risk of hemorrhage is low
  - But more painful and less sensitive than splenic puncture





Indirect evidence

- Blood count:
  - Leucopenia with marked diminution of neutrophils
  - Total count is often < 3000 , may be as low as 1000
  - Erythrocyte count is also low

- Serological tests
  - Nonspecific
    - Aldehyde test
    - Antimony test
    - Complement fixation test with W.K.K. antigen
  - Specific
    - Immunofloroscent test
    - Indirect haemagluttination test
    - Specific complement fixation test

Aldehyde test

- 1 to 2ml of serum + 1-2 drop of 40 % formalin
- If jellification of milky white opacity like white of a boiled egg occurring in 2 to 20 minute – test is considered positive
- Due to an increase in serum gamma globulin
- Positive only after 3 months duration of disease

Antimony test

- To 1-2 ml of serum, add drop by drop 4 % urea stibamine solution in distilled water
- Formation of profuse flocculent precipitate indicates positive reaction
- Less reliable than aldehyde test

Complement fixation test with WKK antigen

- Antigen used is prepared from human tubercle bacillus as suggested by Witebsky, Klinghausen & Kuhn
- Helps in early diagnosis of disease when Aldehyde test is negative
- Positive by 3 weeks of infection

Post kala-azar dermal leishmaniasis (PKDL, Dermal leishmanoid)

- Cutaneous form of leishmaniasis occurring in about 2-10% of kala -azar patients generally one or two year after completion of successful antimonial treatment
- At this time visceral infection disappears but skin lesion persists
- Only found in India & African form of kala-azar
- Also found in spontaneously cured kala-azar

PKDL-Clinical manifestations

- Hypo pigmented patches (macule)
  - Earliest lesions on the trunk & extremities, face is less affected.
- Erythematous patches
  - Early lesion seen on nose, cheek and chin, having butterfly distribution
  - Photosensitive, becoming prominent during mid-day
- Yellow-pink nodules
  - Nodules replace earlier lesions, occasionally develop at the outset.
  - Mostly distributed on skin (mostly of face), rarely on mucus membrane of tongue & eyes
  - Granulomatous, soft and painless yellow to pink nodules of varying size , **do not ulcerate**.

PKDL

Diagnosis & treatment of PKDL

- Demonstration of Amastigote form of *L.donovani* in RE cells of skin in leishman stained smear from biopsy material obtained from nodular lesions.

- Smear from macules are often negative

Diagnosis & treatment of PKDL

- By pentavalent antimonial in double doses used for visceral lesions

- Standard treatment of kala-azar
  - Sodium stibogluconate – 20 mg/kg bodyweight up to 850 mg/day for 30 days
  - Pentamidine 3-4 mg/kg, 1 or 2 times/week for 2 weeks
  - Amphotericin B 0.25 to 1 mg/kg daily slow infusion for 8 days

Leishmania causing old world cutaneous leishmaniasis

- Causative agent
  - *Leishmania tropica*
  - *Leishmania major*
  - *Leishmania aethiopica*
- Responsible for
  - Cutaneous leishmaniasis- *Oriental sore*
  - Also called as –
    - Delhi boil
    - Baghdad boil

Discovery of *Leishmania tropica*

Cunningham	1885	Observed parasite from patient of Delhi boil in Kolkata
Borovsky	1891	Make accurate description of morphology
Luhe	1906	Given name Leishmania tropica

Georgraphical distribution

Life cycle

- Intermediate Host- Sand fly – Phlebotomus
- Definite Host- Man
- Reservoir of infection
  - L.tropica- None
  - L.major- rodents
  - L.aethiopica- rock
- Mode of infection
  - Promastigote present in buccal mucosa enter tissues by bite of infected sand fly or by crushing of the infected sand flies into punctured wound.

Immunity

- CMI develops early and results in elimination of parasites.
- A single attack gives lifelong immunity
- Serum antibodies are not produced
- In non-immune person it produces diffuse cutaneous leishmaniasis (p.e. L.aethiopica)
- Attack lasts for 4-6 months (L.major) sometimes 1-2 year (L.tropica) followed by spontaneous cure in most cases

Clinical features

- Incubation period
  - 2-8 months (may up to 1 or 2 year)
- L.tropica
  - Cutaneous lesion- Oriental sore or Delhi boil
  - Small papule develops at the site of bite which soon becomes a raised nodule 2 to 5 cm in diameter
  - Majority of cases nodules ulcerate, having a clean-cut margin, raised indurated edge surrounded by red areola
  - Limited in number- 2 to 3 or sometimes single & distributed over exposed part of body (face & extremities)
  - Heals spontaneously and in 1-2 years producing a depressed white scar leaving patient immune to reinfection

Oriental sore

- L.major
  - Clinical picture is similar except lesions develop and heal more quickly than those of L.tropica.
  - Ulcer is self healing in 3-6 months
  - Infection gives cross immunity to L.tropica & L.aethiopica
- L.aethiopica
  - Lesion similar to Oriental sore but may give rise to diffuse cutaneous leishmaniasis in patients with poor CMI
  - Incurable condition characterized by formation of disfiguring nodules over the surface of body.

Diffuse cutaneous leishmaniasis

- Characterized by appearance of diffuse nodular infiltrative lesion which are neither destructive nor erosive but most disfiguring
- Starts as single lesion and spreads slowly over face, ears, extremities and buttock, until whole body is affected
- Histologically nodes consists only of histiocytes with relative absence of lymphocytes & plasma cells
- Leishmanin skin test is negative
- Amastigotes are recovered both from blood as well as bone marrow

Diffuse cutaneous leishmaniasis

Laboratory diagnosis

- Microscopy
  - Smear prepared from material obtained by a puncture of indurated edge of the sore or nodule & stained by Leishman stain
  - Amastigotes are found in large numbers inside macrophage
- Isolation
  - Culture into NNN medium will give promastigotes

Laboratory diagnosis

- Skin test (Leishmanin test)
  - Antigen -promastigotes of L.tropica - 10^6 per ml
  - 0.1 ml injected intradermally in inner surface of forearm
  - Area of induration of 5 mm or more after 48-72 hours of inoculation is considered positive
  - Negative leishmanin test is seen in patients of diffuse cutaneous leishmaniasis & active visceral type.

Leishmania causing new world cutaneous & muco-cutaneous leishmaniasis

- Causative agents
  - Cutaneous leishmaniasis
    - L.braziliensis subspecies
      - L.b. guyaensis
      - L.b. panamensis
      - L.b.peruviana
    - L.mexicana
      - L.m. mexicana
      - L.m.amazonensis
      - L.m.pifani
      - L.m.venezuelensis
  - Muco-cutaneous leishmaniasis
    - L. braziliensis subspecies braziliensis

Habitat

- Intracellular parasites inside macrophage cells of skin & mucus membrane of nose and buccal cavity.

- Promastigote forms occur in insect vector- Sand fly (Lutzomyia)

Geographical distribution

- Mainly seen in South & Central America

Epidemiology

- Mainly seen in central & south America
- Reservoir of infection – small forest rodents and dogs
- Transmission- Sand fly – Lutzomyia

Lutzomyia

Life cycle

- Basic life cycle same except that the amastigotes- mononuclear cells of skin & mucus membrane

- Promastigotes- found in mid gut & buccal cavity of sand fly

Pathogenesis

- Similar to that of oriental sore
- Lesions are typically found in skin and mucosa is affected subsequently

Clinical disease

- Cutaneous leishmanisis

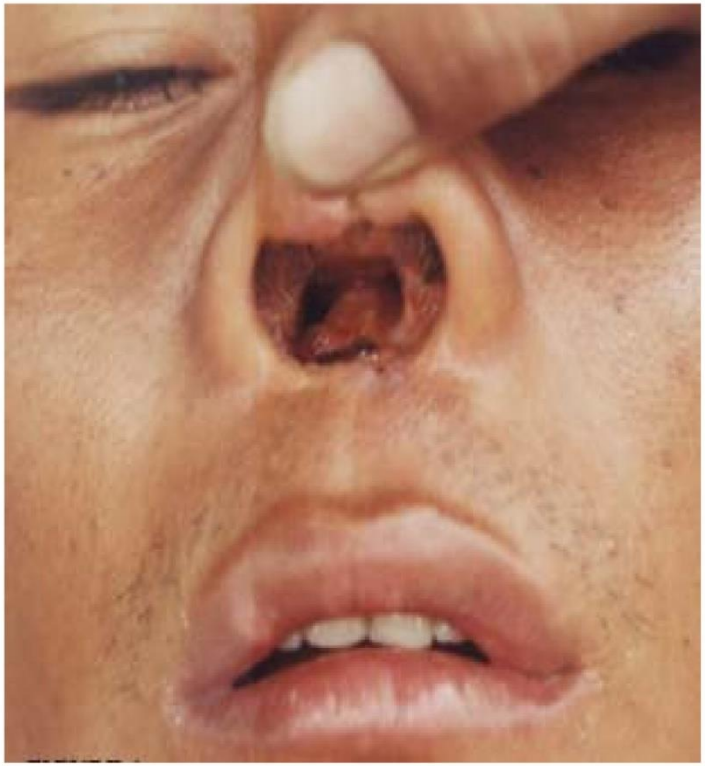
L.b. guyaensis	Forest Yaw-Benign form
L.b. panamensis	Skin ulcer-non healing
L.b.peruviana	ulcer which heals spontaneously after 4 month
L.m. mexicana	Chicler’s ulcer- Benign form

L.b.guyanensis	Benign form (Forest yaw)	Oro-nasal mucosa
L.b.peruviana	Benign form-only dry papule on skin (Uta in peru)	No involvement of mucosa
L.b.pifanoi	Malignant form	Diffuse cutaneous leishmaniasis
L.b.mexicana	Chiclero's ulcer-a benign form without involvement of mucosa	Single cutaneous lesion on ear, face or hand which undergoes spontaneous healing

Muco-cutaneous leishmaniasis - (Espundia)

- A severe and malignant form of cutaneous leishmaniasis similar to oriental sore associated with invasion of oro-nasal mucosa in south America
- Lesion starts as a papulo-pustular swelling in skin localized around mouth, nostrils or eye or widespread on the face, elbows or knees
- Migrate on the mucosal surface of mouth, nose and naso-pharynx causing destructive and mutilating erosions leading to disfiguration often with complete destruction of nasal septum, perforation of palate and damage to tissues of lips and naso-pharynx
- Heals by scarring producing typical tapir nose or camel nose

Espundia




© CD-ROM ILLUSTRATED LECTURE NOTES ON TROPICAL MEDICINE

Laboratory diagnosis

- Microscopy
- Isolation
- Serology
- Skin test


Thank You