Leishmaniases: Principles and unmet needs

BSc course: Global Health

Module: Vector-borne infectious diseases

9 November 2011

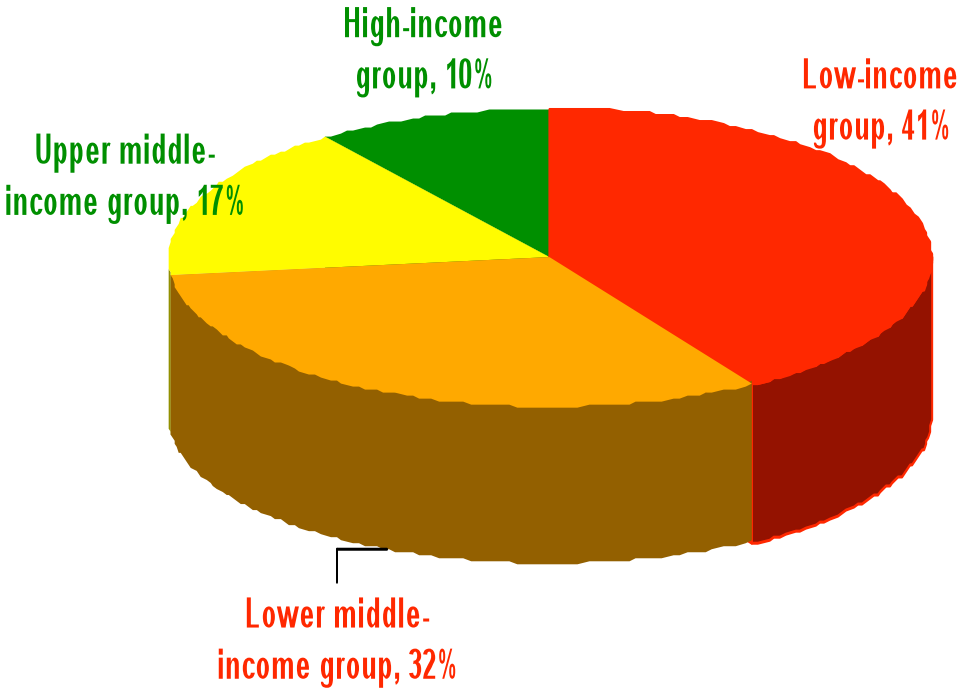
Leishmaniases

- Are a complex of diseases
- Belong to the most neglected tropical diseases (NTD)

NTDs affect poorest people

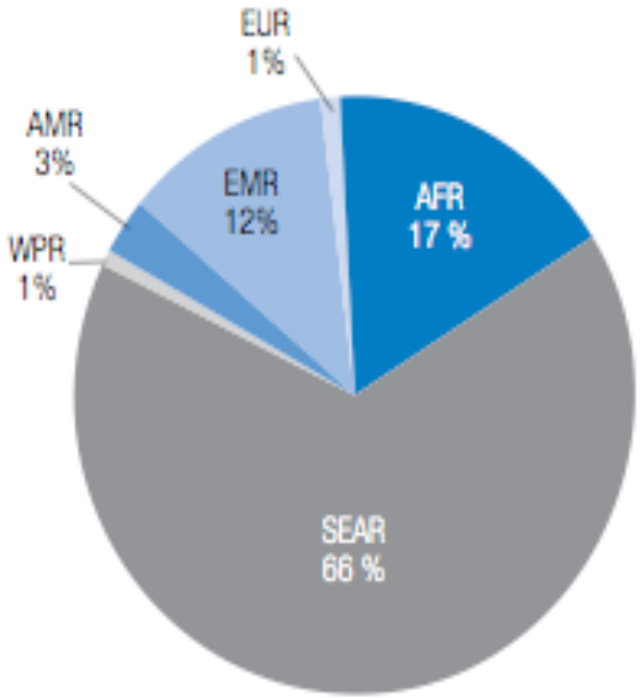
- **2.7 billion people at risk** ***(****living on less than US$2 per day****)***
- **More than 1 billion people affected**
- **Many by one or more neglected tropical diseases**

Distribution of NTDs by income



- More than 70% of countries and territories affected by neglected tropical diseases are low-income and low middle-income countries
- 100% of low-income countries are affected by at least 5 neglected tropical diseases

Burden of leishmaniases – by WHO region, 2009



AFR-African/AMR-The Americas/EMR-Eastern Mediterranean/EUR-European/SEAR-South-East Asia/WPR-Western Pacific

Taken from: “First WHO report on NTDs, 2010

Increasing incidence of leishmaniasis

- 14 October 2010, Infectious Disease Society:

- 13 million Afghans at risk of contracting leishmaniasis
- In 2009: ↑ from 17 000 to 65 000 in Kabul, mainly women and children

- 8 October 2010: International Society for Infectious Diseases:

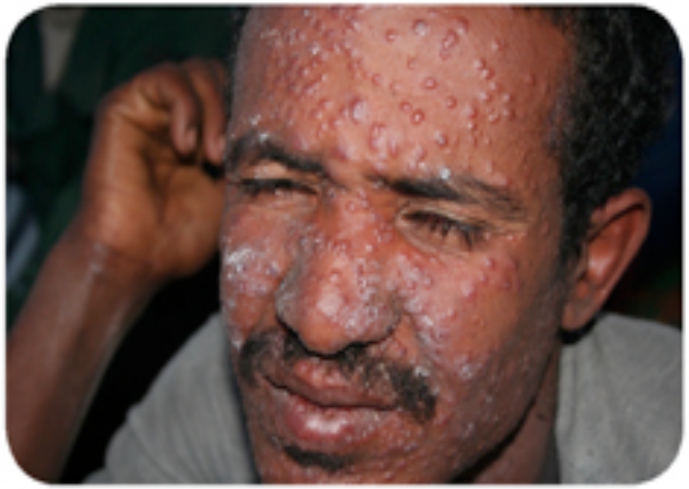
- VL in Sudan has increased 6-fold

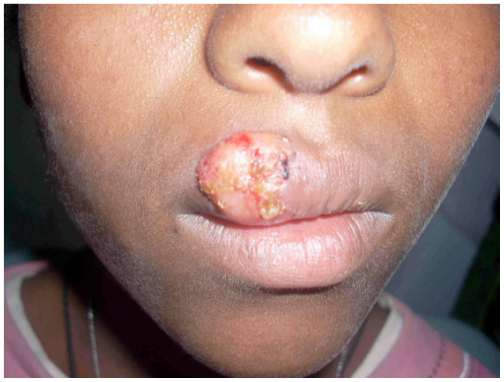
Annual deaths due to NTDs

Revised estimates (The Lancet)

▪Schistosomiasis	150,000 – 200,000
▪**Leishmaniasis**	**100,000**
▪Trypanosomiasis	100,000
▪Hookworm	65,000
▪Ascariasis/Trichuriasis	35,000
▪Dengue (DHF)	20,000
▪Chagas Disease	14,000
▪Leprosy	6,000
Total	**> 500,000**

Tool-deficient NTDs





Killer or severely disfiguring diseases

<u>Complex disease management group</u>

× Complicated and costly

× Difficult to diagnose, dangerous drugs (resistance ↑)

× Highly skilled staff needed

Leishmaniasis

Chagas disease

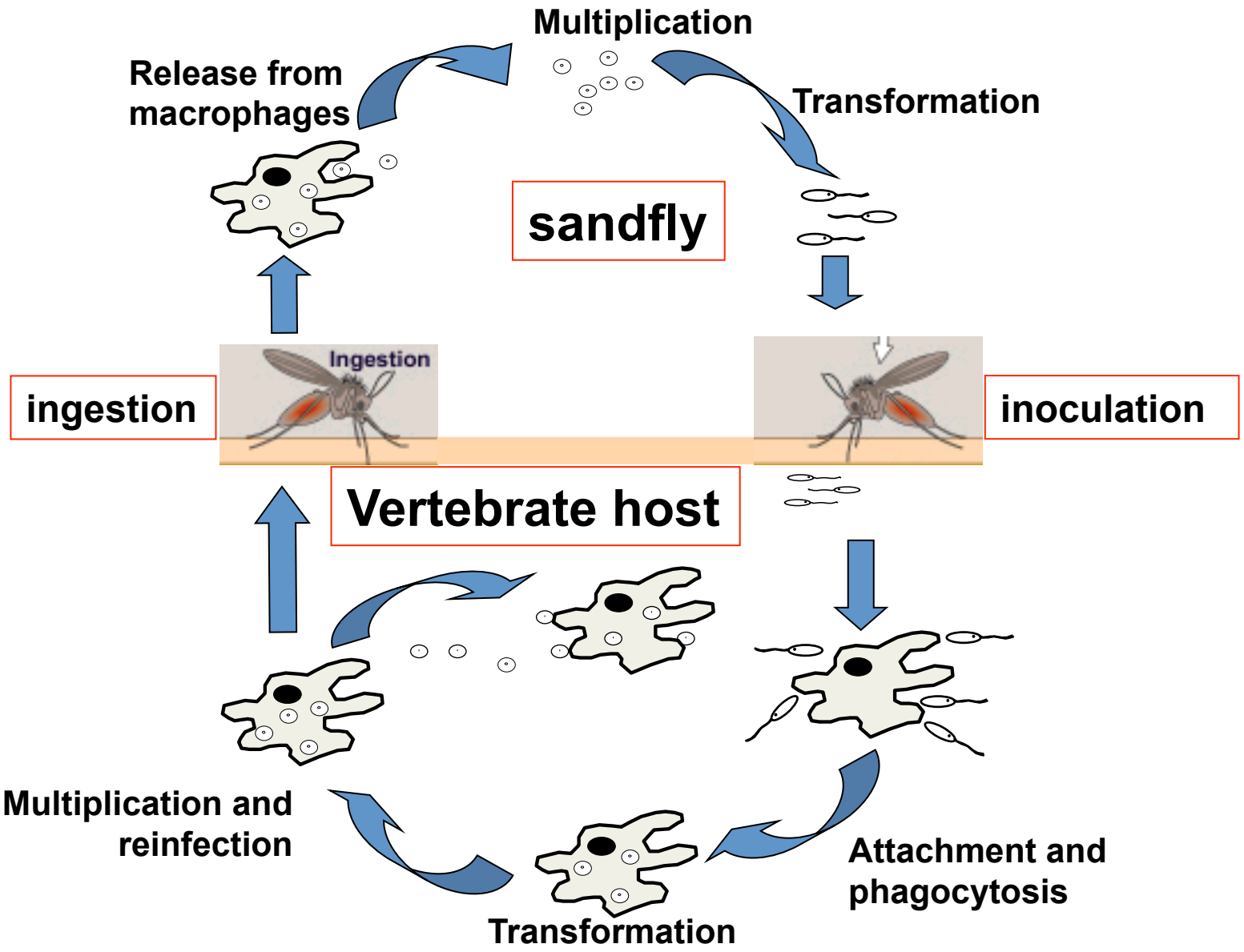
Human African trypanosomiasis

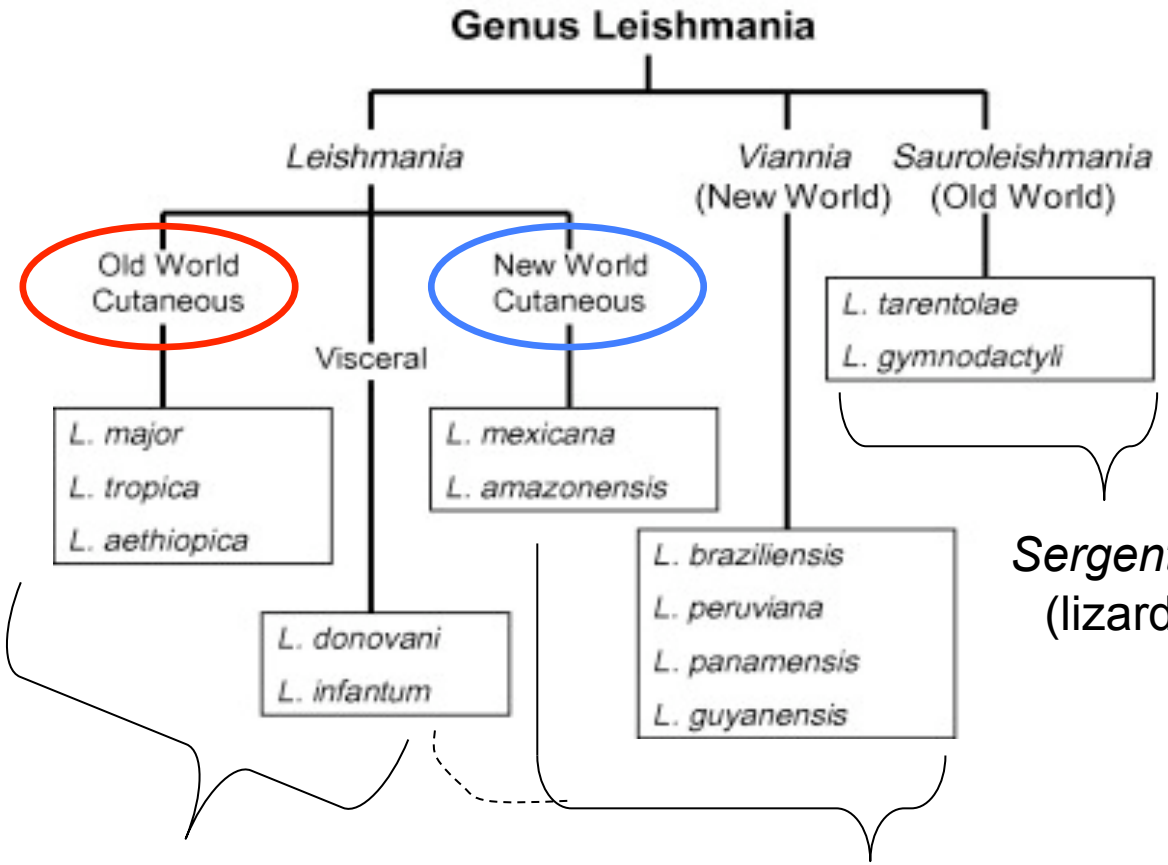
etc

Topics

- Parasite and vector
- Spectrum of disease manifestations
- *Leishmania* HIV co-infections
- Problems in diagnosis and treatment

Life cycle of *Leishmania* parasites



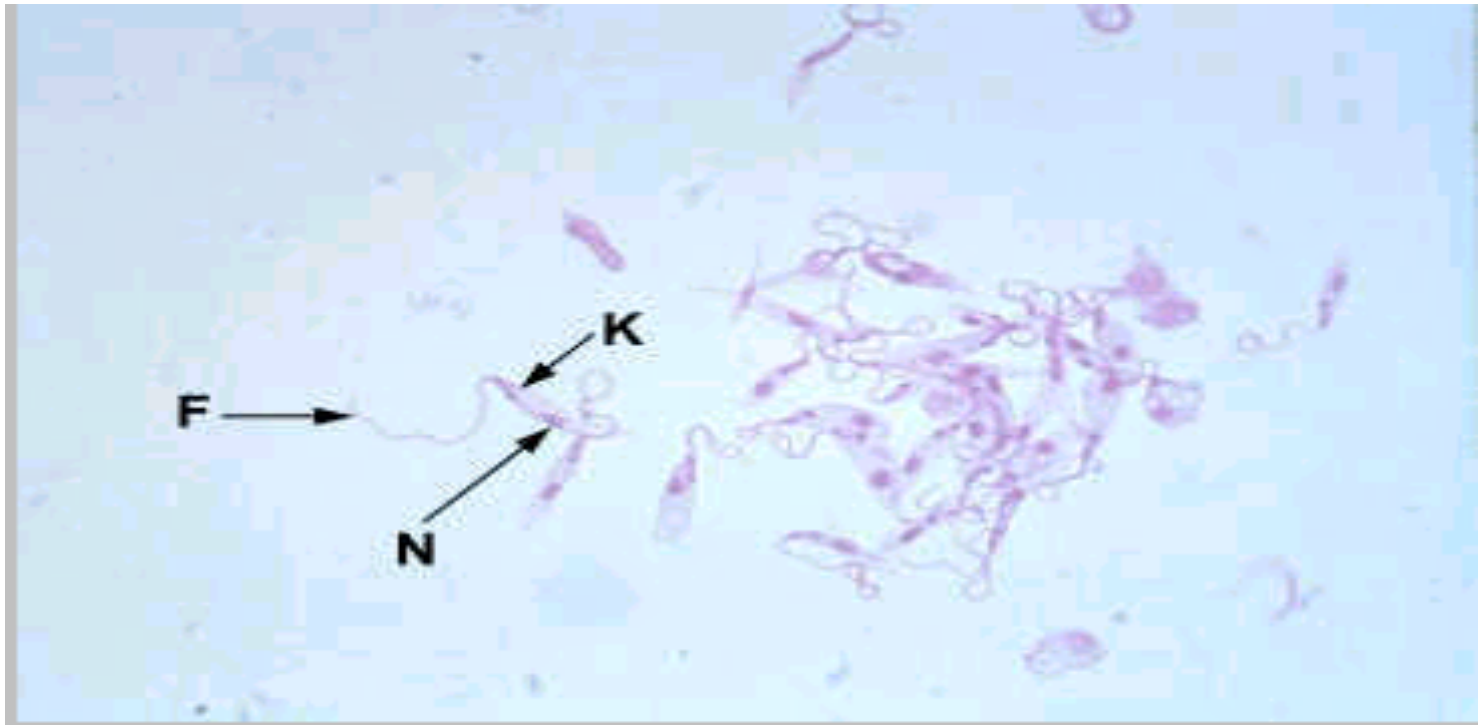


Sergentomyia species
(lizard *Leishmania*)

***Phlebotomus* species**

***Lutzomyia* species**

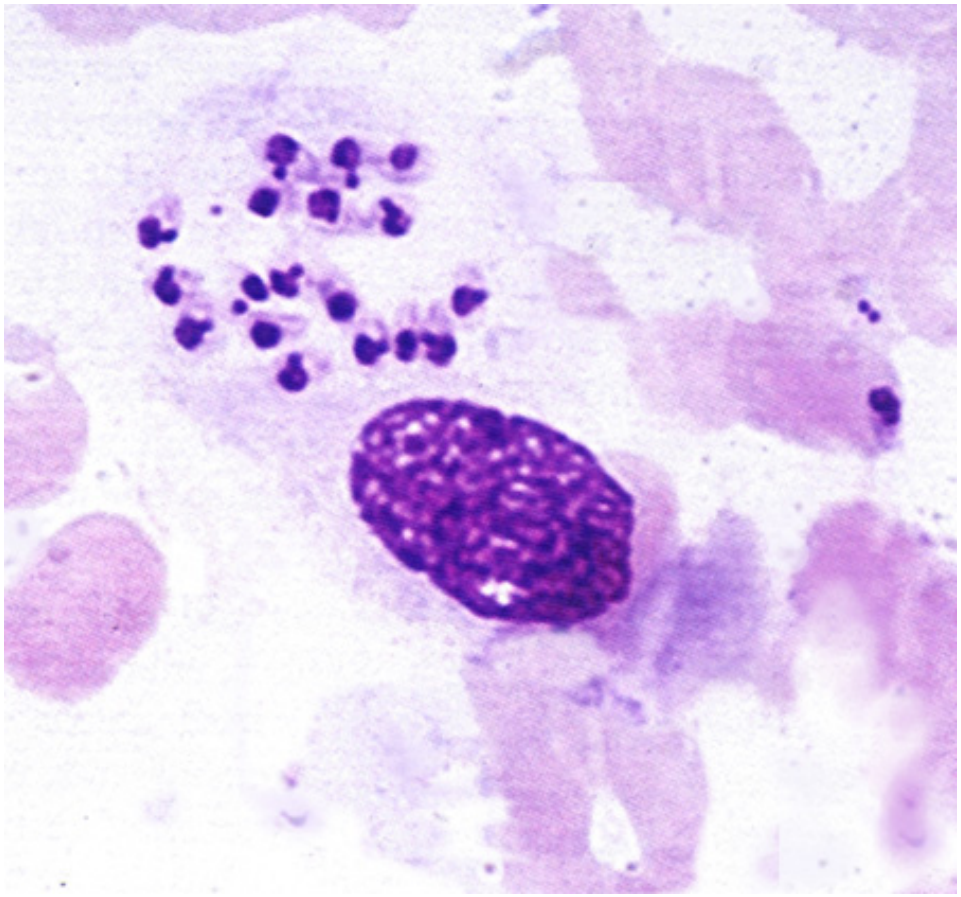
Leishmania promastigotes



Promastigotes of *Leishmania* sp.: in sandfly vector can be cultured

from “Leishmaniasis” Topics in International Health

Leishmania amastigotes



Amastigotes of *Leishmani*a sp.: in human or other vertebrate host's

Female sandflies : vectors of *Leishmania*

Phlebotomus and *Lutzomyia* genera transmit the parasites

Sandflies: 2-3 mm, > 600 species
at least 30 species can transmit *Leishmania*

from “Leishmaniasis” Topics in International Health

Leishmaniases

- parasites, life cycle, vectors
- **The diseases**

Major forms of leishmaniases

- **Visceral leishmaniasis (Kala azar)**: most severe form, fatal if left untreated. Characterised by irregular fever, weight loss, swelling of liver and spleen, anaemia

- **Post Kalar azar dermal leishmaniasis** (PKDL): frequently develops after VL

- **Cutaneous leishmaniasis**: skin lesions on exposed body parts, often self-healing. Can create serious disability and scars. Immunity to reinfection

- **Diffuse cutaneous leishmaniasis:** disseminated lesions, resembles leprosy difficult to treat, no spontaneous healing, frequent relapses

- **Mucocutaneous leishmaniasis**: disfiguring, destroys mucous membranes

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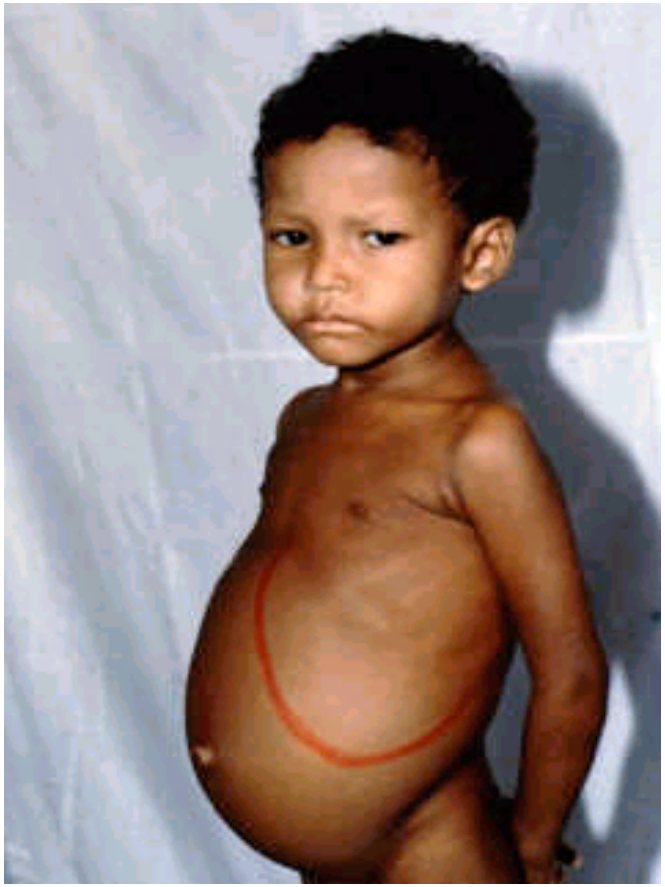
Visceral Leishmaniasis



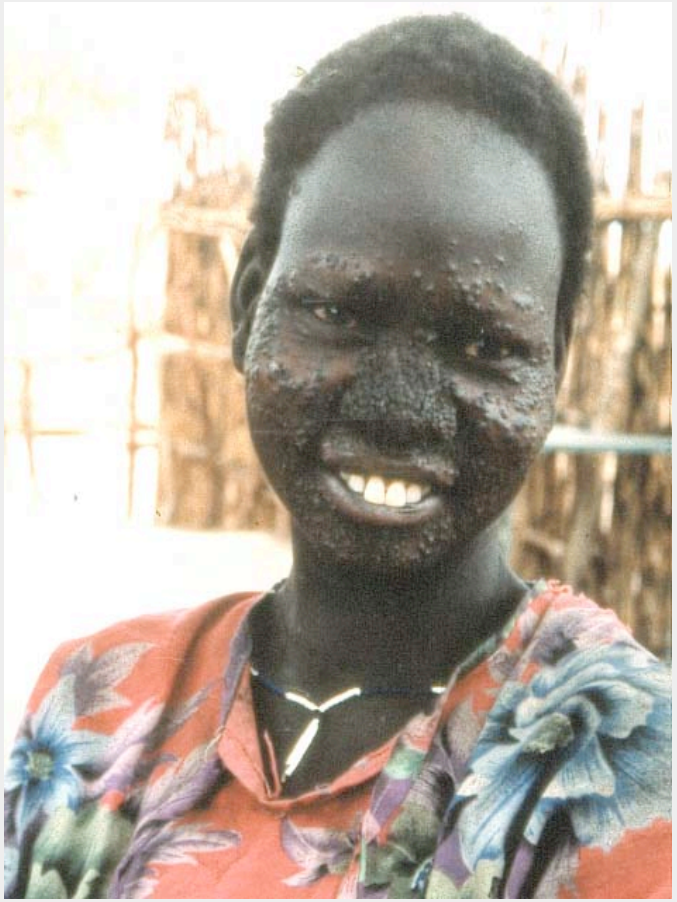
- 30-100 subclinical infections for every overt VL case.
- Lethal if not treated.
- In treated patients, mortality is >10%.
- Increased mortality: jaundice, wasting, severe anaemia and HIV co-infections

Risk factors for development of clinical disease include:

- Malnutrition
- immune suppressive drugs
- HIV co-infections

Fever	95%
Splenomegaly	95%
Uncomfortable spleen	85%
Weight loss	80%
Anaemia	75%
Lymph nodes	75%
Loss of appetite	70%
Cough	75%
Hepatomegaly	60%
Oedema	5%
Diarrhoea	40%
Vomiting	15%
Jaundice	5%

Post Kala-azar Dermal Leishmaniasis (PKDL)



- frequent in Sudan and India
- in ~55% of patients in Sudan, in 5-10% in India
- Occurs during or after treatment,
- after sub-clinical infection
- Lesions start on face, usually around mouth
- Lesions can become nodular
- PKDL can spread to the trunk and limbs.

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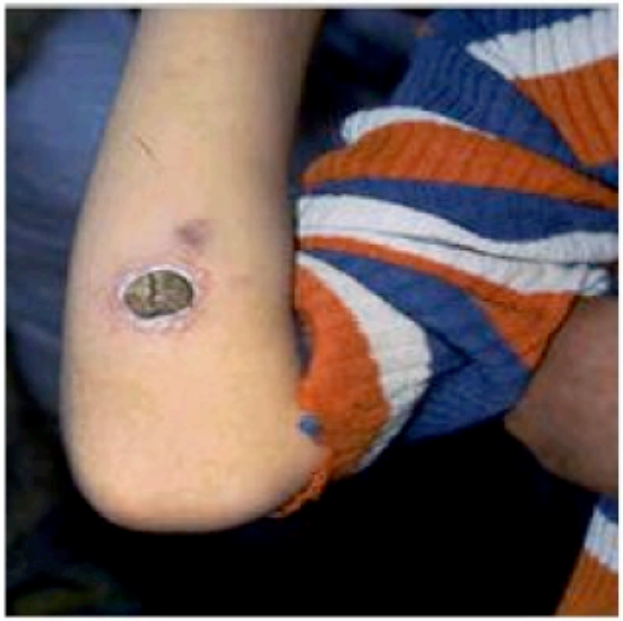
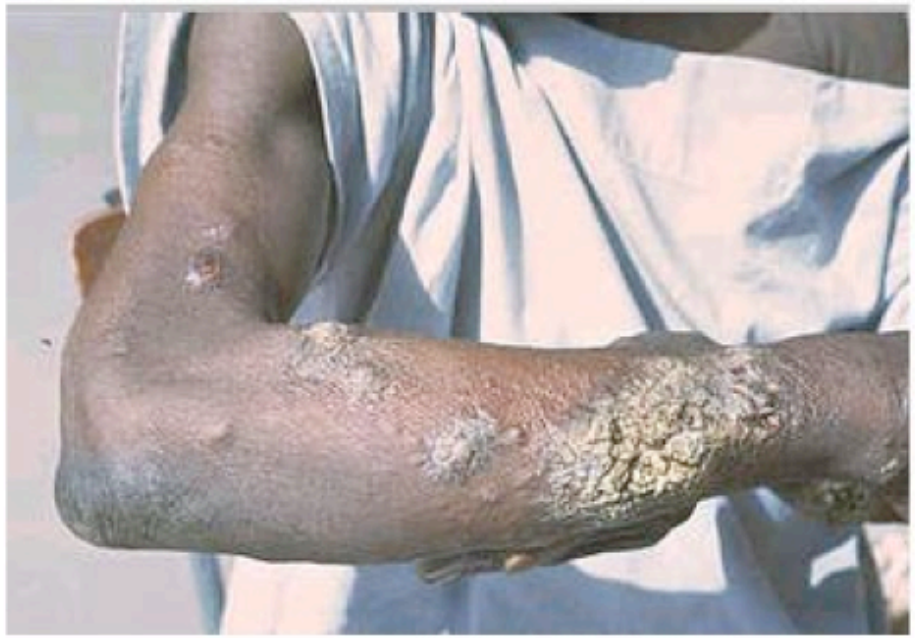
Localised cutaneous leishmaniasis

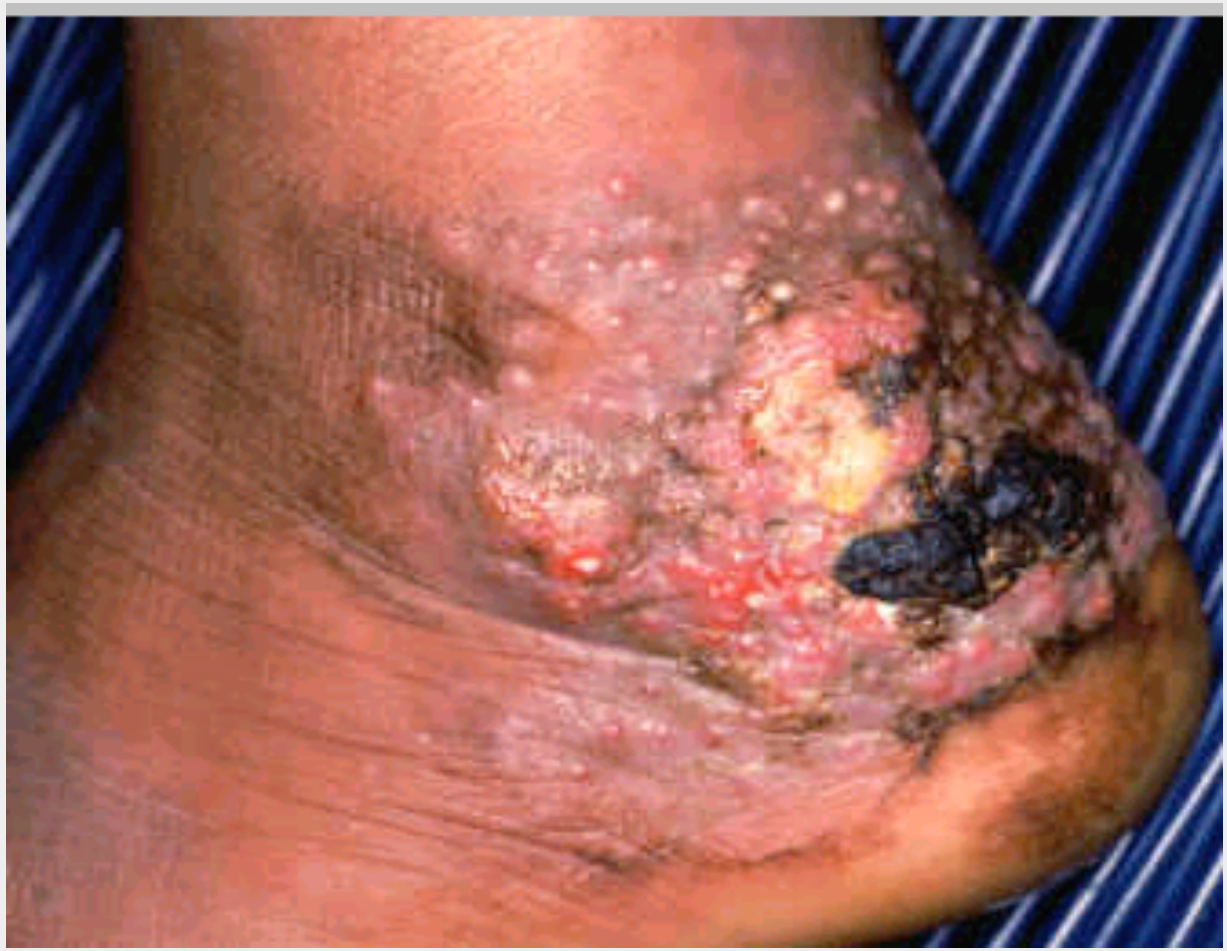
L. tropica: crusted ulcer



Leishmania major:
multiple crusted lesions



Leishmania major: wet lesion



from:
"Leishmaniasis",
Topics in
International Health

Large, irregular ulcer; surrounded by papular and crusted lesions which all contain parasites

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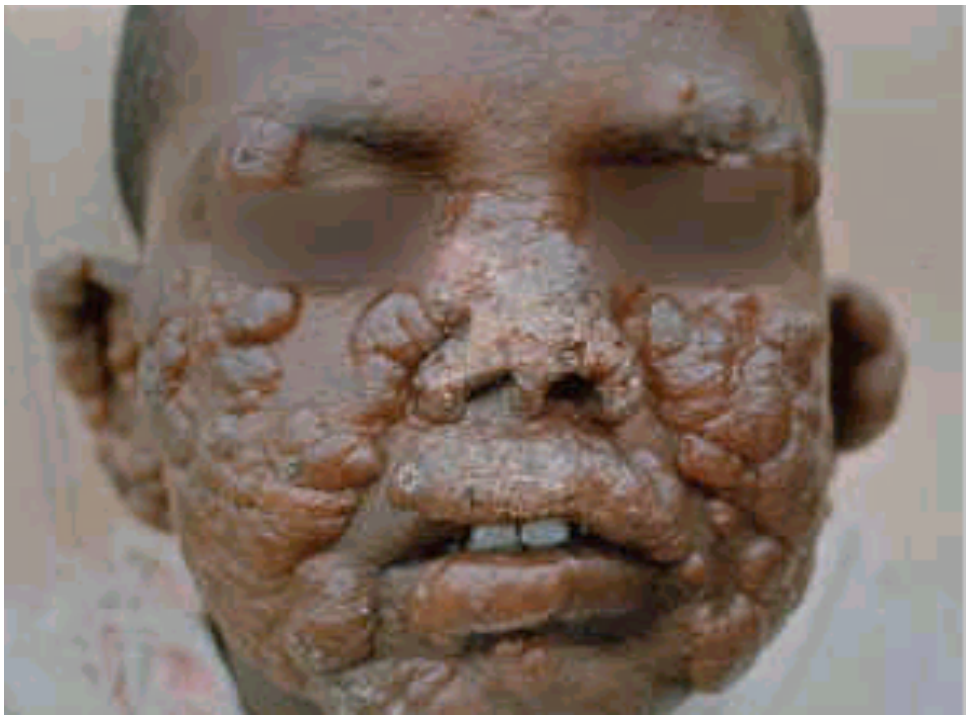
Diffuse cutaneous leishmaniasis

L. aethiopica: disseminated infection



Multiple, nodular non-ulcerating lesions

from “Leishmaniasis” Topics in International Health

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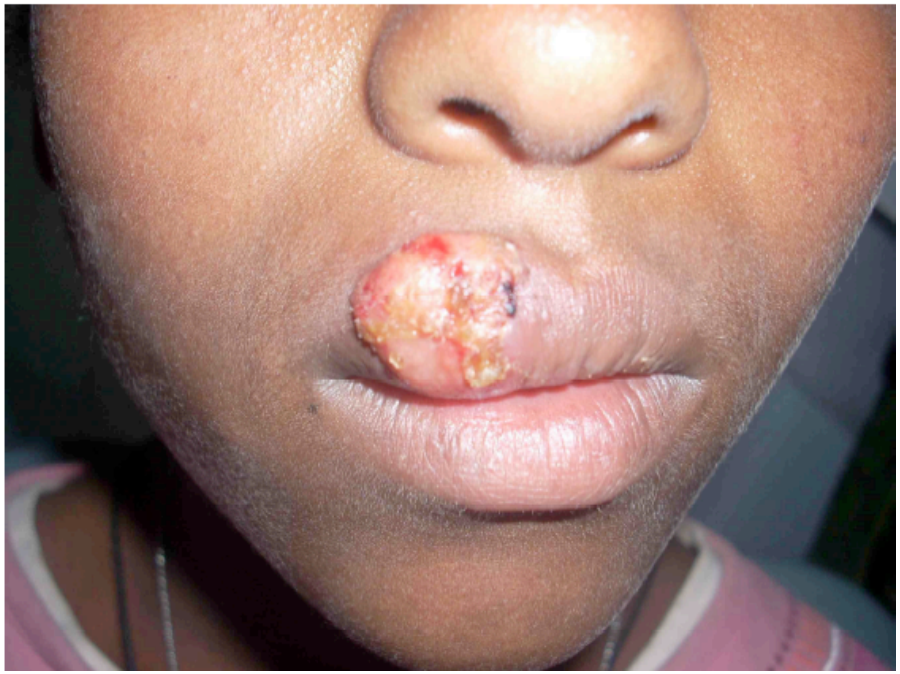
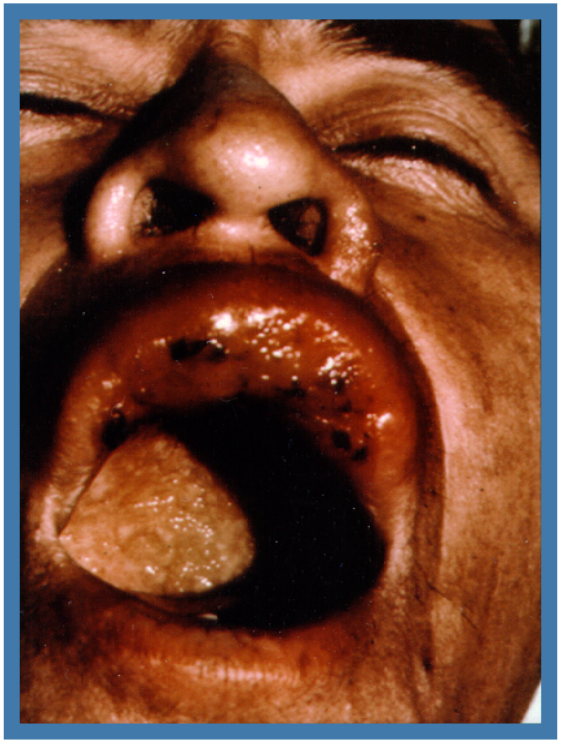
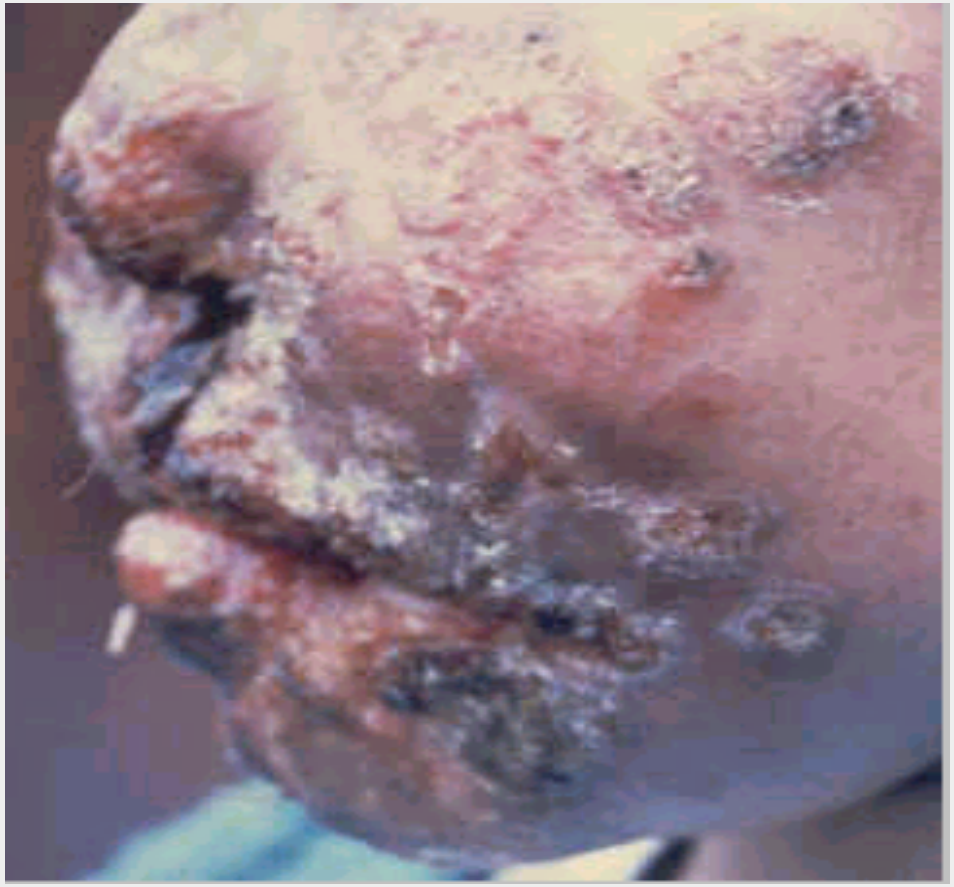
Mucocutaneous leishmaniasis





Addis Ababa University, Leishmania Research & Diagnostic Laboratory, December 2008

Leishmania peruviana: disfiguring crusted lesions



from “Leishmaniasis” Topics in International Health

Leishmaniasis

- The parasites and their vectors
- The diseases
- **Immune response to infection**

Leishmaniases

Factors determining disease manifestation

- parasite species
- vector-derived products
- geographic location
- immune response of the host

(See table 1 in J. Mol. Med., 1998:76:372-390)

Special considerations in parasite immunology

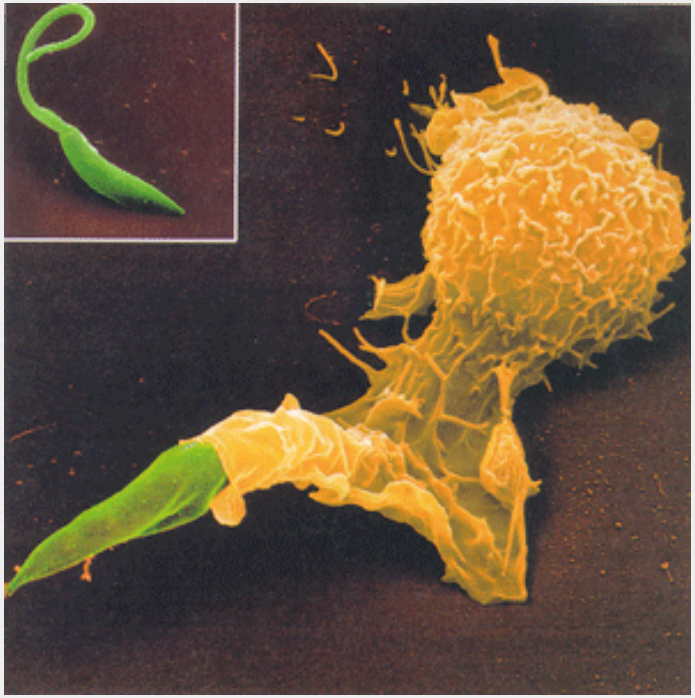
- parasite diversity
- parasite life cycle in host (tissue specificity, migration)
- host-pathogen interactions (evasion/avoidance)
- vector-host interactions
- antigen load (intensity of infection, multiplication)
- concommitant immunity (persistent parasites regulate immunity to new infections)
- pathology

Fundamental principle of immuno-regulation of leishmaniasis:

- replication inside MΦ
- parasite elimination by MΦ
- cell-mediated immunity: T cells instruct MΦ
- T cells regulate immune responses
- T cell memory protects against reinfection

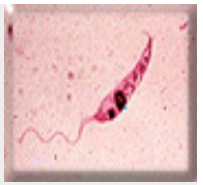
Leishmania are obligate intracellular parasites

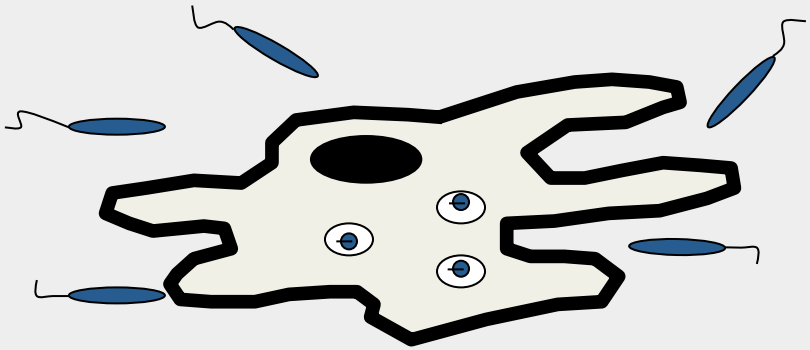
Main host cells: macrophages



Killers

Hosts





Classical activation

Alternative activation

IL-12

IL-4

T-bet
Th1

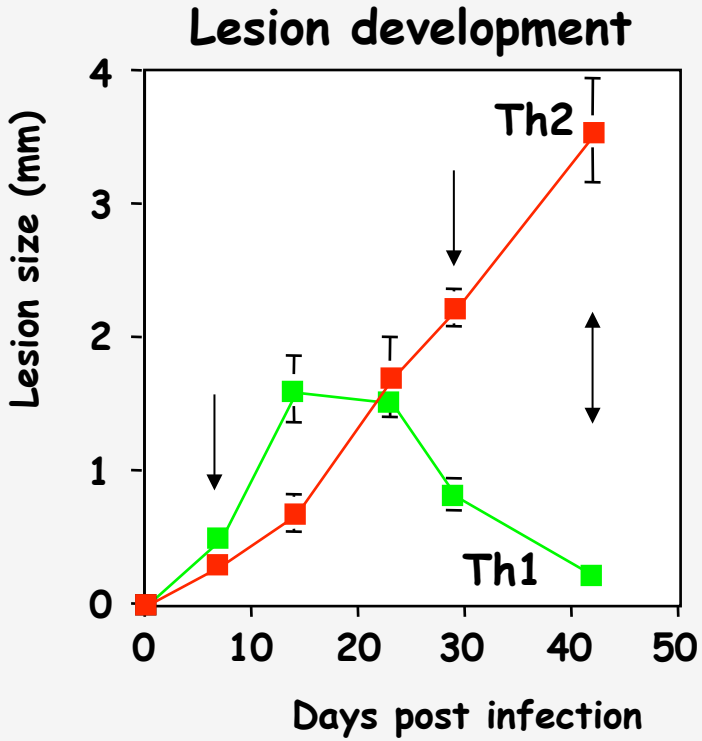
GATA-3
Th2

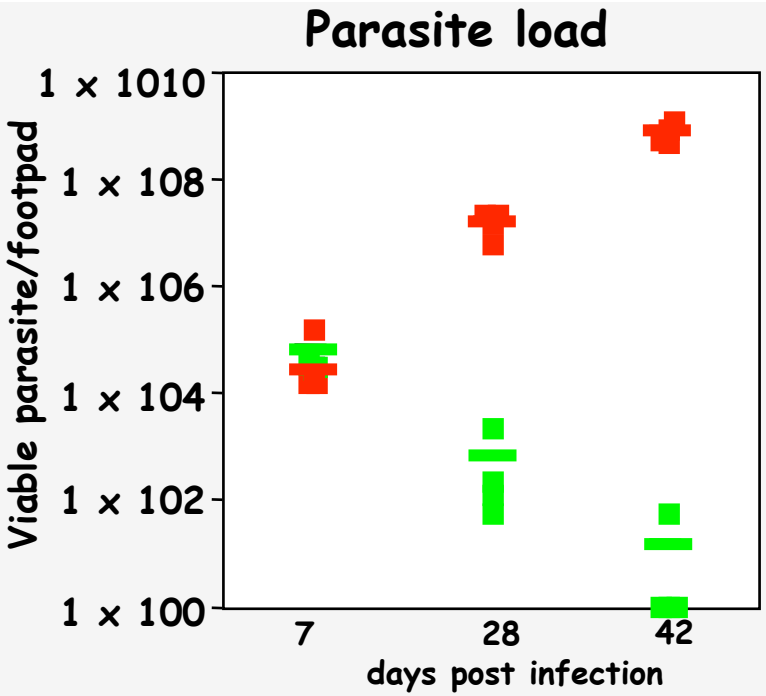
HEALING

IL-2
IFN-γ
TNF-β

IL-4
IL-5
IL-10
IL-13

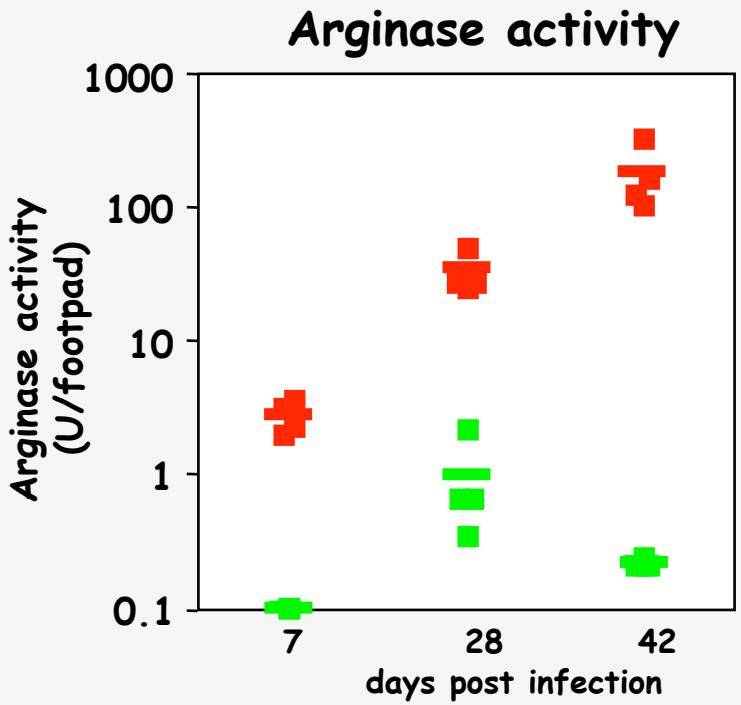
NON-HEALING



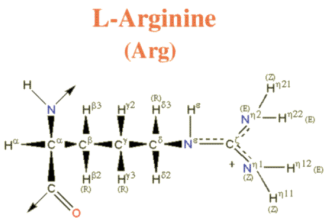


BALB/c

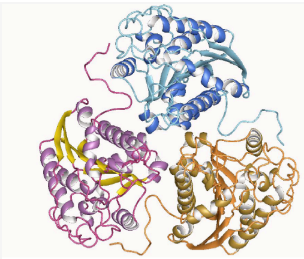
CBA



=> Uncontrolled parasite replication correlates with high arginase activities



Arginase isoforms



Arginase I (Liver-type arginase)

- Cytosolic
- Hepatocytes, RBC

- Inducible in - macrophages, dendritic cells

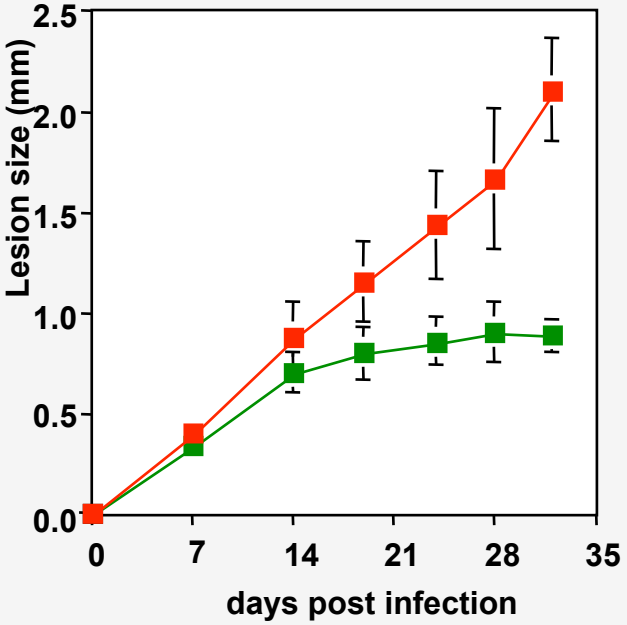
- Constitutively expressed in neutrophils
- Arginase I -/- mice: not viable
- Human arginase 1 deficiency
  (Progressive dementia, spasticity, short stature)

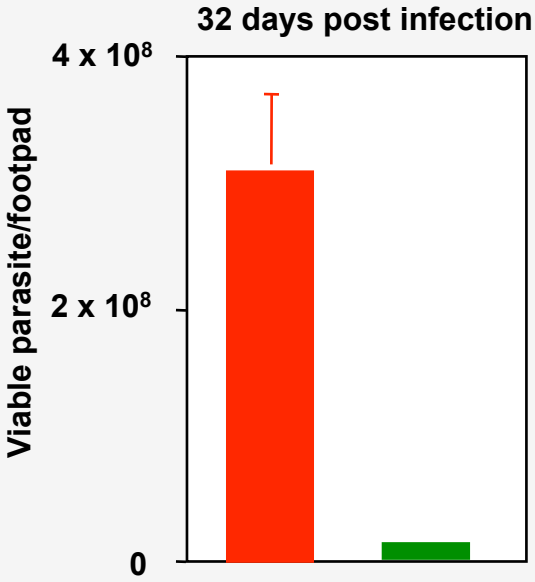
Arginase II (kidney-type arginase)

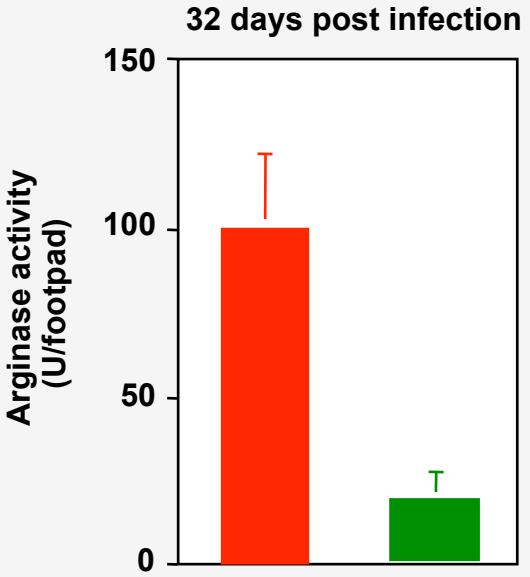
- Mitochondrial
- Kidney, brain, prostate
- Constitutively expressed

Does modulation of arginase activity *in vivo* alter *L. major* infections?

Lesion development in BALB/c mice treated with nor-NOHA, a competitive inhibitor of arginase







control infected BALB/c

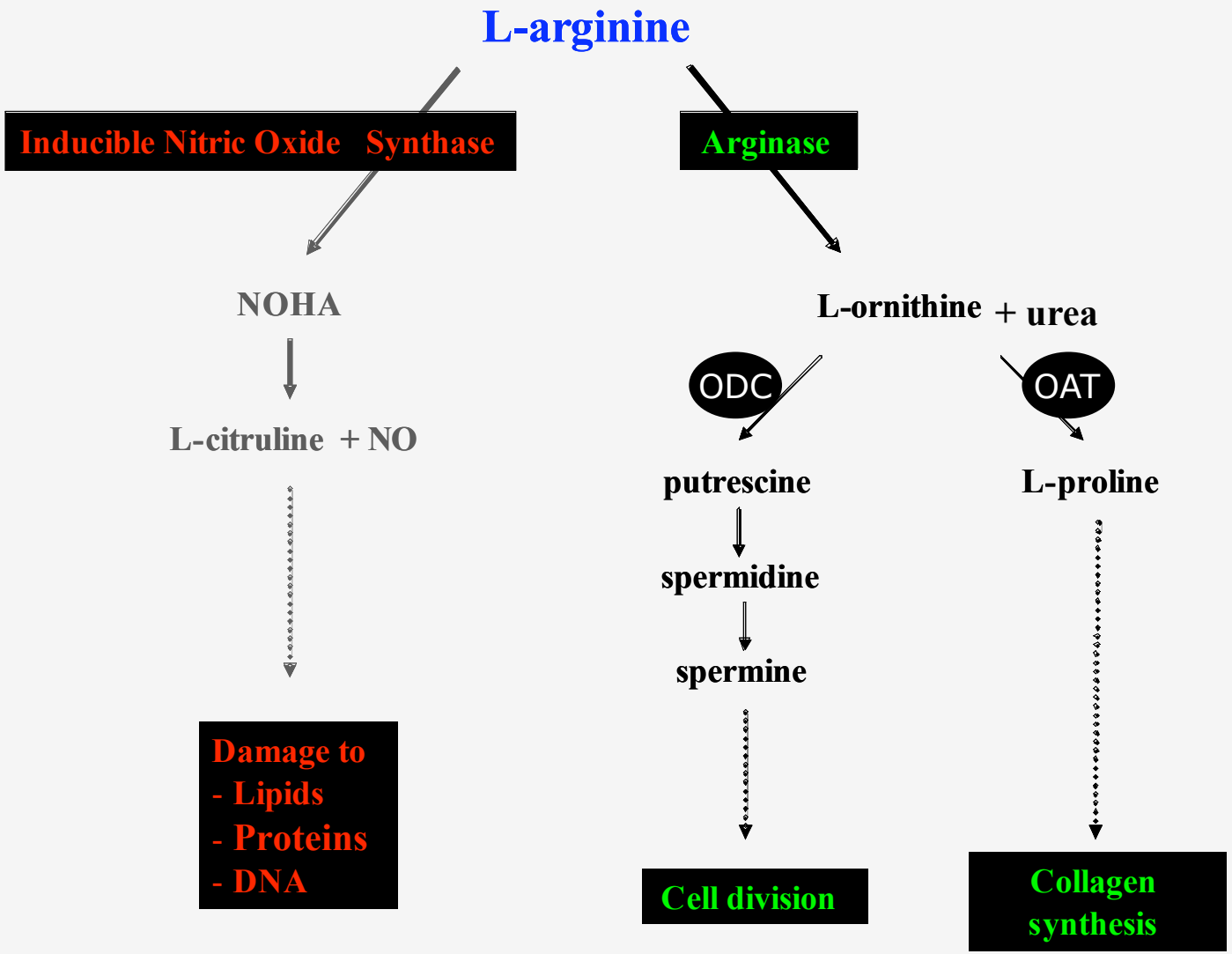
nor-NOHA treated BALB/c

=> more efficient control of
- Lesion development and pathology
- Parasite replication

Why is the L-arginine metabolism relevant in leishmaniasis?

What are the consequences of arginase activity?

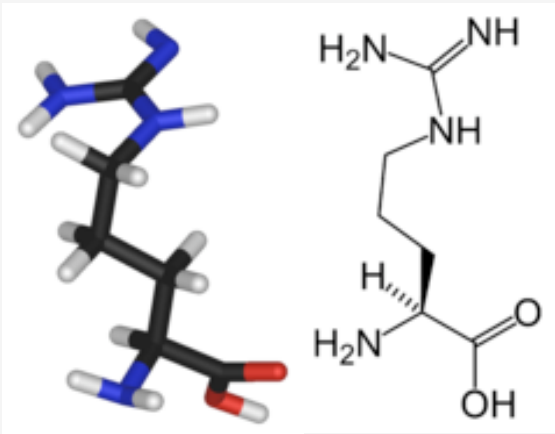
L-arginine metabolism

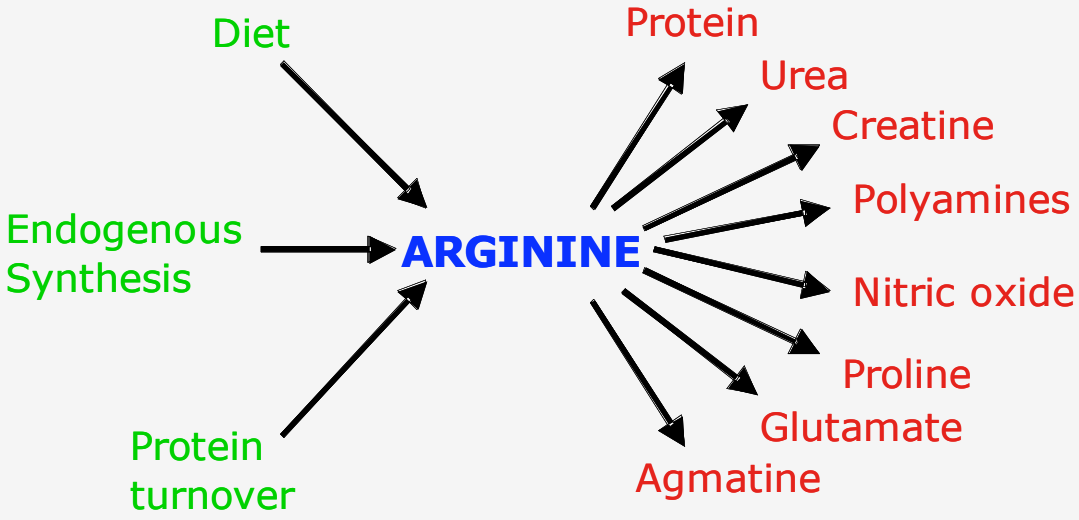


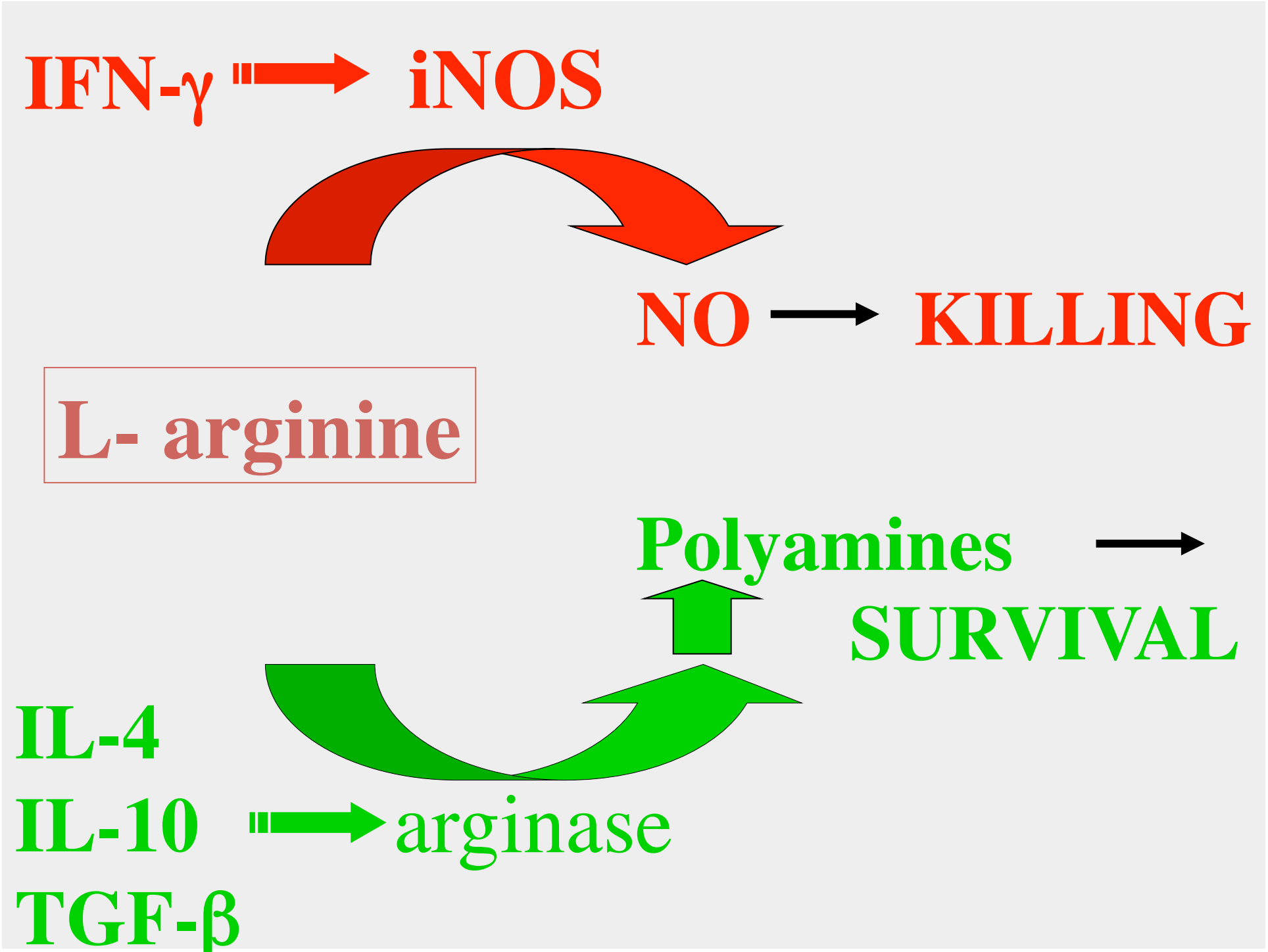
L-arginine (2-amino-50guanidvaleric acid)

- Isolated from lupin seedlings in 1886
- western diet L-arginine average intake of 5.4g/day
- L-arginine is a semi-essential amino acid

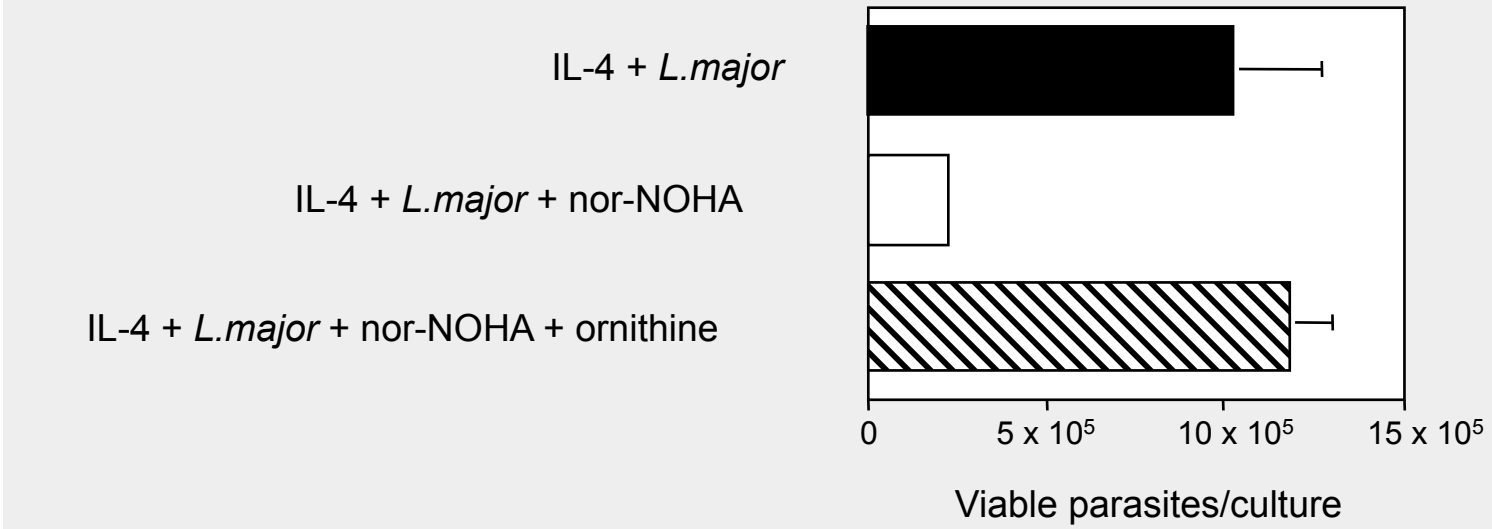
L-arginine (ARG)

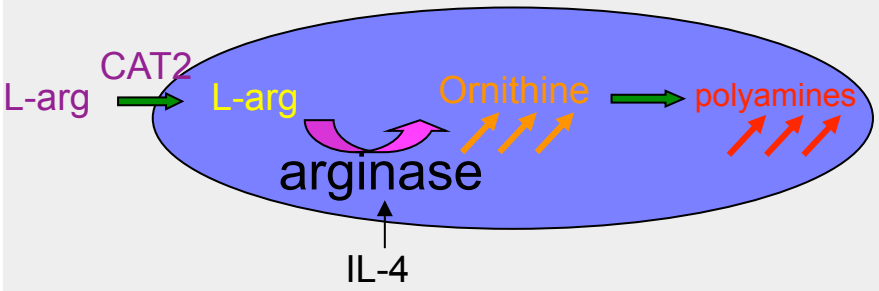


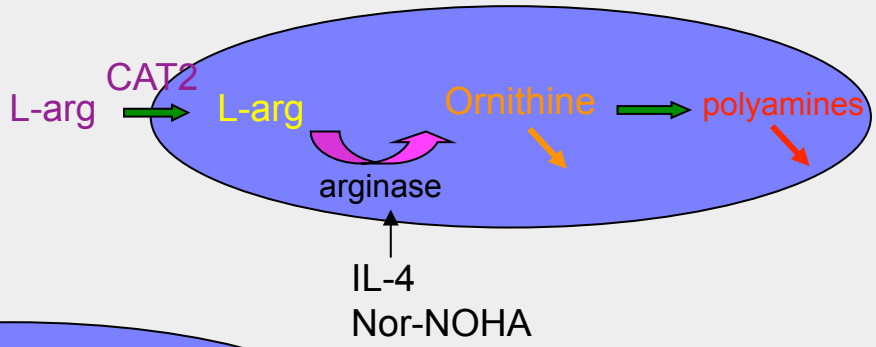


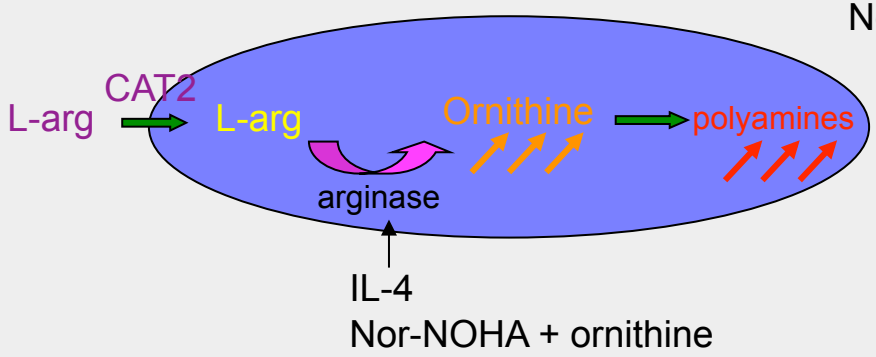

IFN-γ
iNOS
NO
KILLING
L- arginine
Polyamines
SURVIVAL
IL-4
IL-10
TGF-β
arginase

Ornithine rescues parasite growth









Summary:

- Leishmaniases = spectrum of diseases
- Most neglected tropical diseases
- Related to poverty
- Leishmania parasites are transmitted by sand flies
- Leishmania parasites modulate immune responses of the infected hosts
- Exploit metabolism of the host

Disease burden estimates (1)

600 000 infections are officially reported each year.

Declaration is compulsatory in 32 of the 88 countries affected
=>
substantial number of cases are never reported

- **2-fold to 40-fold underreporting** (Singh,SP, Trop Med Int Health, 11:899, 2006; Copeland, HW, Am J Trop Med Hyg, 43:257, 1990; Bora, D, J CommDis, 26;120, 1994)

- **91% of all VL death unrecognised (1999-2002)** (Collin, SM, Trop Med Int Health, 11:509, 2005)

- **20% of all VL patients (the majority female) in one village in India died before the disease was diagnosed** (Barnet, P, Am J Trop Med Hyg, 73:720, 2005))

Disease burden estimates (2)

Leishmaniases are in the top league of the world's most important vector-borne diseases and accounts for ~ 2 million DALYs annually

Social burden

Social stigma associated with the deformities and disfiguring scars caused by this disease.

Leishmaniasis-related disabilities impose a great social burden, especially for women: **In some parts of the world they have reduced health care access, heightened social isolation from the disfiguration caused by CL, can prevent woman to touch her children, enter into marriage or remain married.**

Leishmaniasis-related disabilities impair economic productivity. On several occasions, epidemics have significantly delayed the implementation of development projects.

Leishmaniasis impedes socioeconomic development

Table 3.1.1 Estimated number of disability-adjusted life years (DALYs) (in thousands) by cause (neglected tropical disease), and by WHO region (excluding the European Region)[a], 2004

Neglected tropical disease	World[b]	WHO region				
		African	Americas	Eastern Mediterranean	South-East Asia	Western Pacific
Human African trypanosomiasis	1 673	1 609	0	62	0	0
Chagas disease	430	0	426	0	0	0
Schistosomiasis	1 707	1 502	46	145	0	13
Leishmaniasis	1 974	328	45	281	1 264	51
Lymphatic filariasis	5 941	2 263	10	75	3 525	65
Onchocerciasis	389	375	1	11	0	0
Leprosy	194	25	16	22	118	13
Dengue	670	9	73	28	391	169
Trachoma	1 334	601	15	208	88	419
Ascariasis[c]	1 851	915	60	162	404	308
Trichuriasis[c]	1 012	236	73	61	372	269
Hookworm disease[c]	1 092	377	20	43	286	364

[a] Source: *The global burden of disease: 2004 update* (1).
[b] Because estimates from the European Region were omitted from the table, numbers for the regions may not always add up to the world's total.
[c] Soil-transmitted helminthiases.

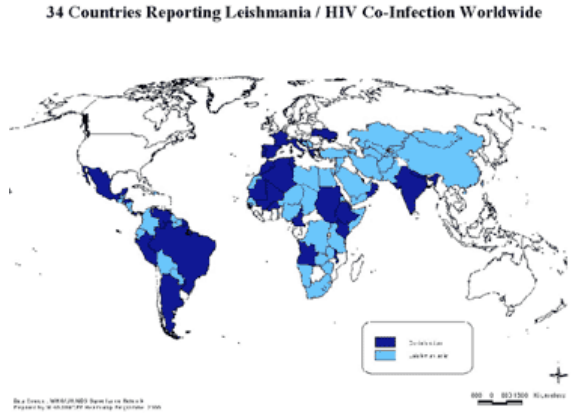
MAGNITUDE OF THE PROBLEM

Leishmaniases are diseases affecting the poorest of the poor.
Malnutrition is a major risk factor => epidemic flourish under conditions of famine and mass population movement.

- **Sudan (1984-1994)**
- **Sudan (1997)**
- **Eritrea and Ethiopia (1998)**
- **Brazil (1999)**
- **Afghanistan (2002)**

Leishmaniasis and HIV co-infections

Co-infection reported in 34 countries in Africa, Asia, Europe and South America.



➢ **Europe: 70% of VL cases are associated with HIV infection (drug users).**
ART: reduced incidence of VL
reduced relapse rates
prolonged the intervals between relapses
improved survival.

➢ **Brazil: high parasite load**
parasite dissemination to unusual sites
lower cure rates
greater susceptibility to drug toxicity
increased drug resistance
higher rates of death
higher rates of relapse

➢ **Ethiopia carries the greatest burden of HIV-VL co-infection: high rates of relapses**
high rates of mortality

• HIV infection can lead to reactivation of latent Leishmania infection or to symptomatic VL at initial infection

• 100-1000 x greater risk for HIV+ individuals to develop the disease as compared to HIV- individuals
• VL accelerates the onset of AIDS: - increased HIV replication
- cumulative immunosuppression

Why is disease control difficult?

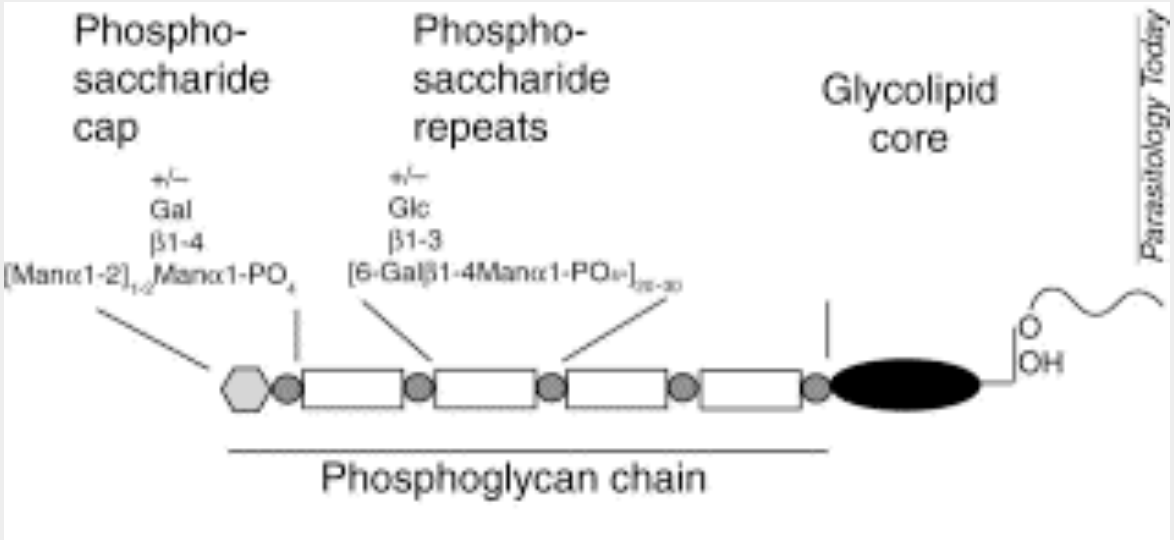
- **BIOLOGICAL REASONS**:
- Escape mechanisms
- Complexity of host-parasite interaction
- Contribution of vector-derived compounds
- Zoonosis
- Drug efficacy differs (AmB: India – Africa)

- **ECONOMICAL REASONS:**
- Poverty
- Tool-deficient

Complement

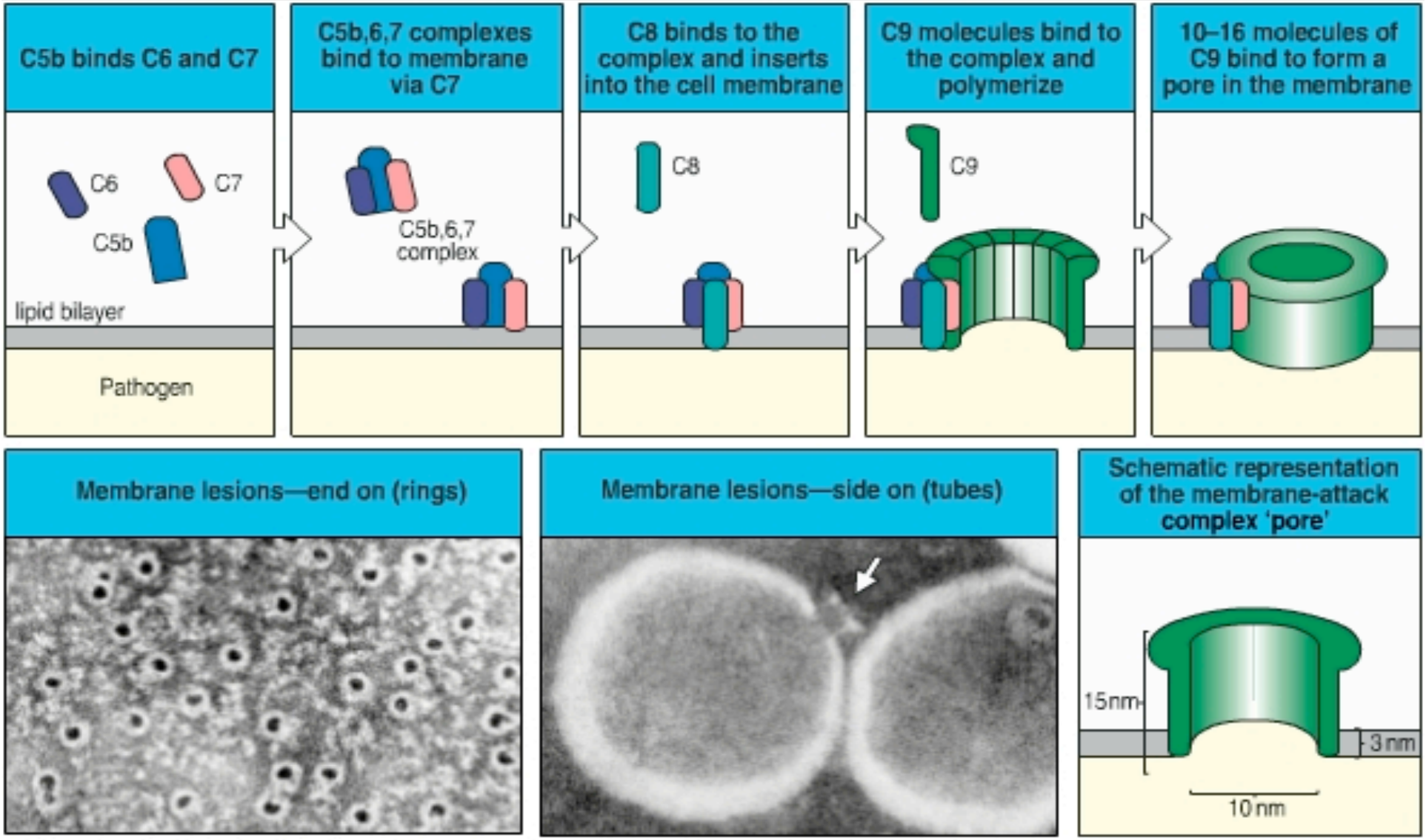
- Can kill microorganism by the formation MAC, resulting in holes in the cell surface
- Infective *Leishmania* avoid complement activity by altering structure of LPG: sugars are added, elongating LPG => the lytic C5-C9 complex can no longer insert into the membrane

Phosphoglycans of *Leishmania*



Ilg, 2000, Parasitology Today (now Trends in Para), 16, 489-497

Formation of membrane attack complex



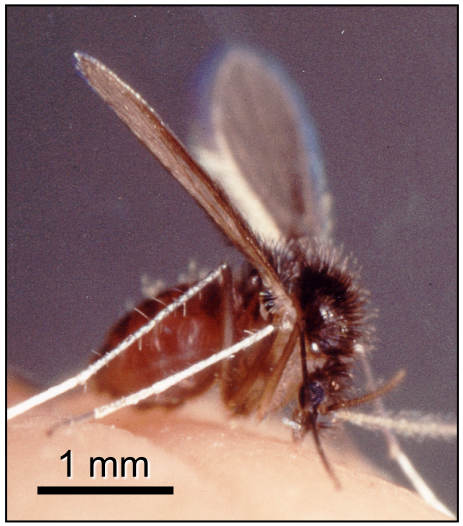
Leishmaniases

Factors determining disease manifestation

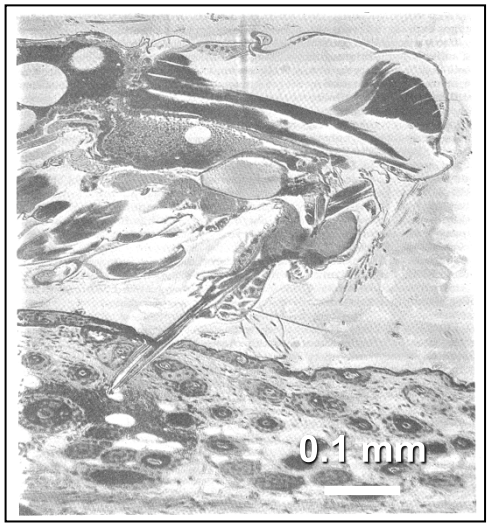
- parasite species
- vector-derived products
- geographic location
- immune response of the host

(See table 1 in J. Mol. Med., 1998:76:372-390)

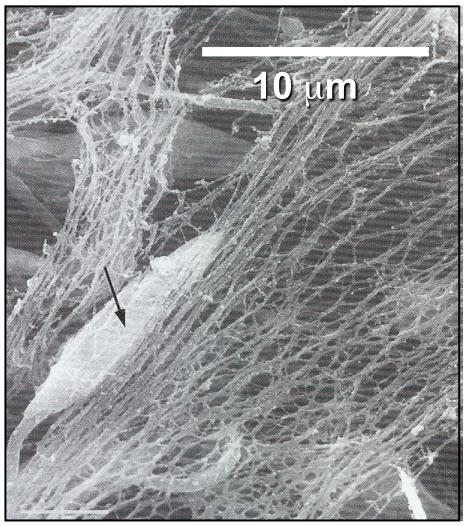
Leishmania transmission by sand fly bite



Sand fly feeding on knuckle



Cross-section of sand fly feeding on 'pool' of blood in dermis



Metacyclic promastigote trapped in gel that blocks sand fly gut

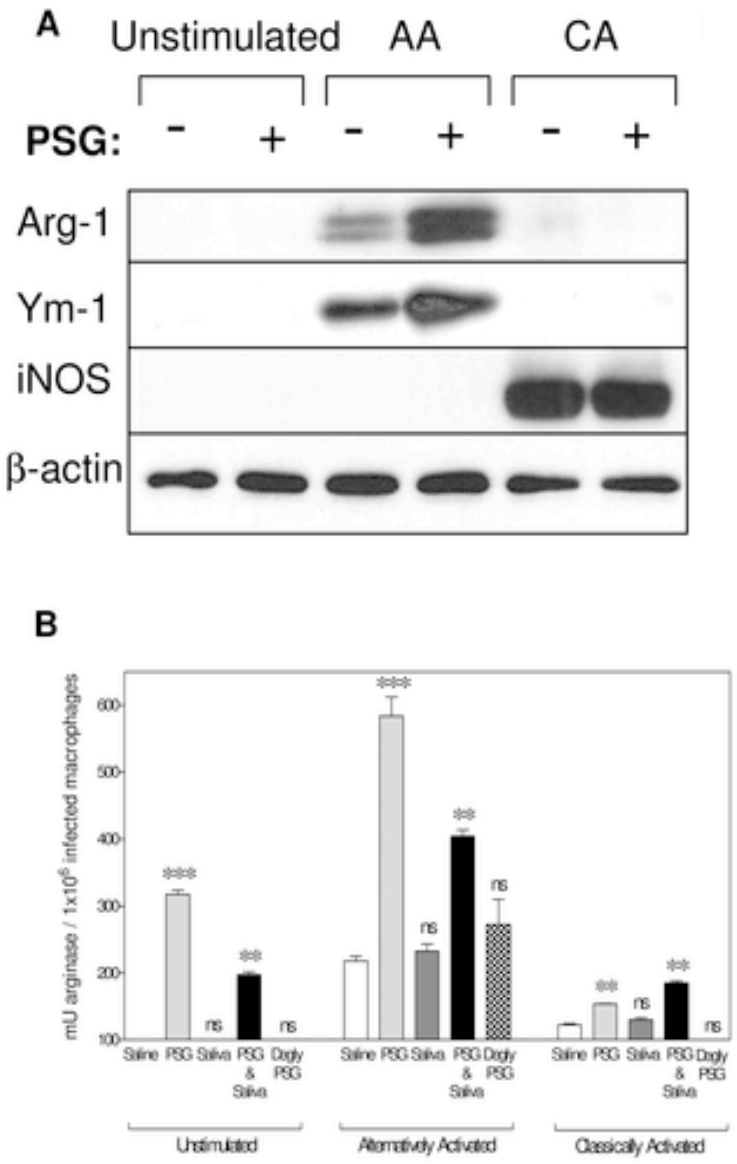
- Female sand flies transmit leishmaniasis when they blood feed.
- Sand flies secrete saliva to help blood feeding by preventing clotting and promoting vasodilation.
- *Leishmania* develop and multiply entirely in the sand fly gut.
- Sand flies deliver average dose of **1,000 metacyclics per bite**.
- **Transmission is very efficient** (i.e. Infected sand flies in endemic areas are rare, typically 0.001%)..
- *Leishmania* **block the sand fly gut by secreting a phosphoglycan gel**.
- **Blockage forces fly to regurgitate parasites**, **sand fly saliva** and **parasite gel**.
- **Immunomodulatory properties of gel and saliva promote infection and exacerbate disease**.

Rogers et al, 2004. Nature; Rogers et al, 2007 & 2009 PLoS Pathogens

Promastigote secretory gel

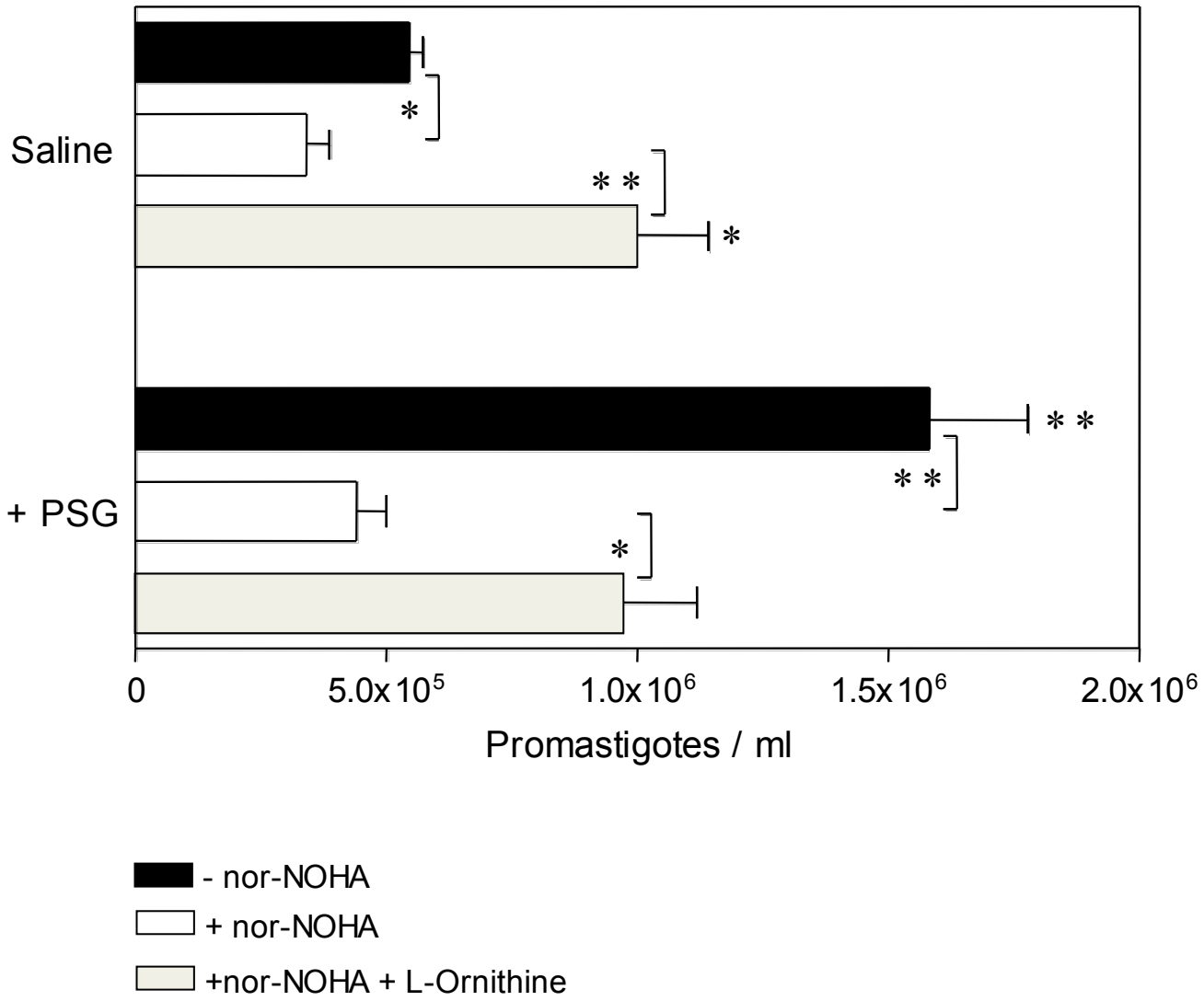
- The establishment of infection during natural transmission of Leishmania parasites by their sand fly vectors is not only supported by the co-transmission of sand fly derived saliva
- PSP which is produced by the parasite during its development in the vector is also transmitted during natural transmission and helps the parasite to establish infection in the mammalian host

How do components delivered by the sand fly vector influence parasite growth and L-arginine metabolism of host macrophages *in vitro* and *in vivo*?


A
Unstimulated
AA
CA
PSG:
-
+
-
+
-
+
Arg-1
Ym-1
iNOS
β-actin
B
mU arginase / 1x10^6 infected macrophages
600
500
400
300
200
100
***
**
ns
Saline
PSG
Saliva
PSG & Saliva
Dgly PSG
Unstimulated
Alternatively Activated
Classically Activated

48 hr viable infection of BALB/c **bone marrow** macrophages

MOI 1:1 +/- 0.25 μg L. mexicana PSG



Why is the treatment of leishmaniases and the control of these diseases problematic?

Drugs:
inadequate, toxic, parenteral, long courses, resistance patterns

Diagnostics:
invasive, non-predictive, complex, poor biomarkers

Vaccines:
complex, stage dependent,

What are the problems?

Treatments are inadequate or inaccessible

- Ineffective (resistance)
- Toxic
- Expensive
- Painful to deliver
- Difficult to follow up
- Not adapted to patient's needs
- Not registered in endemic regions

Risks and costs of drug development:

- The mean overall investment required for the introduction of a new drug (NCE) is currently above US$800-1000 million
- For a single chemical entity, the cost of completing basic, preclinical and clinical studies required to demonstrate efficacy and safety in humans is estimated at US$50-100 million
- Conclusion: The cost of introducing a NCE is mostly associated with project failures, particularly in the late clinical stages of development, giving an equivalent failure rate of 95%

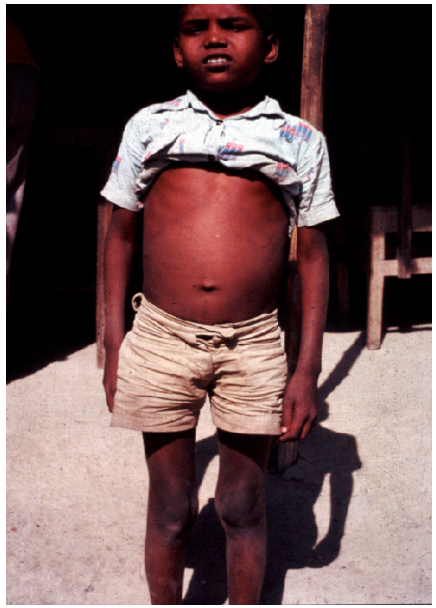
Table 3.2.2.1 Cost-effectiveness of controlling neglected tropical diseases[a]

Disease	Intervention	Cost per DALY averted (US$)
Chagas disease	Vector control	317
Lymphatic filariasis	In implementation units (districts) where prevalence is greater than 1%, annual mass drug administration to treat the entire at-risk population for 5–7 years: ivermectin and albendazole in Africa, and diethylcarbamazine and albendazole in onchocerciasis-free countries:	5–10
	• to interrupt transmission and achieve elimination of the public-health problem	35
	• to initiate morbidity control, surgery and lymphoedema management	1–4
	To provide salt fortified with diethylcarbamazine (China)	59–370
	Vector control	
Schistosomiasis	Mass school-based treatment with praziquantel and albendazole combined with schistosomiasis treatment	10–23
	Mass school-based treatment with praziquantel alone	410–844
Trachoma	Trachoma control based on SAFE strategy (Surgery, Antibiotic treatment, Face washing and Environmental control)	5–100
Onchocerciasis	Community-directed treatment programmes with ivermectin	9
Soil-transmitted helminthiases (hookworm, roundworm, and whipworm)	Mass school-based treatment with albendazole or mebendazole	2–11
Leprosy	Case-detection and treatment with multidrug therapy using donated drugs	46
	Prevention of disability	1–122
Dengue fever control	Case-management	716–1757
	Environmental control	more than 2440
Leishmaniasis	Case detection and treatment; vector control.	11–22
Human African trypanosomiasis	Case-finding and treatment:	
	• with melarsoprol	Less than 12
	• with eflornithine	Less than 24

[a] Source: Reproduced with permission from Conteh L et al. (4).

visceral leishmaniasis - drug combinations

- What is possible ? Not co-formulations but co-administrations based upon 3 new treatments introduced since 2000 …..
  - First oral drug miltefosine
  - Highly potent liposomal amphotericin B
- First trial by Sundar (2008) – 1 dose AmBisome + 7 days miltefosine ……97% cure
- Clinical trials by DNDi (results ……January 2010)
  - AmBisome + paromomycin
  - AmBisome + miltefosine
  - paromomycin + miltefosine



Significance: 28 day course to 7 day course of treatment