Les interactions parasites eucaryotes-hôtes:

l'exemple de *Leishmania*

Geneviève Milon

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Leishmania major : first recognized as the etiological agent of "transient pathogenic processes" in humans

Human cutaneous Leishmaniasis

How does "*Leishmania* development" result in transient skin lesion ?

Does it assess direct and or indirect pathogenic processes?

Are they other mammal hosts than the human ones ?

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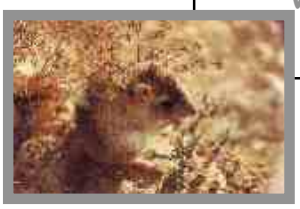
Leishmania major within its mammalian hosts : first recognized as the etiological agent of "transient pathogenic processes" when the latter deploy

Human cutaneous Leishmaniasis

How does "*Leishmania* development" result in transient skin lesion ?

Does it assess direct and or indirect pathogenic processes?

Yes there are other mammal hosts wild rodents : no lesion detectable



Psammomys obesus

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Leishmania major within its mammalian hosts

Asymptomatic parasitism also noticed

In these wild rodents: asymptomatic parasitism deploys

Therefore what do we need to consider ?

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For further deciphering this complexity
what do we need to consider ?

The features of the natural ecosystems on
which relies the *Leishmania/L.* perpetuation:

a first example anchored to *L.major*
in its natural ecosystem

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Leishmania major in its natural ecosystem

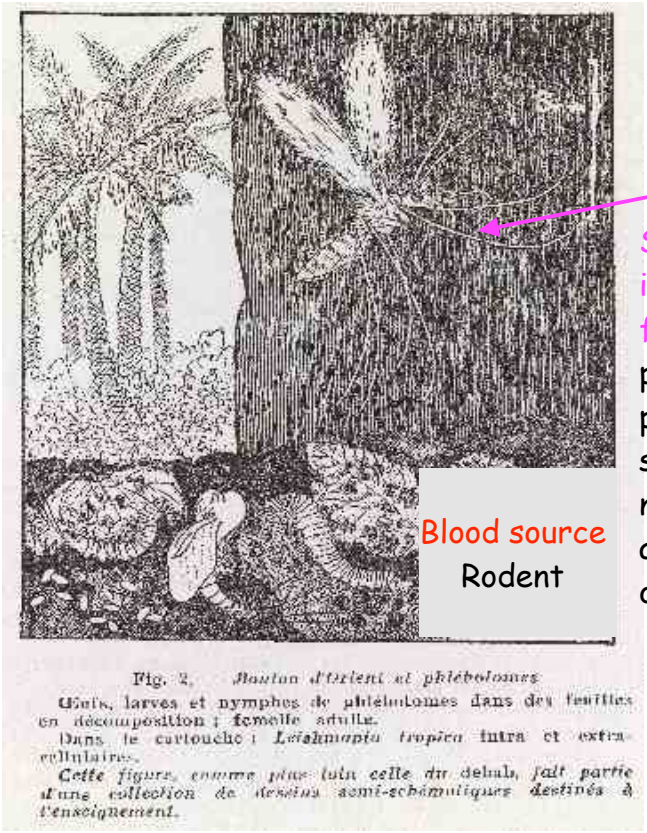

Fig. 2. *Bouton d'Orient et phlébotomes*

Œufs, larves et nymphes de phlébotomes dans des feuilles en décomposition ; femelle adulte.

Dans le cartouche : *Leishmania tropica* intra et extra-cellulaires.

Cette figure, comme plus loin celle du début, fait partie d'une collection de dessins semi-schématiques destinés à l'enseignement.

Sand fly , an adult insect blood-feeding female : the production of its progeny relies on a singular life trait namely the blood of a vertebrate most often a mammal

E and E.Sergent

Alger

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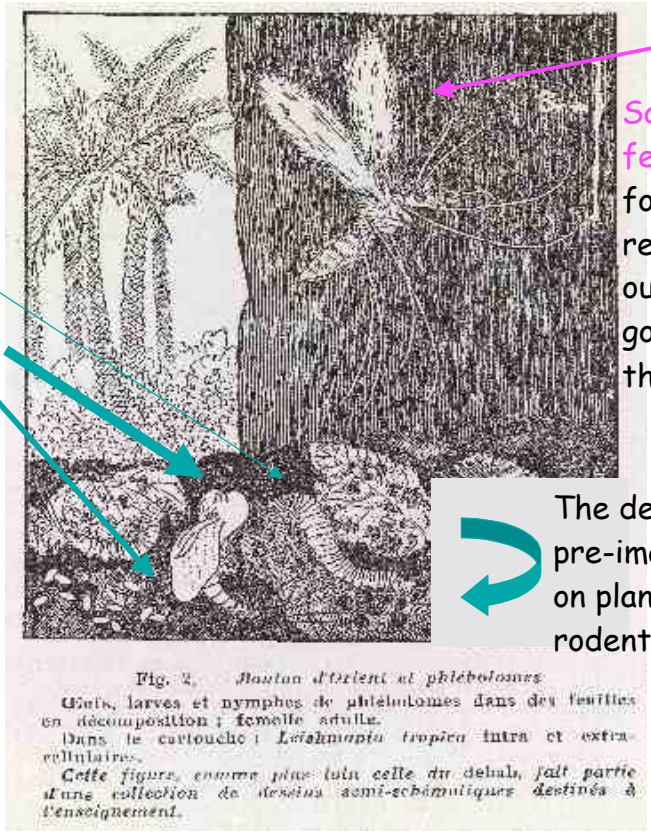
Leishmania major in its natural ecosystem

Insect pre-imaginal developmental stages

2

3

1



Sand fly , an adult female :once it has found its blood resource, what is the outcome of the gonotrophic cycle ie the progeny ?

The development of the pre-imaginal stages relies on plant remnants and rodent feces

E and E.Sergent

Alger

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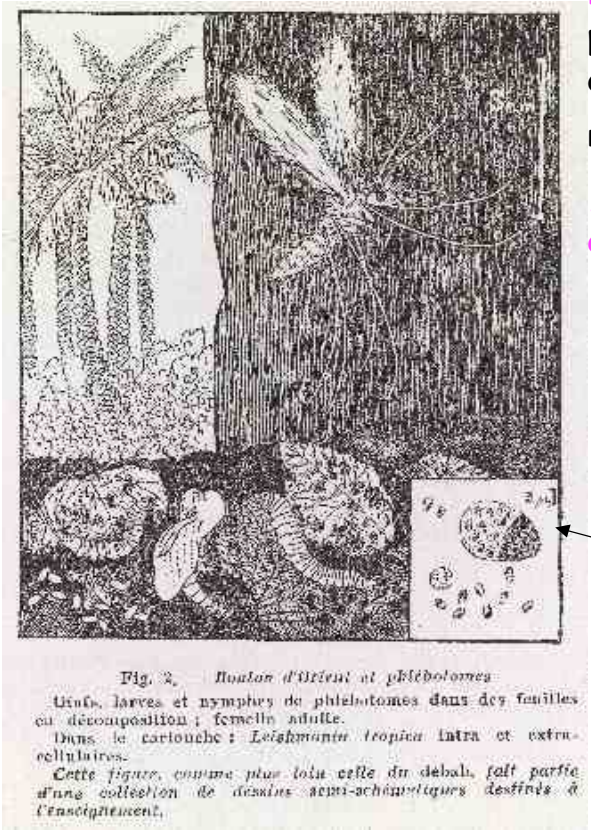
Leishmania major in its natural ecosystem

E.Sergent

Alger



Female sand fly the perpetuation of which relies on blood-feeding ,the blood resources being rodents , canids, humans ,avian

Transmissible *Leishmania* amastigotes could be hosted within cells present in the dermis of lesion –free

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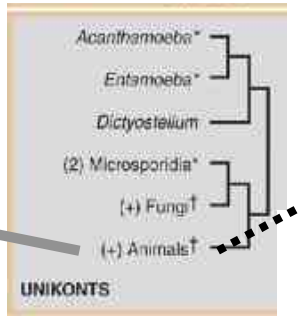
Thus it was possible to establish that *Leishmania* major does subvert two organisms from different taxa as hosts

Purified amastigotes post the release from macrophages

Promastigotes

Leishmania major

Psammomys obesus



Blood- feeding sand fly

K.Chaudhary &D S Roos *Nature Biotechnology* **23**, 1089 - 1091 (2005)

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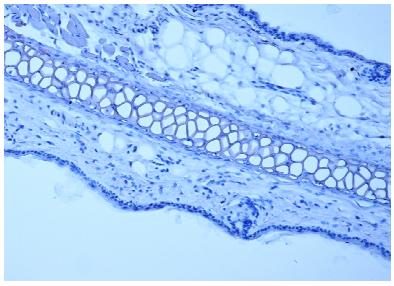
The mouse model
designed with the objectives to mimic the features of the natural ecosystem

-Low dose 10 to 1000

-Metacyclic promastigotes *Leishmania major*

-In the dermis (ear)



Belkaid,*Sacks* Milon

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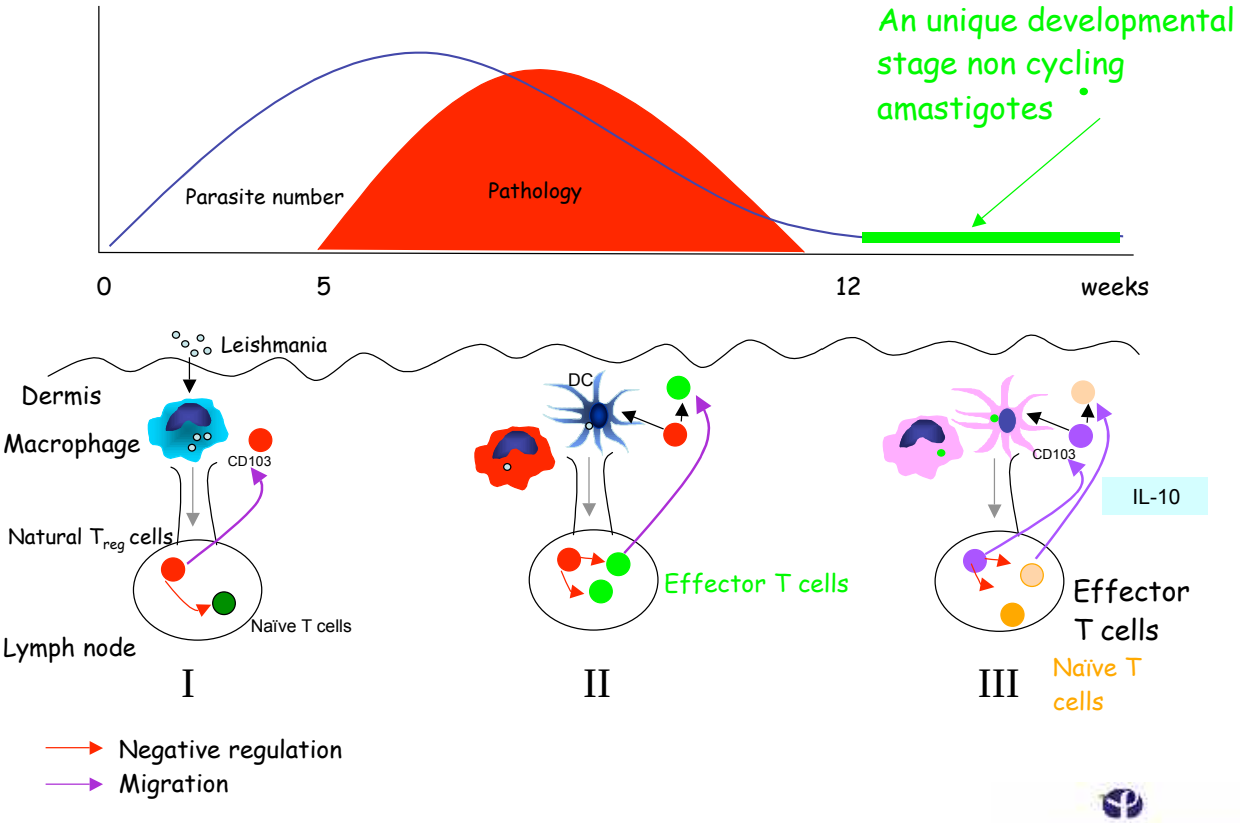
A fascinating example of tissue remodeling imposed by *Leishmania*



Adapted from Y Belkaid et al

The features of the natural ecosystems on which relies the *Leishmania/L.* perpetuation:

a second example *L.amazonensis*

The context: *Leishmania amazonensis* developmental biology

Proechymis spp.
(natural host)

Mammal
-insect blood source
-parasite host

Lutzomyia flaviscutellata
sand fly

Blood-feeding insect
-parasite host
-parasite vector

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The context: *Leishmania amazonensis* developmental biology

Proechymis spp.
(natural reservoir)

Amastigote
within a parasitophorous vacuole
dermal macrophage

Promastigote
extracellular

Lutzomyia flaviscutellata
sand fly

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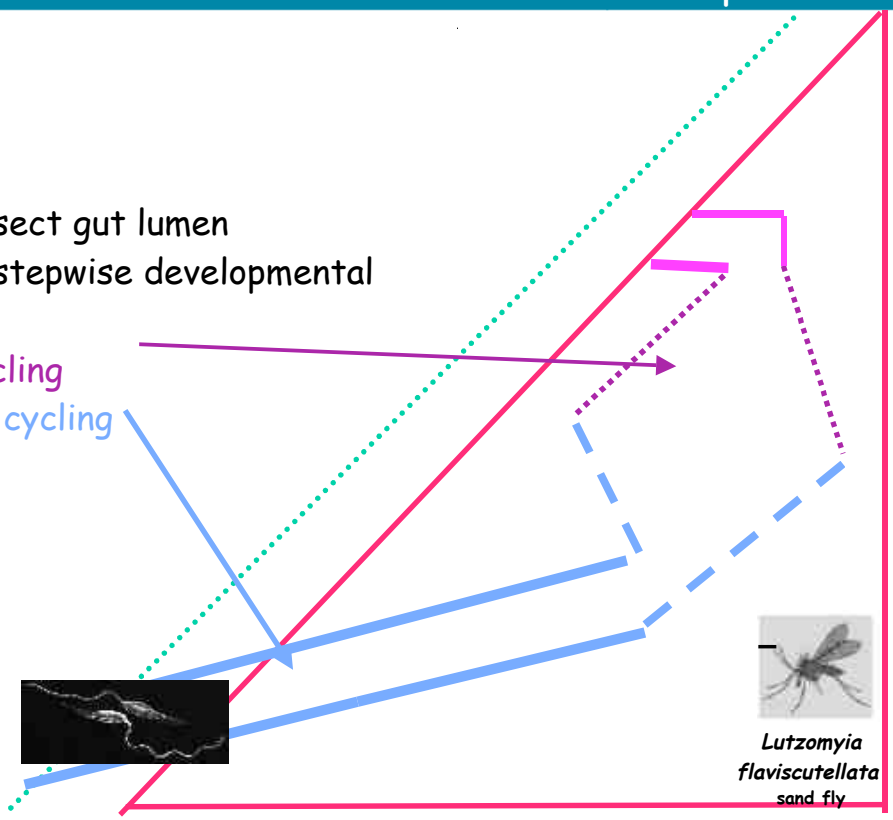
The context: *Leishmania amazonensis* developmental biology

In the insect gut lumen
complex stepwise developmental
programs
cell -cycling
non cell cycling



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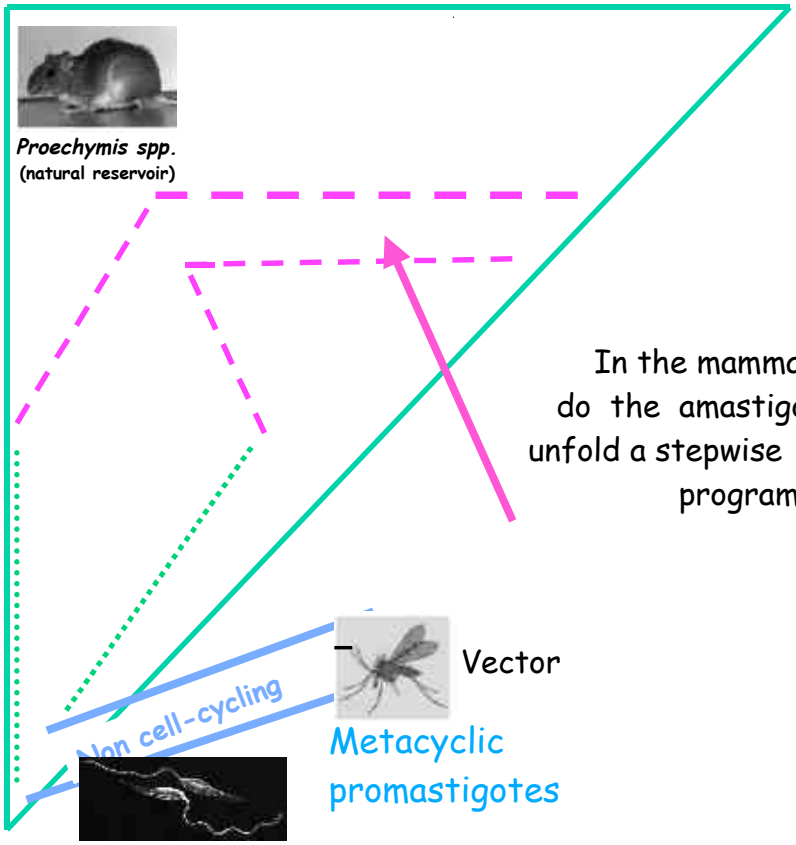
The context: *Leishmania amazonensis* developmental biology



In the mammal host
do the amastigotes also
unfold a stepwise developmental
program

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Leishmania amazonensis developmental biology

Proechymis spp.
(natural host)

Mammal
- -insect blood source
- -parasite host

Cell-cycling *Leishmania* amastigotes within macrophages: an intimate relationship

Cell-cycling

Dermal M_

Courtesy of Thierry Lang

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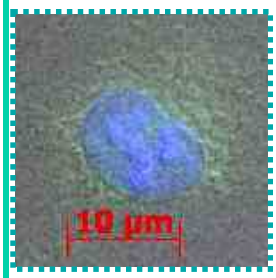
L.amazonensis within mouse macrophages

BALB/c mouse

Mammal: laboratory mouse

-source of bone marrow progenitors responsive to CSF-1



Bone marrow derived CSF-1R^{+} macrophages

This homogenous macrophage population is exposed to carefully prepared amastigotes

Day 0 : 4 amastigotes / macrophage

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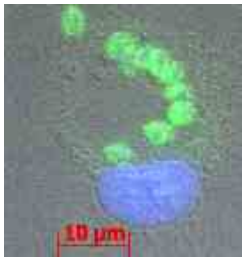
L.amazonensis within mouse macrophages

BALB/c mouse

Bone marrow derived

CSF-1R^{+} macrophages



DAY 1

Are cell- cycling *Leishmania* amastigotes re-programming the otherwise versatile macrophages as *bona fide* host cells ?

Insights from a transcriptional analysis of macrophages hosting this *Leishmania amazonensis* developmental stage

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Affymetrix core facility at Génopole - PF2

GeneChip Mouse430_2

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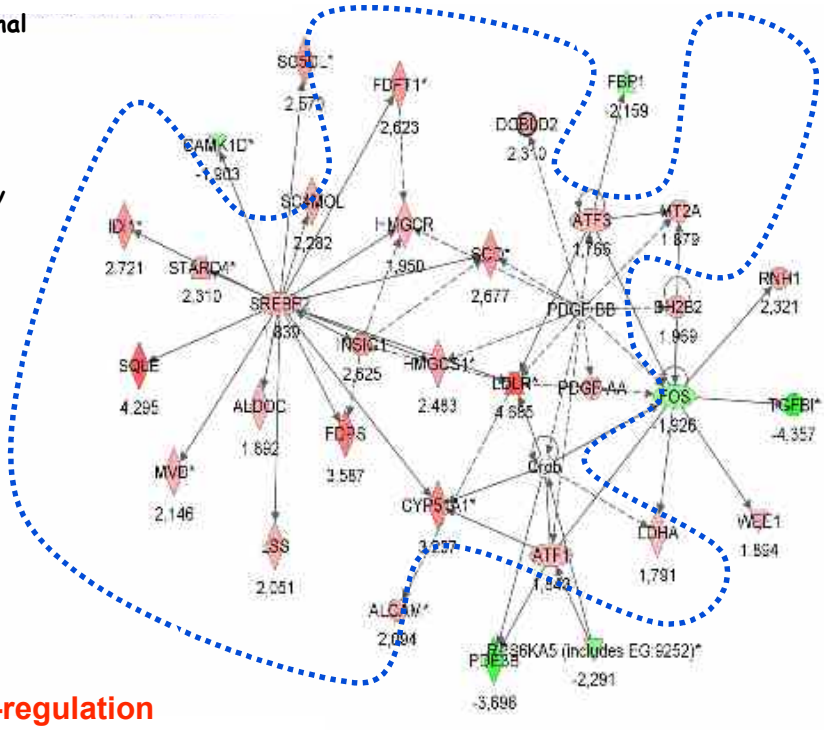
Biological interaction network analysis

INGENUITY SYSTEMS

Ingenuity Pathway Analysis (IPA) Software

- Transcriptional regulator
- Enzyme
- Transporter/ Receptor
- Phosphatase
- Kinase
- Other molecule
- Direct interaction
- Indirect interaction



Red nodes = up-regulation
Green nodes = down regulation

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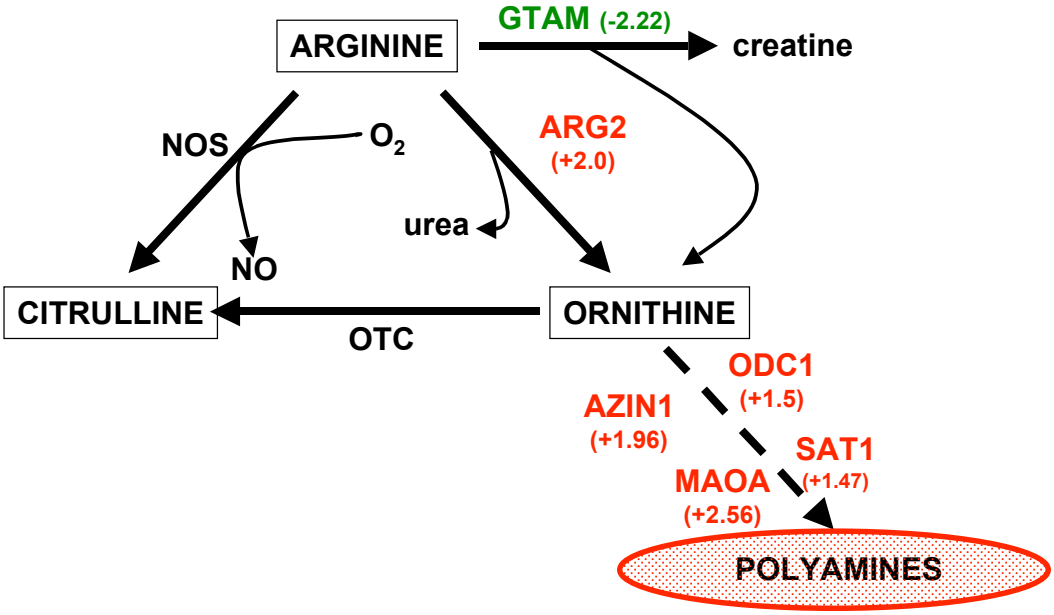
Polyamine pathway overrides iNOS pathway



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Macrophages subverted as bona fide host cells

Cell-cycling amastigotes within macrophages :

Exploit the sterol and fatty acid pathways to multiply efficiently

Override iNOS pathway to produce polyamines thus favoring their growth

Create a safe niche :

prevention of macrophage apoptosis

prevention of inflammatory signalling

prevention of T-lymphocyte stimulation

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Generation of other tools

Luciferase transgenic *L.amzonensis*

Metacyclic promastigotes

In vitro culture of promastigotes from frozen amastigote stocks

Intradermal inoculation (ear) low dose of promastigotes

C57BL/6 mice

BALB/c mice

Lecoeur , Goyard , Milon and Lang,

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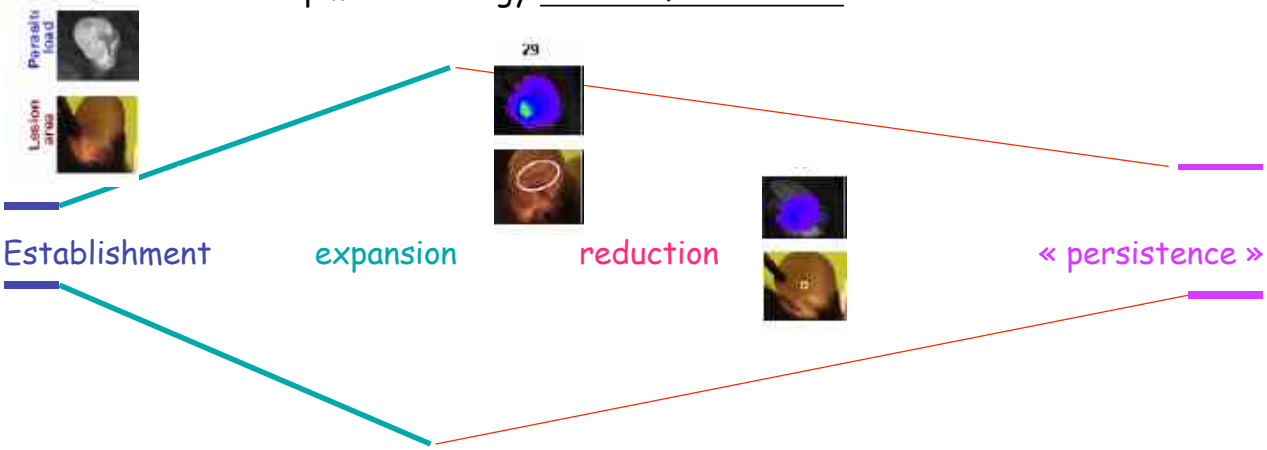
The mouse-based models designed with the objectives to mimic as closely as possible the developmental biology of *Leishmania*

Parasite developmental biology at site of inoculation



Real time dynamic imaging of both

-parasite developmental biology and tissue remodelling

-host signatures at the tissue level - fed by *in vitro* a,nd *ex vivo* approaches-

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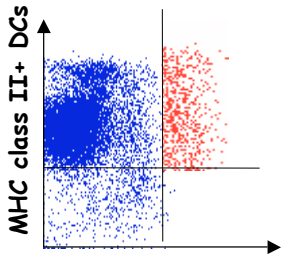
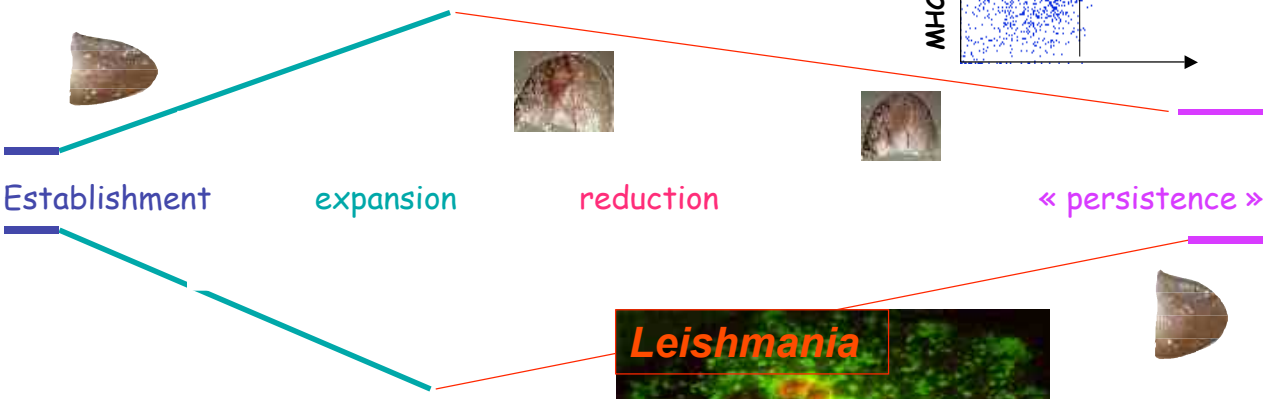
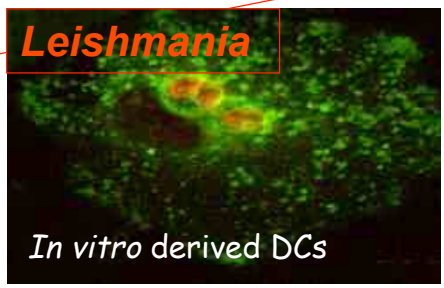
The mouse-based models designed with the objectives to mimic as closely as possible the developmental biology of *Leihsmania*







Bone marrow derived

-macrophages

-dendritic cells

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Perspectives back to in *vivo/ex vivo* settings

Proechymis spp.
(natural reservoir)

?

In the mammal host
do the amastigotes also
unfold a stepwise developmental
program with the persisting parasite
being no more cell- cycling?

Vector

Non cell-cycling

Metacyclic
promastigotes

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Perpectives

Mammal

Non cell-cycling within dermal dendritic leukocytes ?

Cell-cycling

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Perpectives

BALB/c mouse

Mammal

Non cell-cycling within dermal dendritic leukocytes ?

Cell-cycling

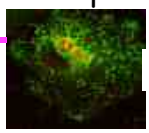
Biosafety level 2 containment cell sorting core facility

Leishmania amazonensis developmental biology

34/35°C

Proechymis (natural reservoir)

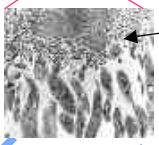
Mammal
- -insect blood source
- -parasite host



Non cell-cycling amastigotes?

Within the bloodmeal

Midgut



Leishmania

Dermal macrophage

- Metacyclic promastigote vector
- Procyclic promastigote host

Blood –feeding insect

26°C

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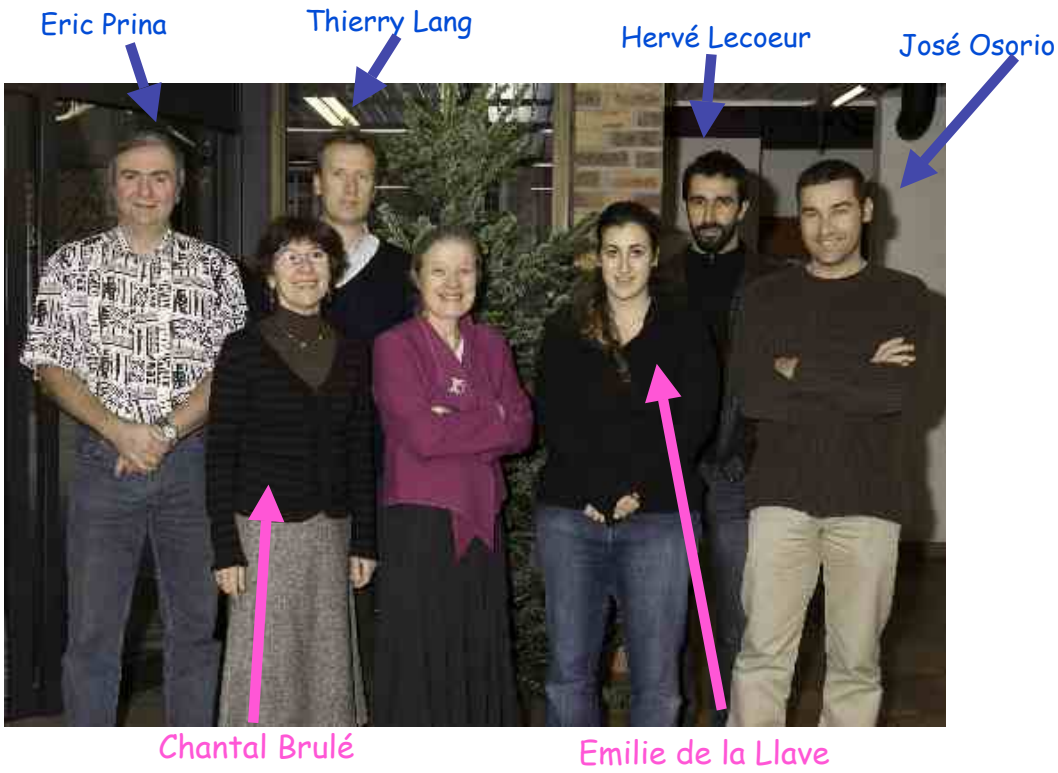

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