3 December 2012

Leishmaniases and immunodeficiency

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Major diseases caused by parasites + prevalence

Organism	Disease	Infected (millions)	At Risk (millions)
Protozoa			
Plasmodium spec.	Malaria	300-500	>2000
Leishmania spec	Leishmaniasis	>10	~ 350
Trypanosoma cruzi	Chagas Disease	16-18	>90
Trypanosoma brucei.	Sleeping sickness	unknown; $3x10^5$ new cases/year	
Helminths			
Schistosoma spec.	Schistosomiasis	200	>600
Wuchereria bancrofti	Lymphatic filariasis	120	1100
Brugia malayi			
Onchocerca volvulus	Onchocerciasis (river blindness)	18	90
Gastrointestinal nematodes	Gastrointestinal Conditions and damage, anaemia, malnutrition	>1000	>3000

From W. Harnett, Biologist, 49:222-226; 2002

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

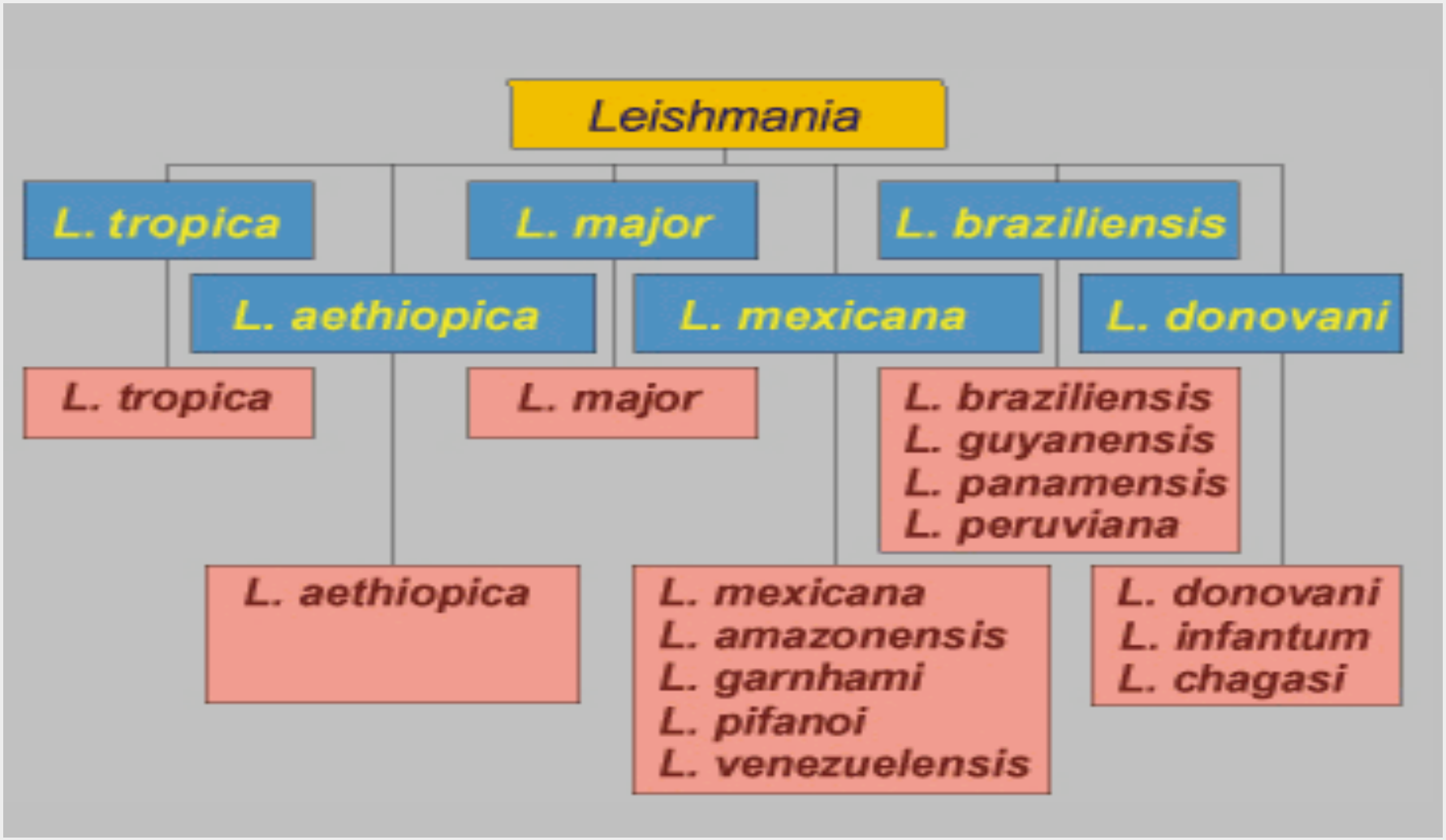
Leishmaniasis

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

Leishmania

- protozoan parasites
- > 20 *Leishmania* species pathogenic for humans
- **2 stage life cycle** (simplified)
- **promastigote**: in sandfly vector, mobile, flagellated
- **amastigote**: in infected mammals, intracellular, resorbed flagellum, ovoid, 2-6 mm, central nucleus
- Metacyclogenesis = differentiation non-infectious → infectious form

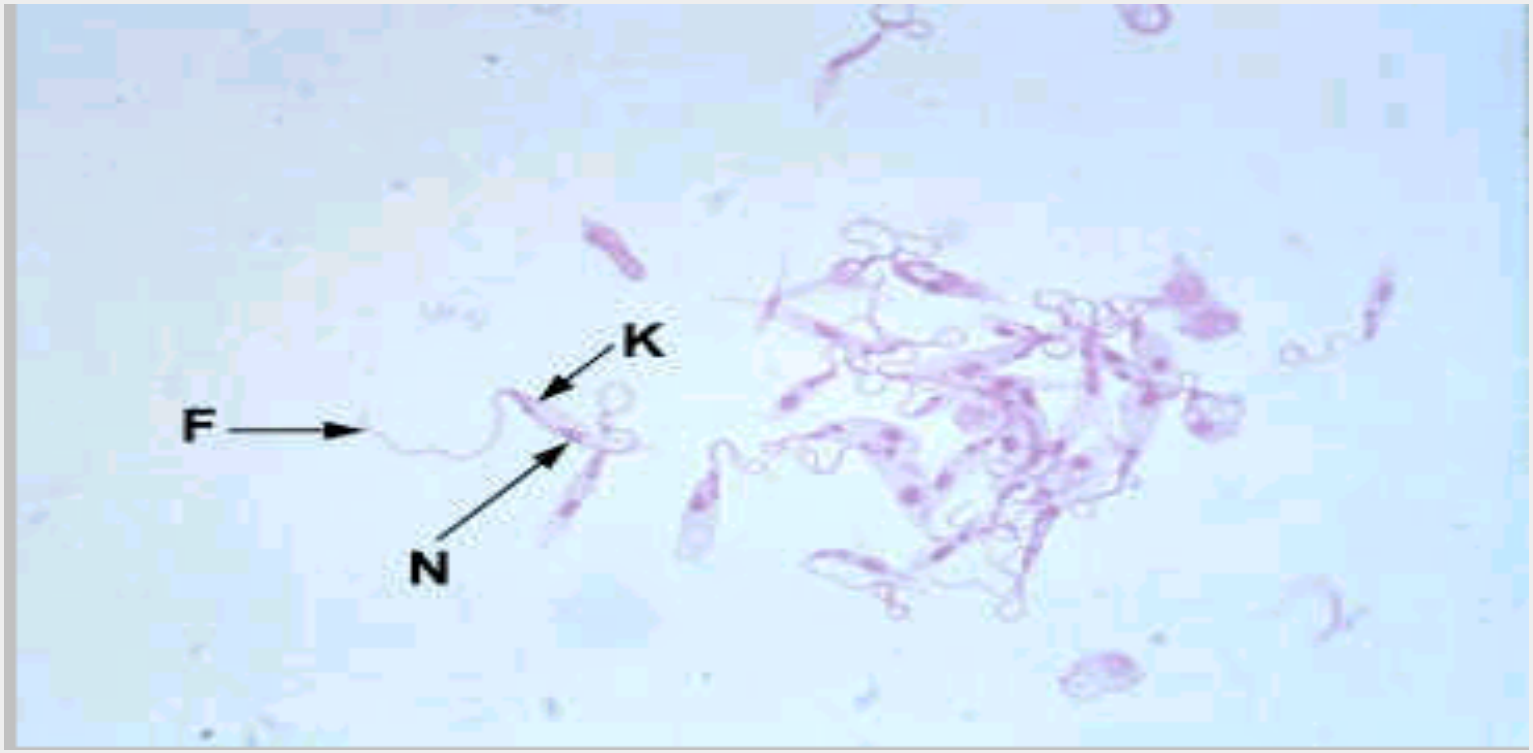
Classification of *Leishmania* species that can infect humans



***Leishmania* species: divided into "species complexes" (n = 6)**

1.) Phylum: Euglenozoa
2.) Class: Kinetoplastidae
3.) Order: Trypanosomatidae
4.) Family: Trypanosomatidae
5.) Genus: Leishmania

Leishmania promastigotes



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

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

from "Leishmaniasis" Topics in International Health

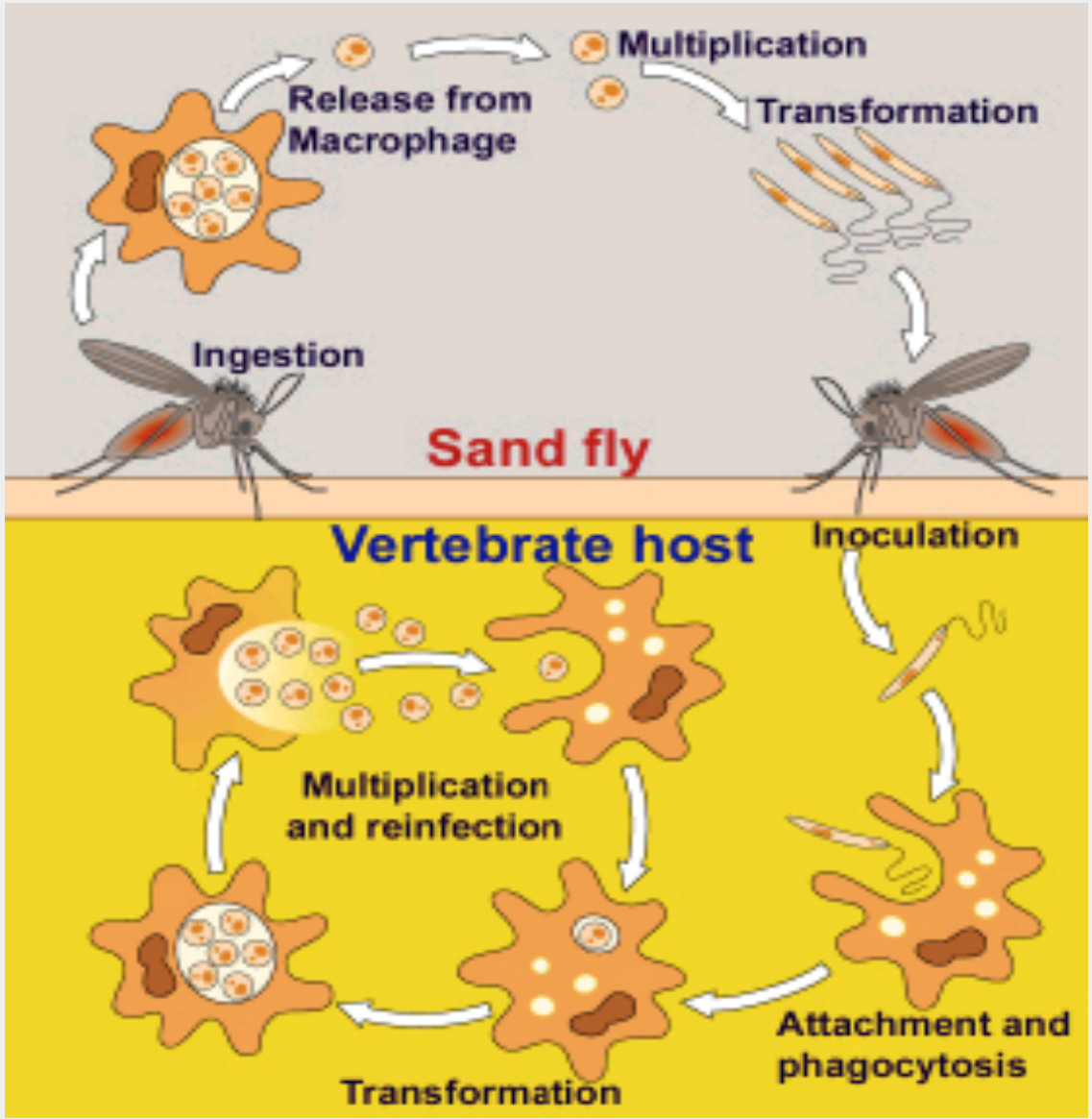
Female sandflies : vectors of *Leishmania*

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

The life cycle of *Leishmania* parasites



from “Leishmaniasis” Topics in International Health

Leishmaniases are:

- Zoonotic = transmitted from animals to humans (most forms)
- Anthroponotic = transmitted from human to human via vectors
- Reservoir hosts: domestic (dogs) and wild animals (rodents, opossum, anteater, humans, etc.)
- Reservoir hosts provide source of infection for vector in natural environment.
- Usually, wild animals do not present clinical symptoms whereas dogs can die from leishmaniasis

Leishmaniasis

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

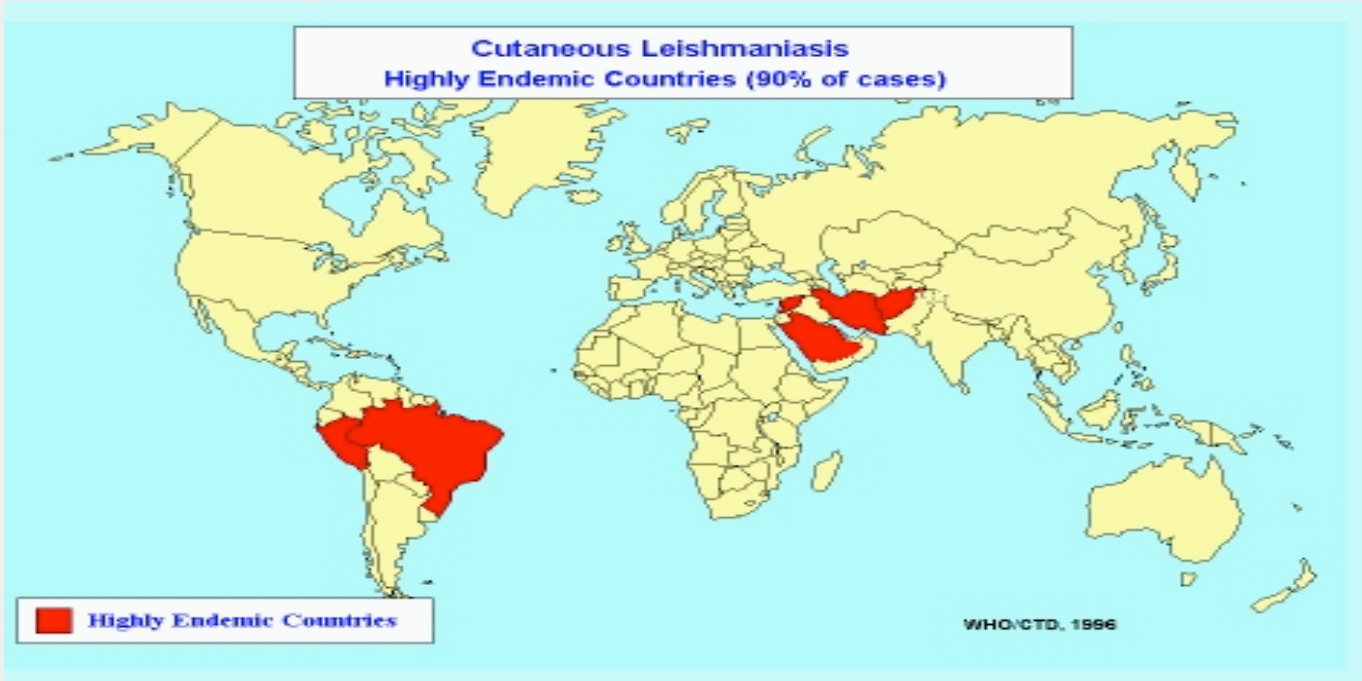
Leishmaniases

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)

Distribution of leishmaniasis



88 countries

>20 Leishmania species

350 million people at risk

~ 2 million new cases/year (1.5 CL, 0.5 VL)

Major forms of leishmaniases

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

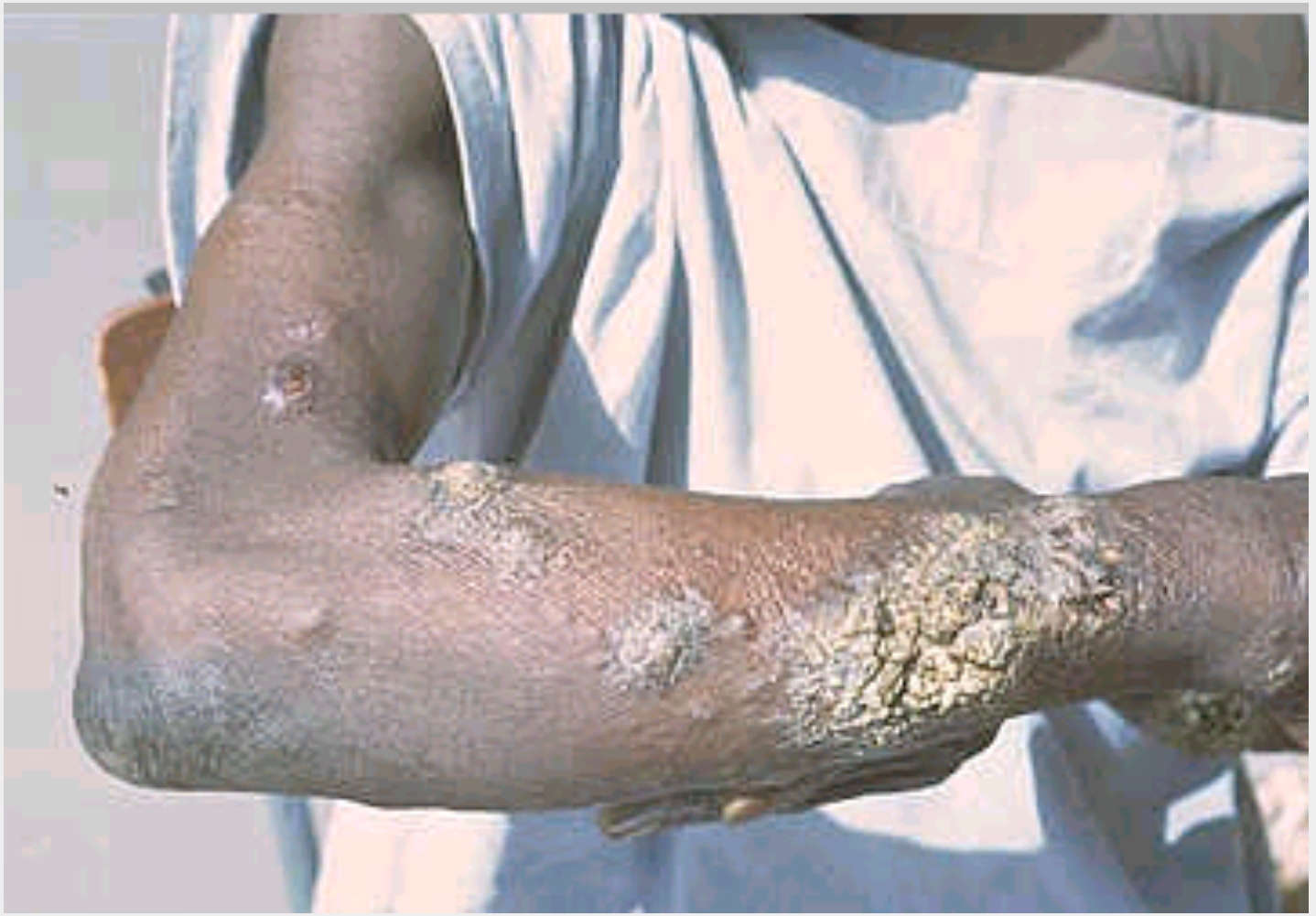
- **Mucocutaneous leishmaniasis**: disfiguring, destroys mucous membranes

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

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

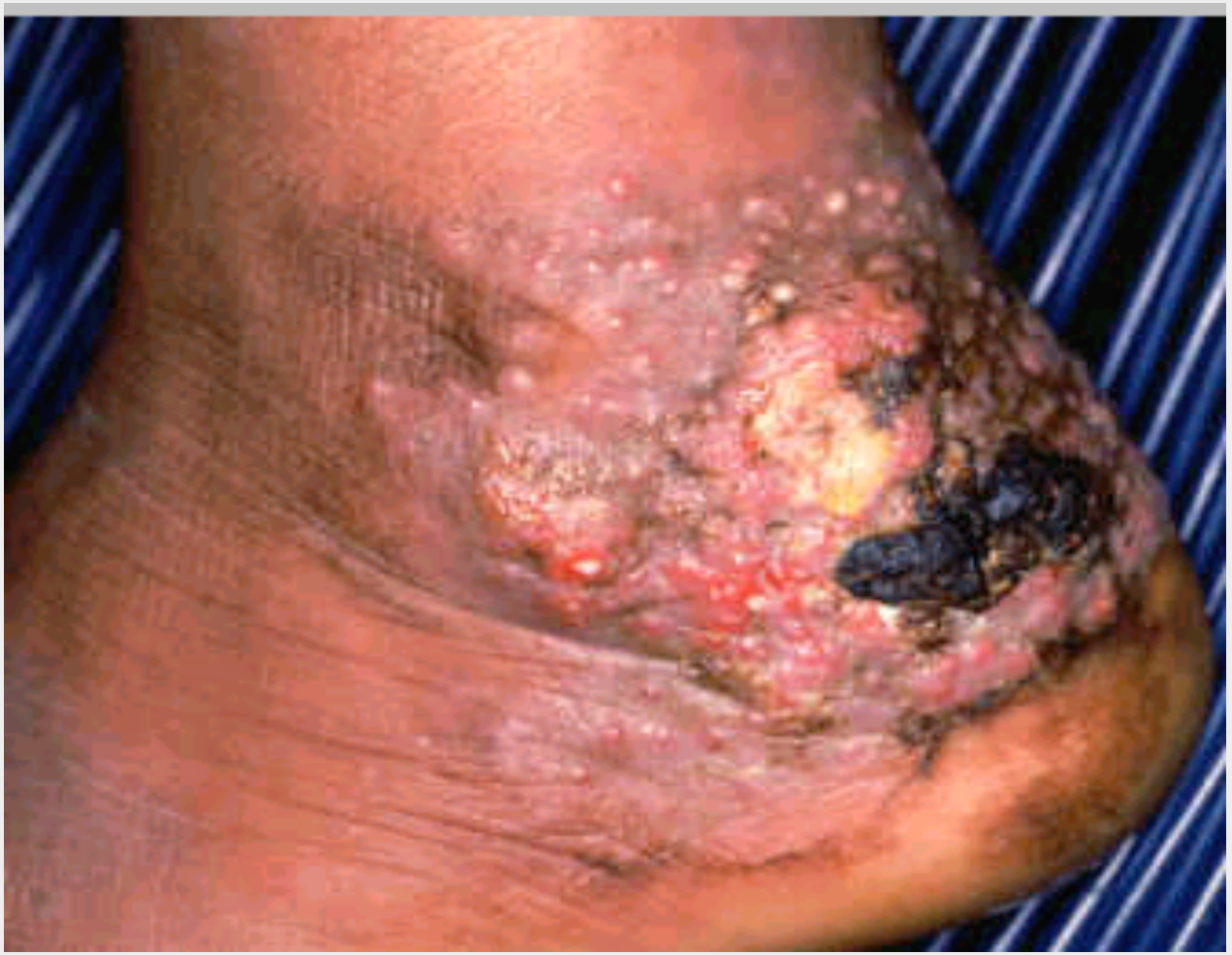
- **Post Kalar azar dermal leishmaniasis** (PKDL): frequently develops after VL

Leishmania major: multiple crusted lesions



(Northern Nigeria), taken from “Leishmaniasis” Topics in International Health

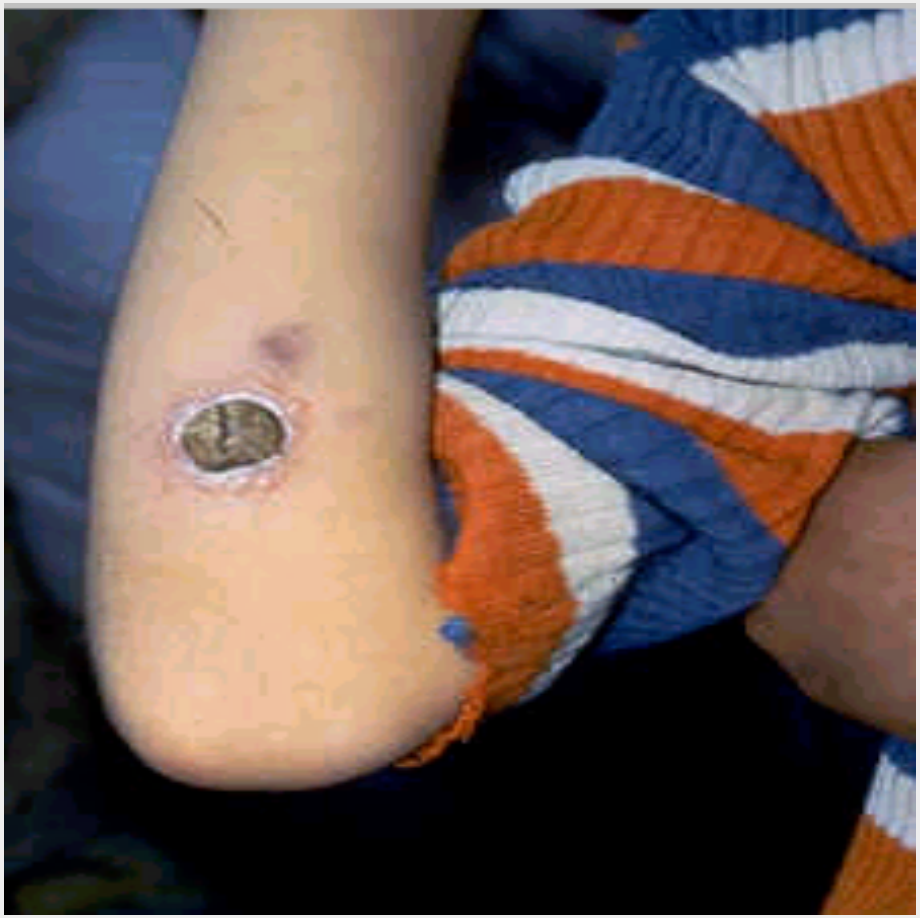
Leishmania major: wet lesion



from:
"Leishmaniasis",
Topics in
International Health

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

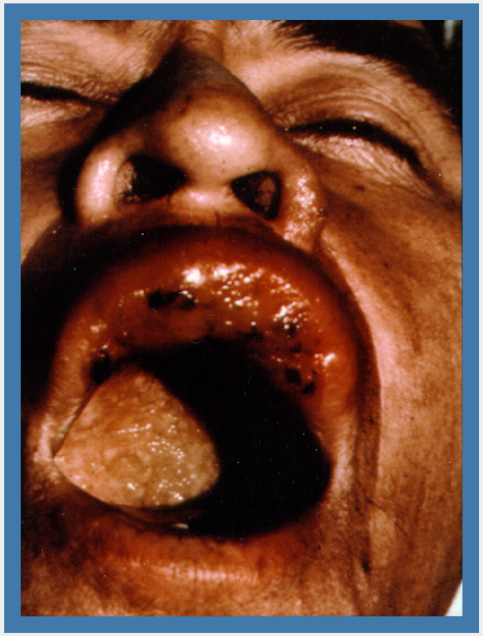
L. tropica: crusted ulcer



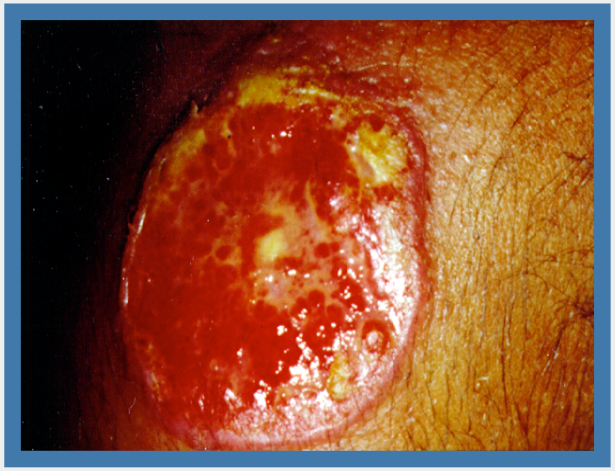
In the process of healing the cutaneous ulcer becomes dry (evolution from granulomatous inflammation to fibrosis)

Aleppo, Syria, taken from: “Leishmaniasis”, Topics in International Health

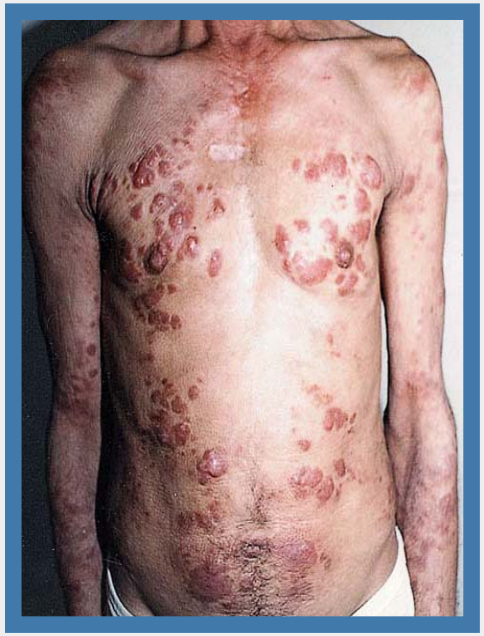
American Tegumentary Leishmaniasis



Mucosal Leishmaniasis



Localised cutaneous Leishmaniasis



Diffuse-cutaneous Leishmaniasis

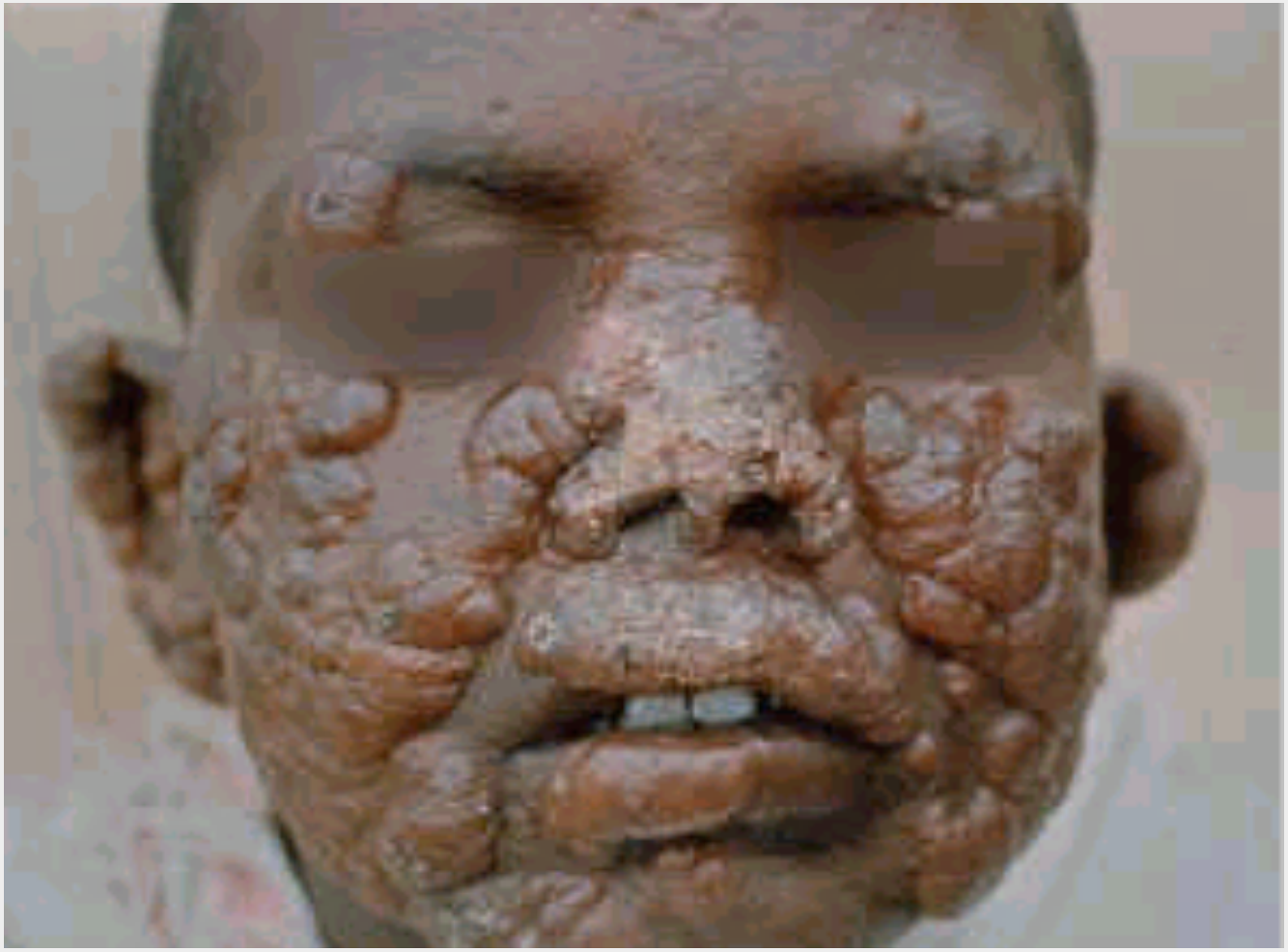
Parasite in Lesions

+	++	++++

Leishmanin skin test - DTH

++++	++	negative

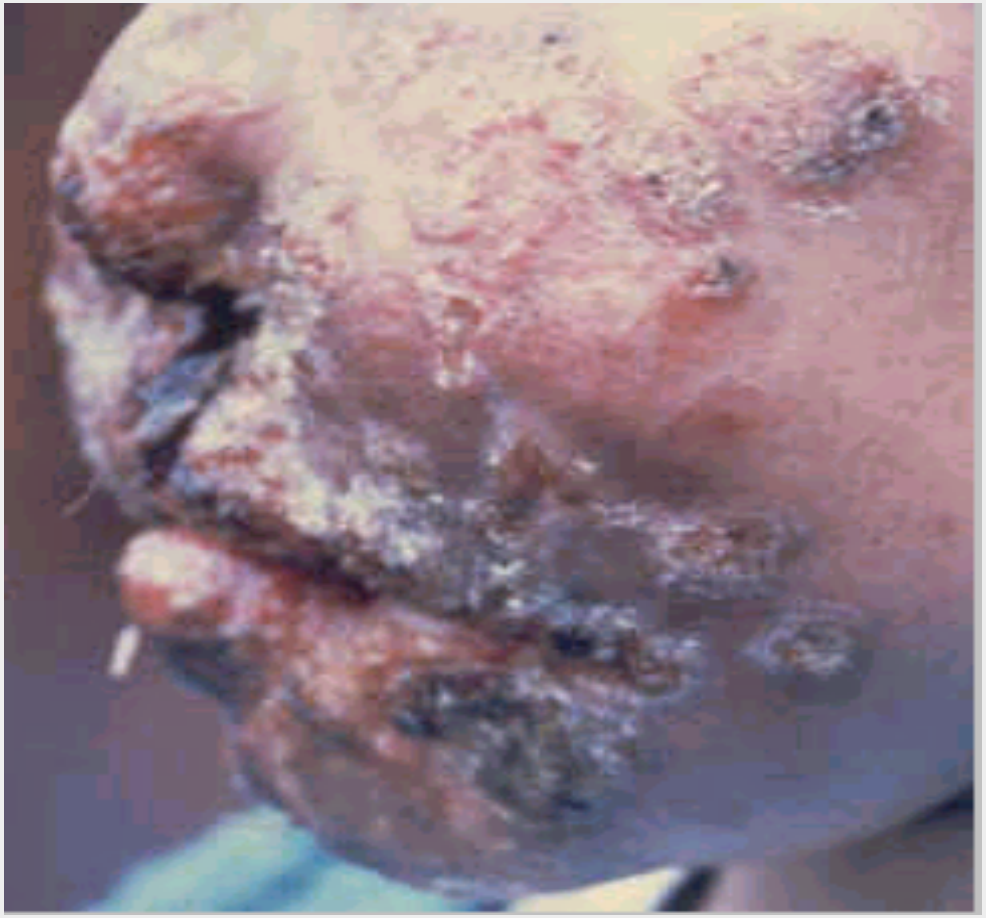
L. aethiopica: disseminated infection



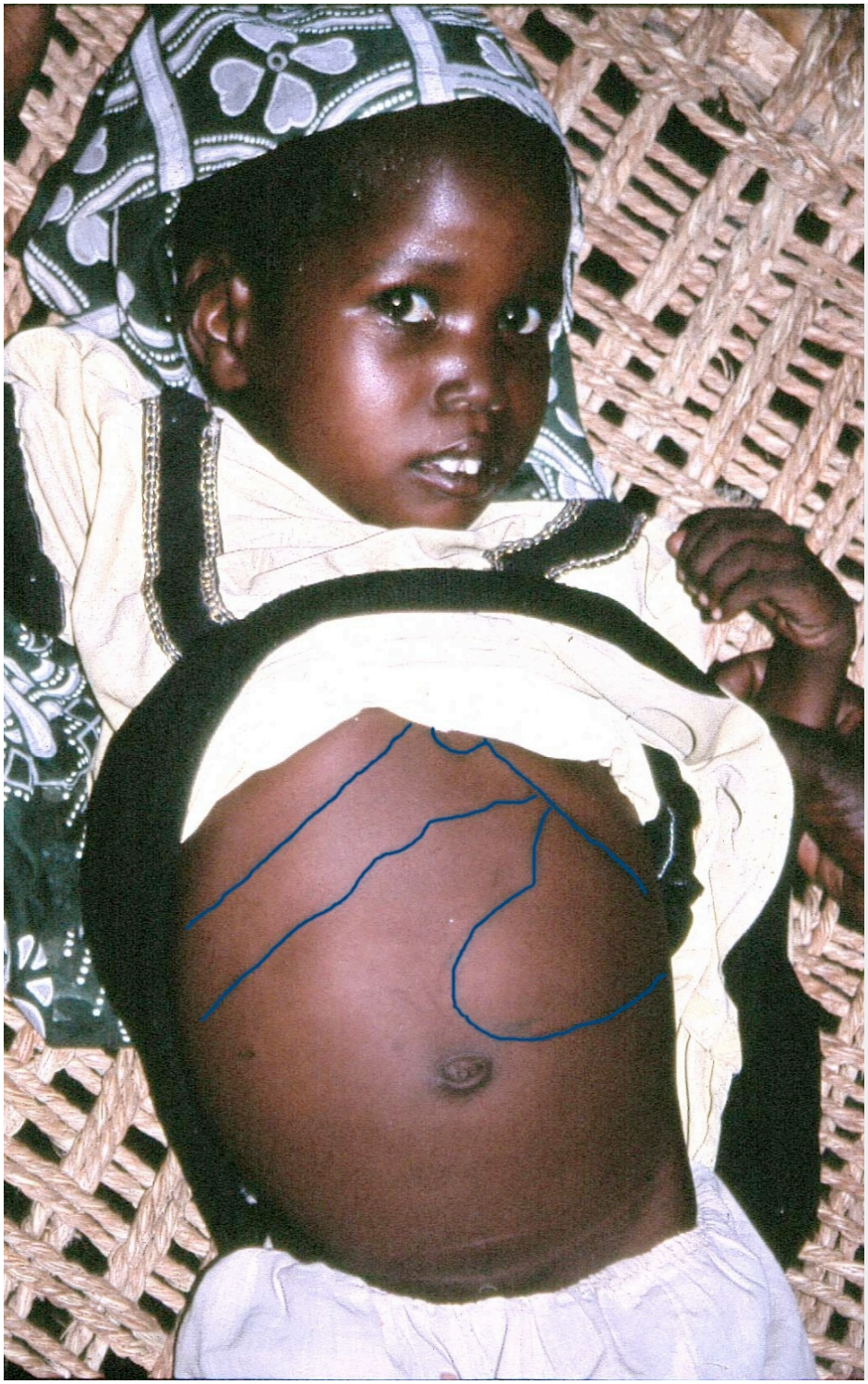
Multiple, nodular non-ulcerating lesions

from “Leishmaniasis” Topics in International Health

Leishmania peruviana: disfiguring crusted lesions



from “Leishmaniasis” Topics in International Health



Visceral leishmaniasis (VL)

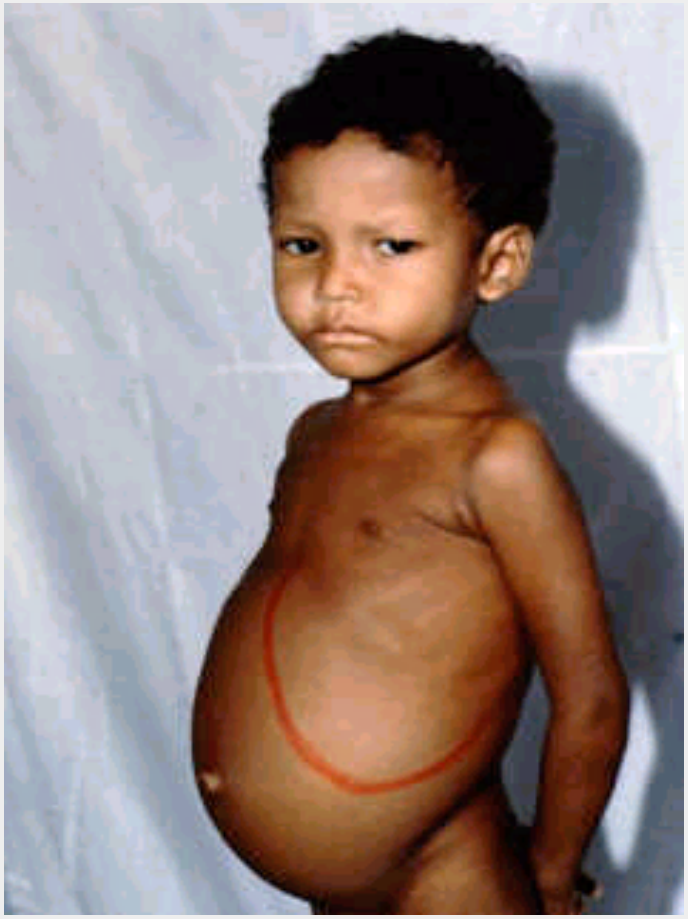
30-100 subclinical infections for every overt VL case

VL = **Kala azar**

Not severely ill yet

Risk factors for development of clinical disease include:

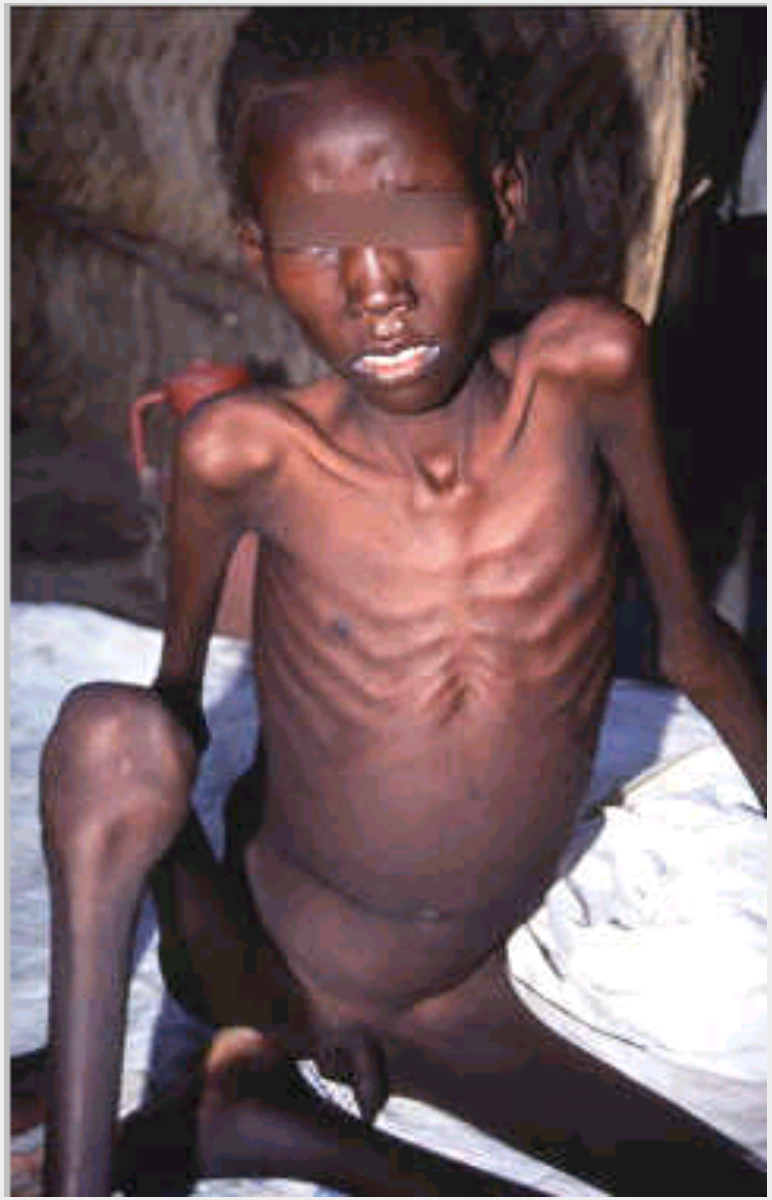
- Malnutrition
- immune suppressive drugs
- HIV co-infections



Spleen: heavily infiltrated with parasitized MΦ. MΦ accumulate, block blood vessels, cause expansion of spleen.
Liver: parasitized Kupffer cells proliferate, cause congestion of veins, form granulomas.

Visceral leishmaniasis

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%



VL, due to *L. donovani*, Sudan

Visceral leishmaniasis:

- 90% of VL cases in:
  India, Bangladesh, Nepal
  Sudan, Ethiopia and Brazil

HIV-VL co-infections:
↑notably in India,Brazil, Ethiopia

Disease burden:
1 980 000 disability-adjusted life years

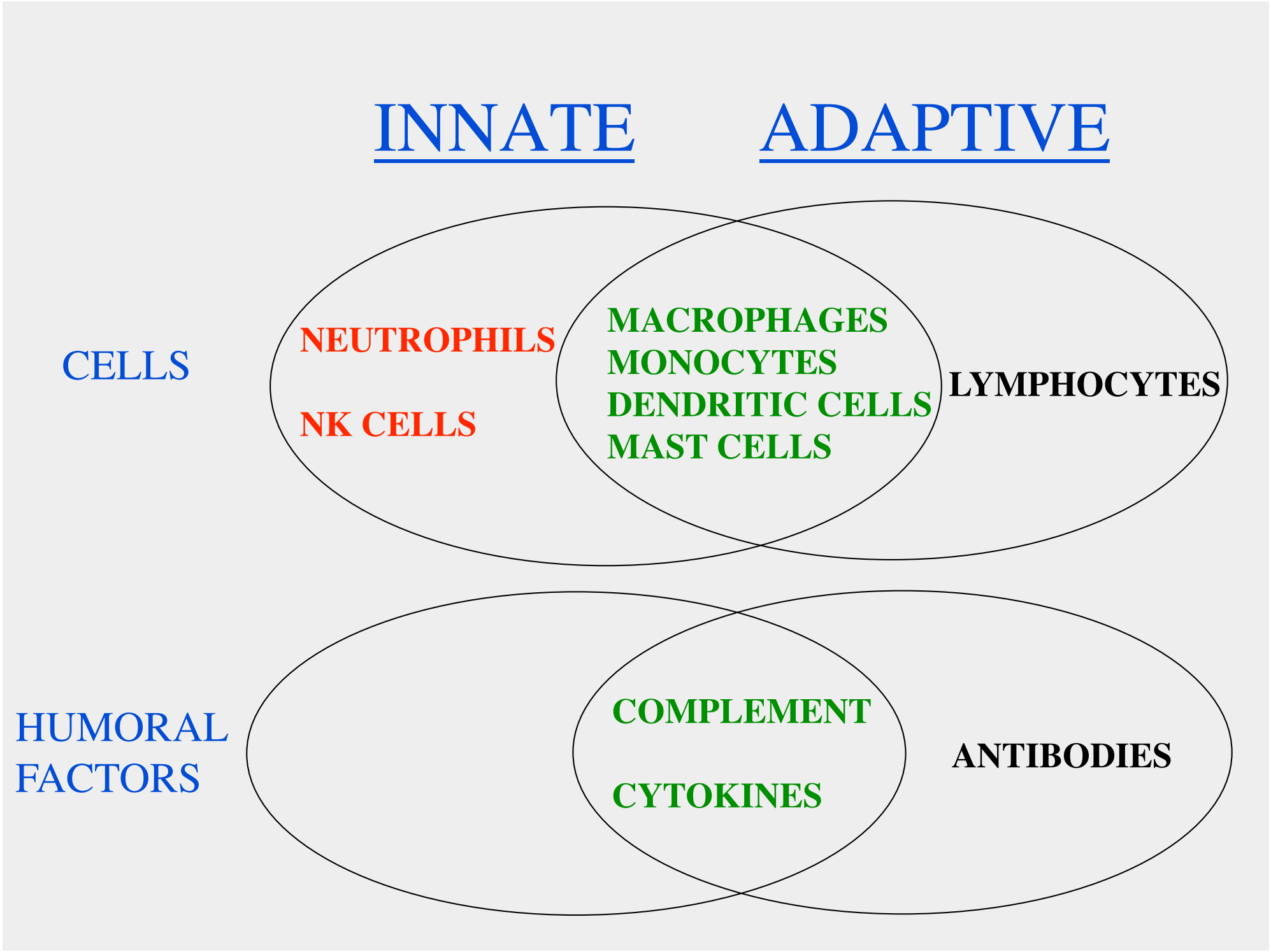
from: "Leishmaniasis", Topics in International Health

Leishmaniasis

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

Fundamental principle of immuno-regulation of leishmaniasis:

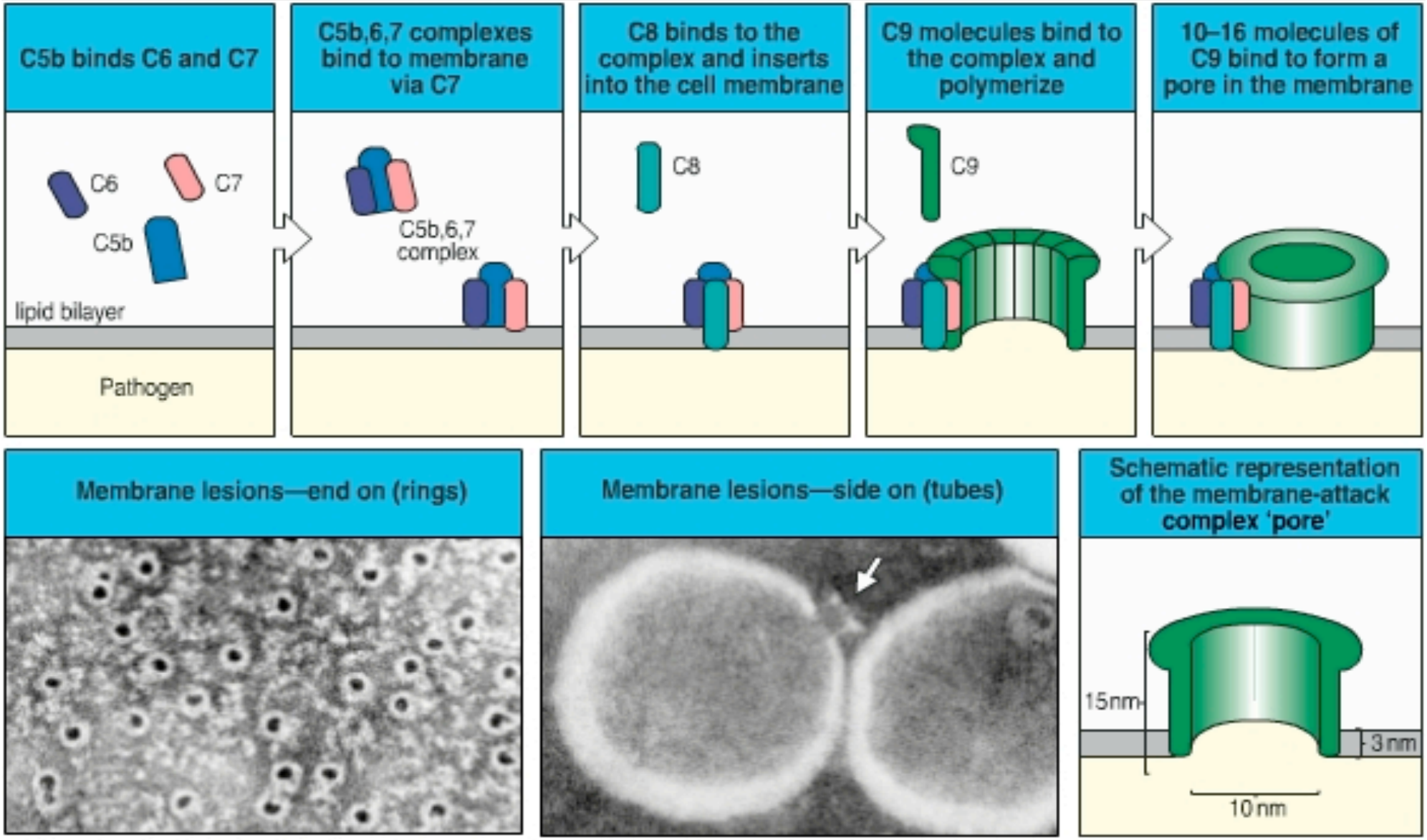
- replication inside MΦ
- parasite elimination by MΦ
- cell-mediated immunity: T cells instruct MΦ
- T cells regulate immune responses
- T cell memory protects against reinfection


INNATE
ADAPTIVE
CELLS
NEUTROPHILS
NK CELLS
MACROPHAGES
MONOCYTES
DENDRITIC CELLS
MAST CELLS
LYMPHOCYTES
HUMORAL FACTORS
COMPLEMENT
CYTOKINES
ANTIBODIES

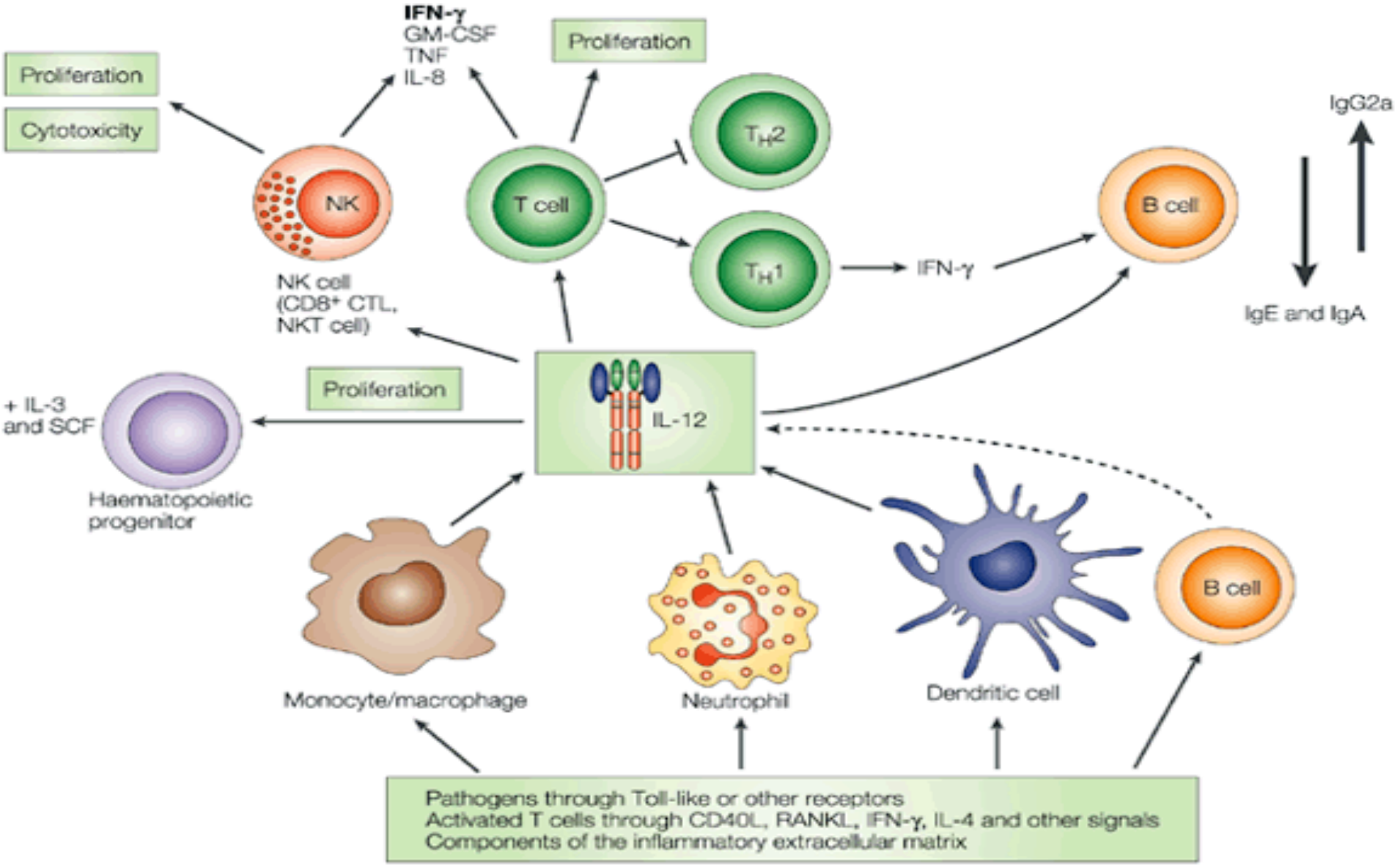
Complement

- 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

Formation of membrane attack complex



Innate and adaptive immunity

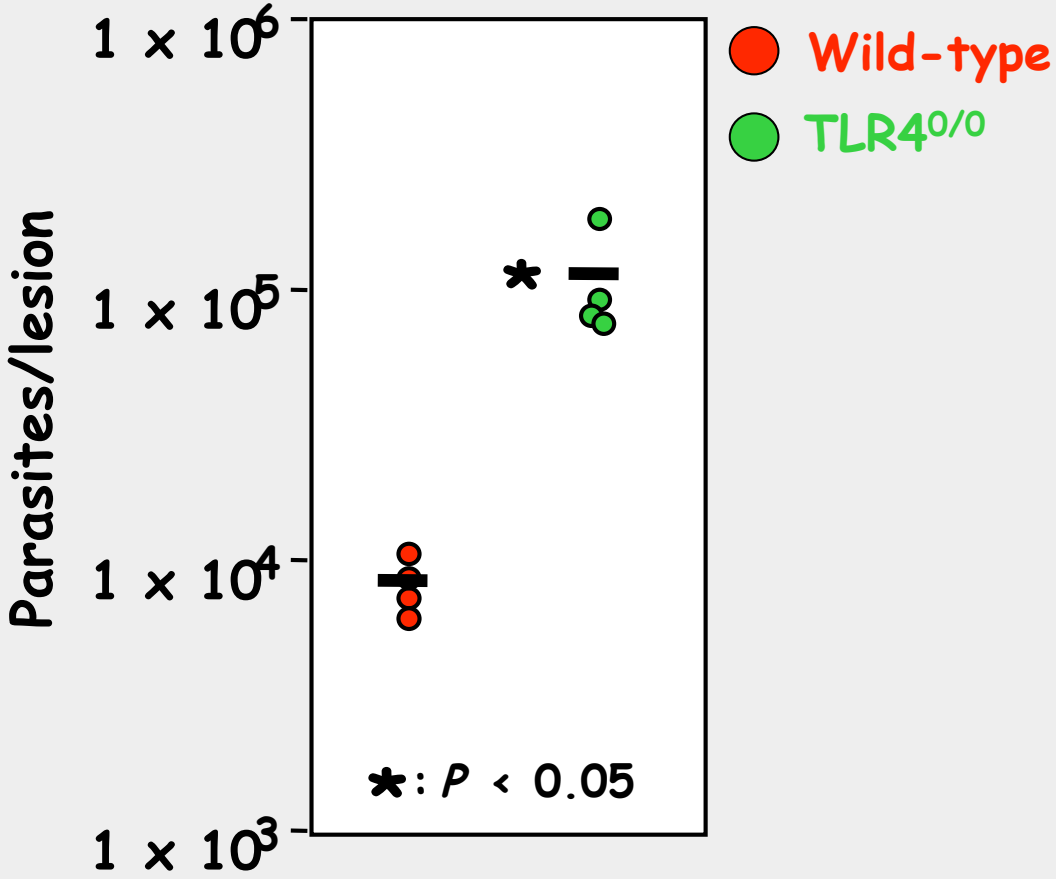


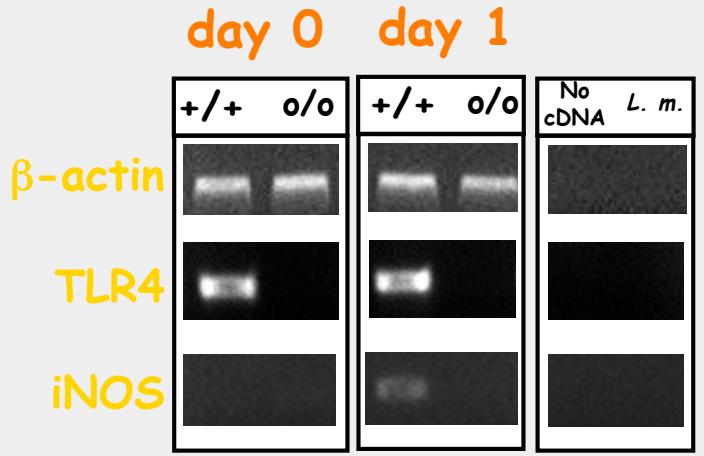
fromTrinchieri,3:133-146, 2003

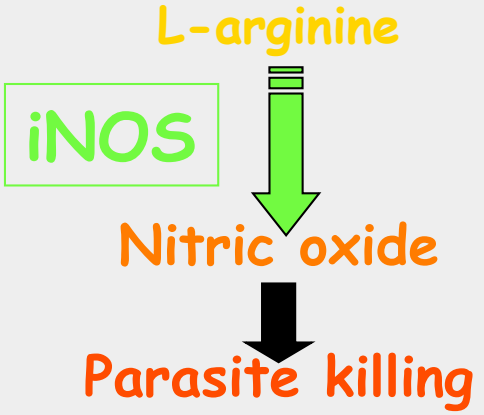
Nature Reviews | Immunology

Parasite load in the footpads of *L. major* infected TLR4$^{0/0}$ and wild-type C57BL/10 mice

24 hours post infection



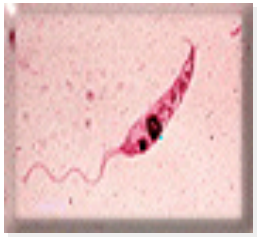


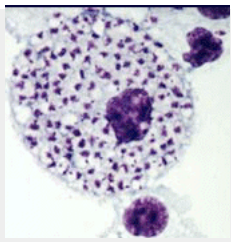


In the absence of TLR4

=> higher parasite load at the site of infection

=> no induction of iNOS at the site of infection





Toll Like Receptors in Leishmania infection

- MyD88-/- become susceptible (Muraille et al, JI 2003)

- several TLRs are involved: TLR4 (Kropf et al, IAI 2004)

- LPG is recognised by TLR2

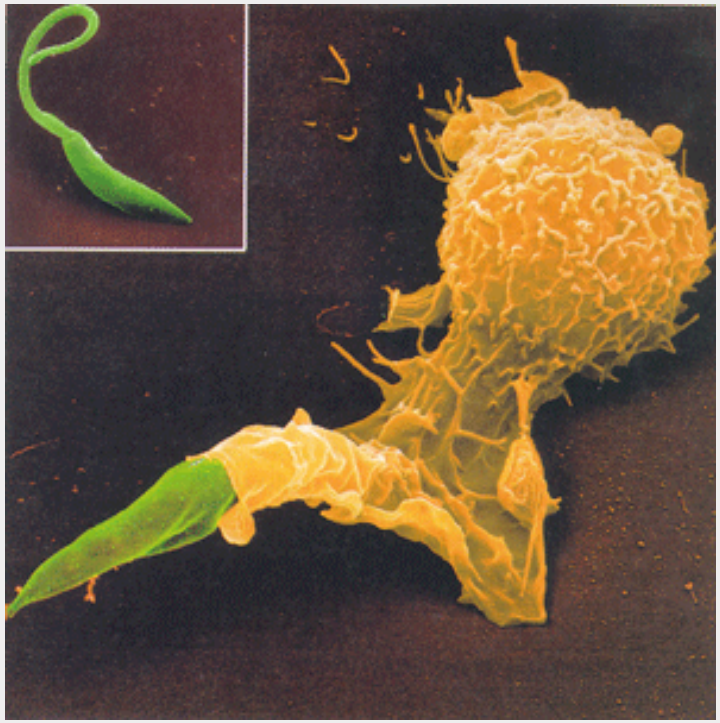
  (Becker et al, Mol Biochem Parasitol. 2003; de Veer et al, EJI, 2003)

- activation of NK cells requires TLR9

  (Schleicher et al, JExpMed 2007)

Leishmania are obligate intracellular parasites

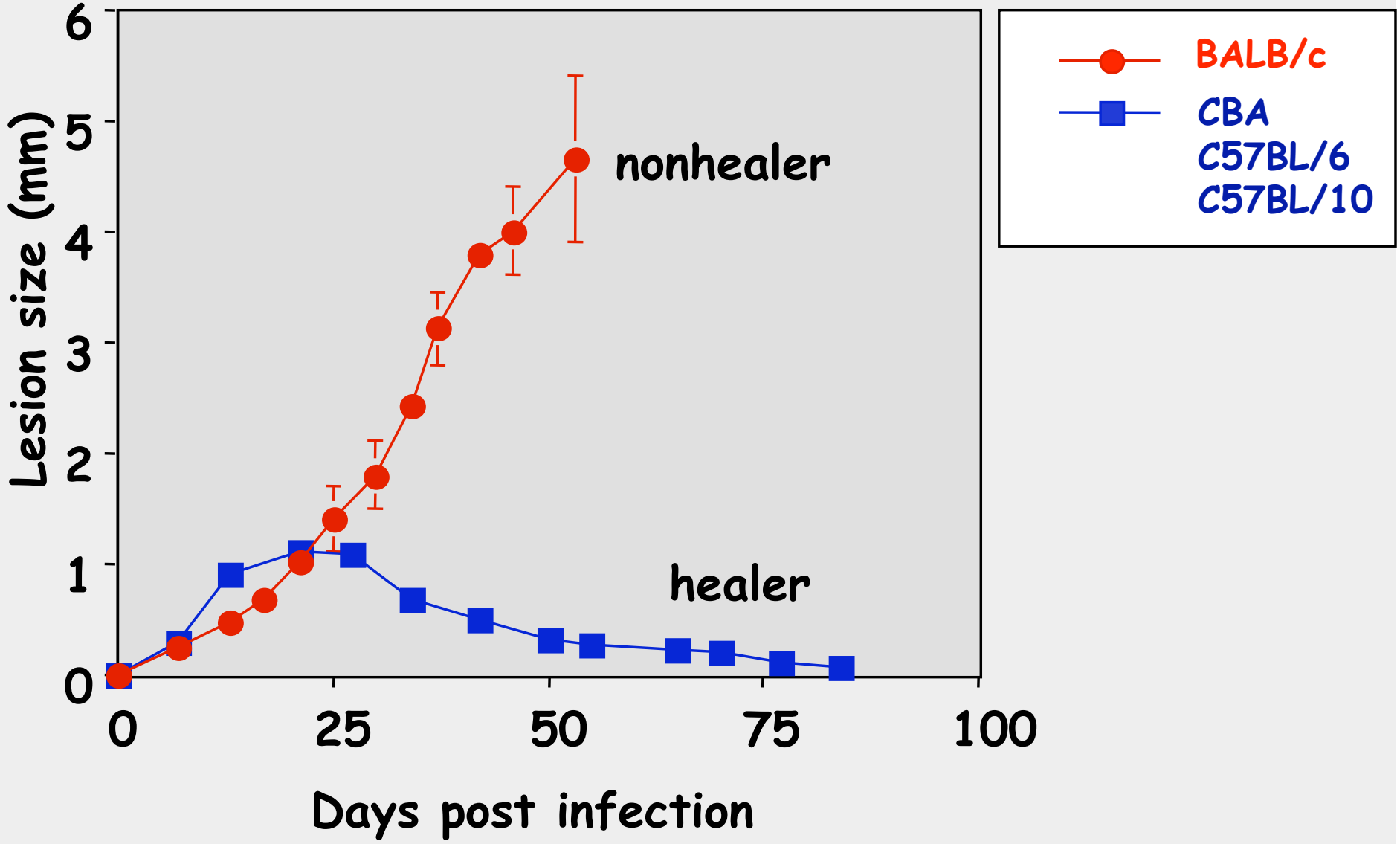
Main host cells: macrophages

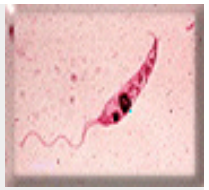


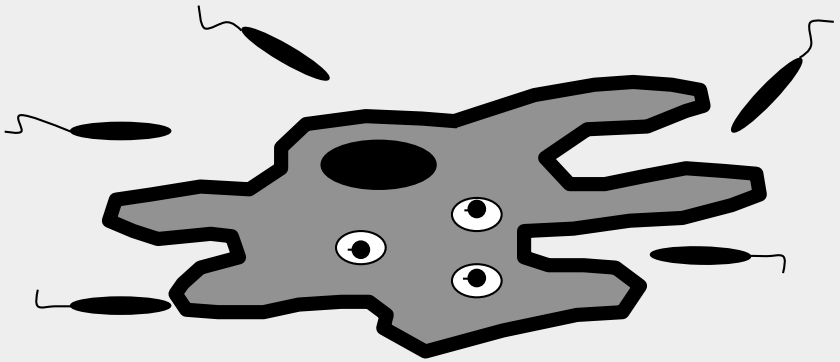
Killers

Hosts

Lesion development after infection with *L. major* parasites in different inbred strains of mice







Classical activation

IL-12

IL-4

Alternative activation

T-bet
Th1

GATA-3
Th2

HEALING

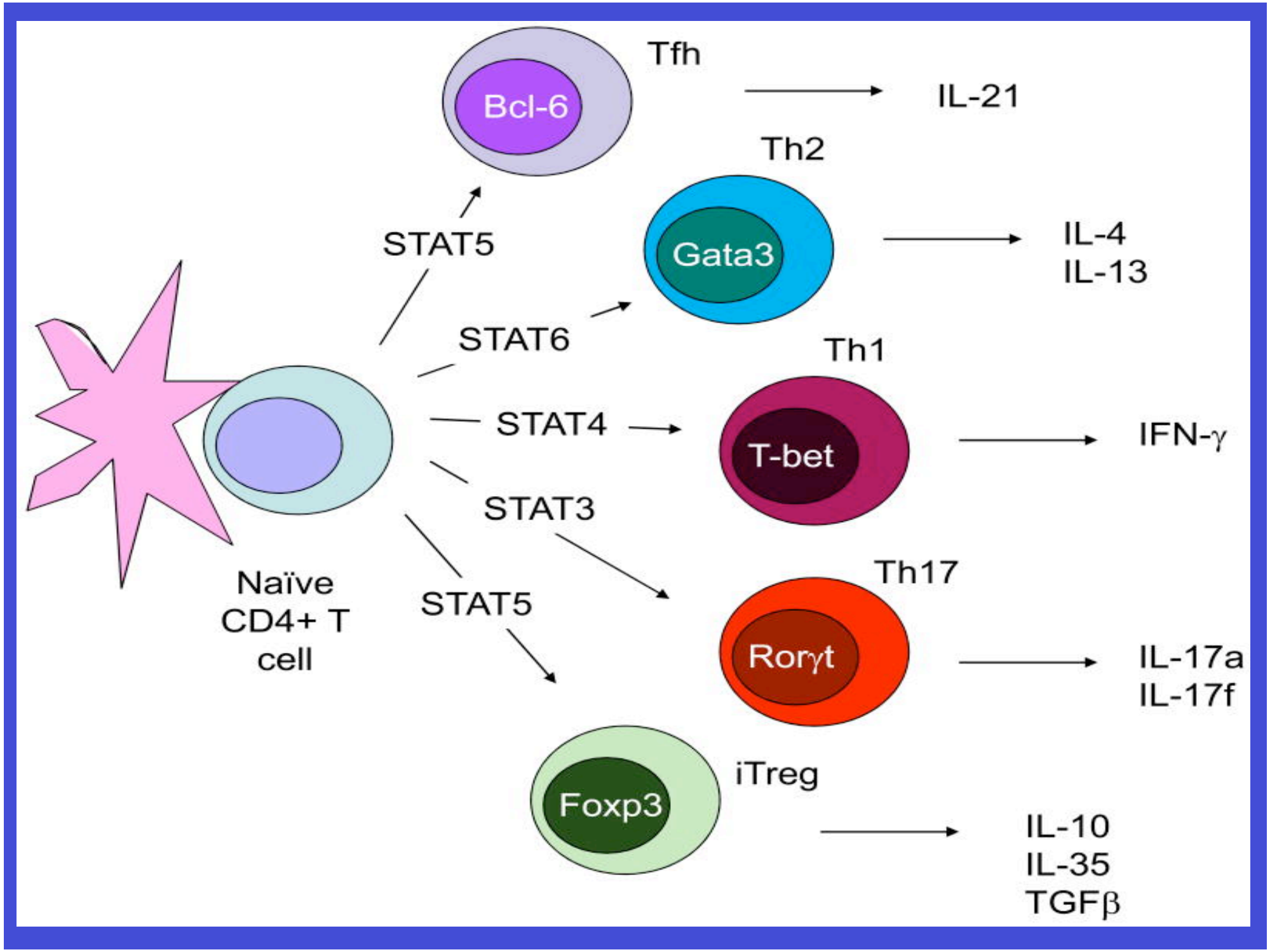
IL-2
IFN-γ
TNF-β

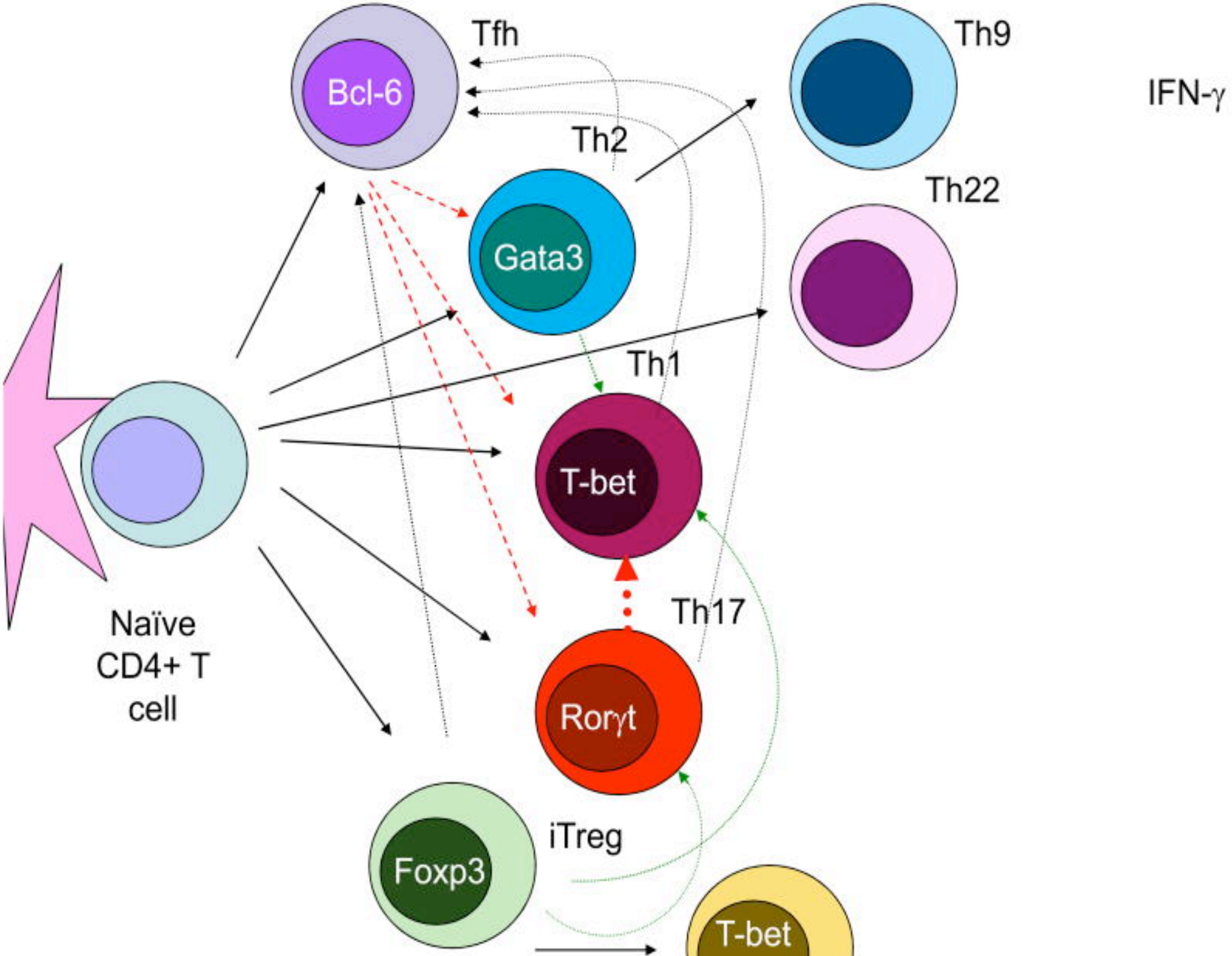
IL-4
IL-5
IL-10
IL-13

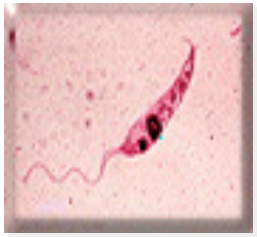
NON-HEALING

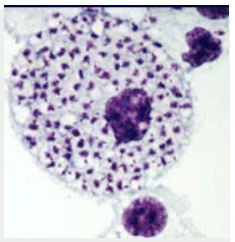
$CD4^+$ T cell differentiation

- Central theme of immune regulation.
- Th1 and Th1 polarisation and cross-regulation was demonstrated in experimental leishmaniasis
- CD4+ T cell subsets are likely to be more plastic and flexible
- Th subset differentiation is not pre-determined, depends on signals that drive cells towards either subset.

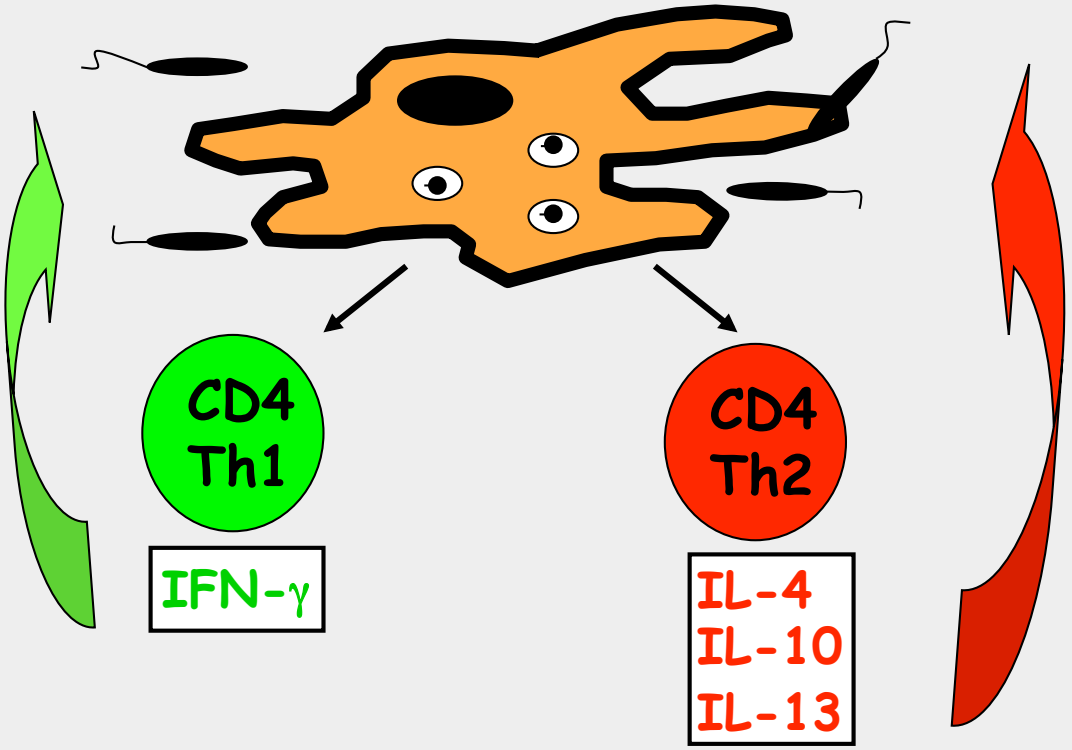

Tfh
Bcl-6
IL-21
Th2
STAT5
Gata3
IL-4
IL-13
STAT6
Th1
STAT4
T-bet
IFN-γ
STAT3
Naïve
CD4+ T
cell
STAT5
Th17
Rorγt
IL-17a
IL-17f
iTreg
Foxp3
IL-10
IL-35
TGFβ


Tfh
Bcl-6
Th2
Gata3
Th9
IFN-γ
Th22
Th1
T-bet
Naïve
CD4+ T
cell
Th17
Rorγt
iTreg
Foxp3
T-bet





ADAPTIVE IMMUNE RESPONSE



Classical activation

Parasite killing

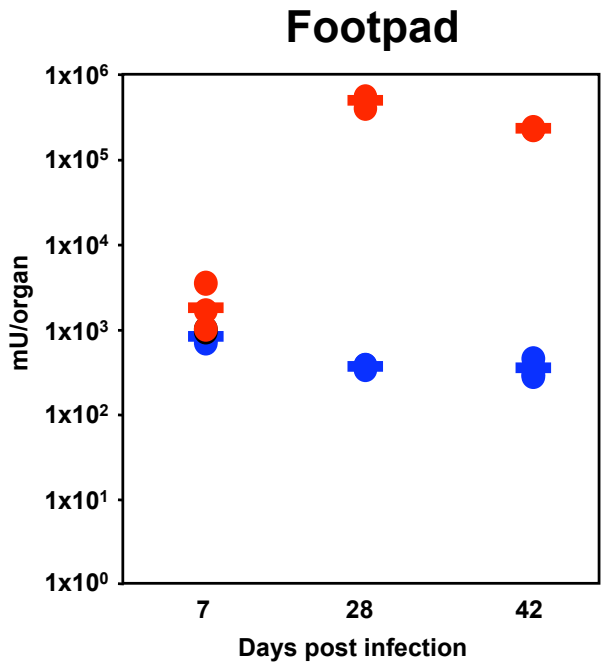
HEALING

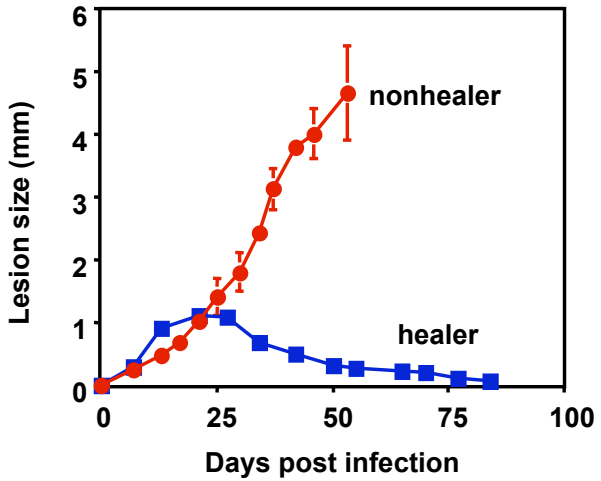
Alternative activation

Parasite multiplication

NON-HEALING

Arginase activity in the lesions of healer and nonhealer mice





nonhealer

healer

=> High arginase activity is expressed at the site of pathology

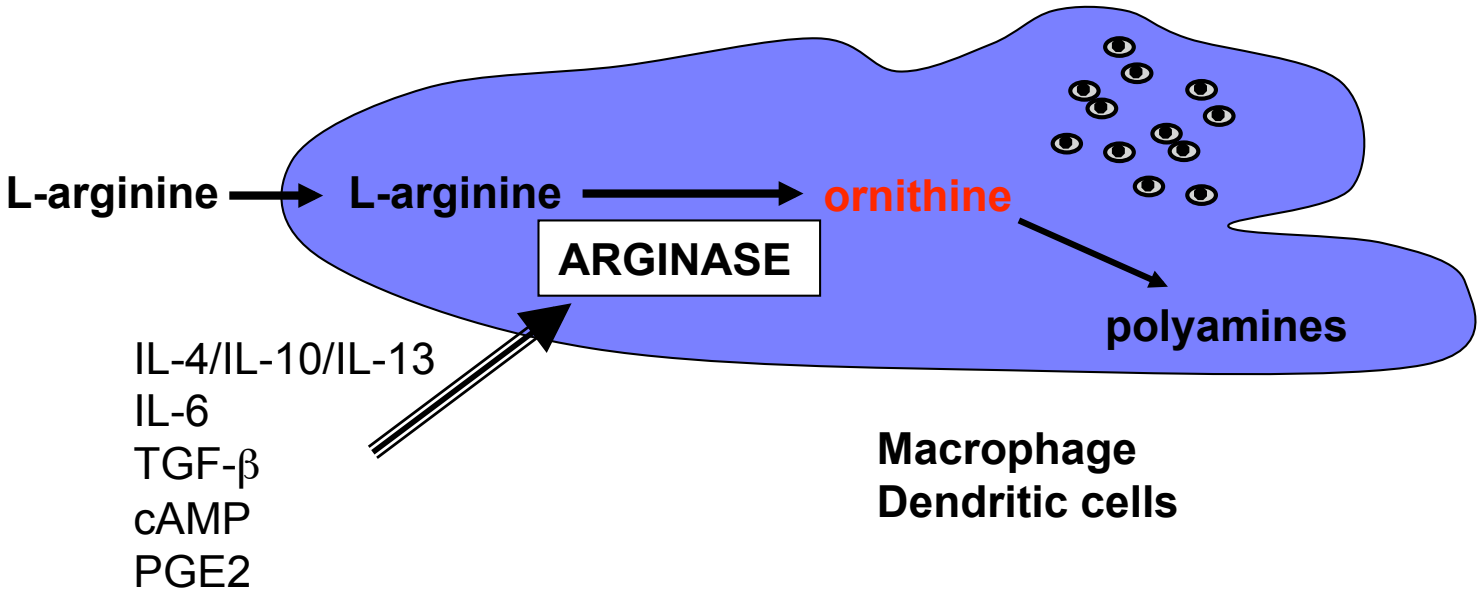
Arginase

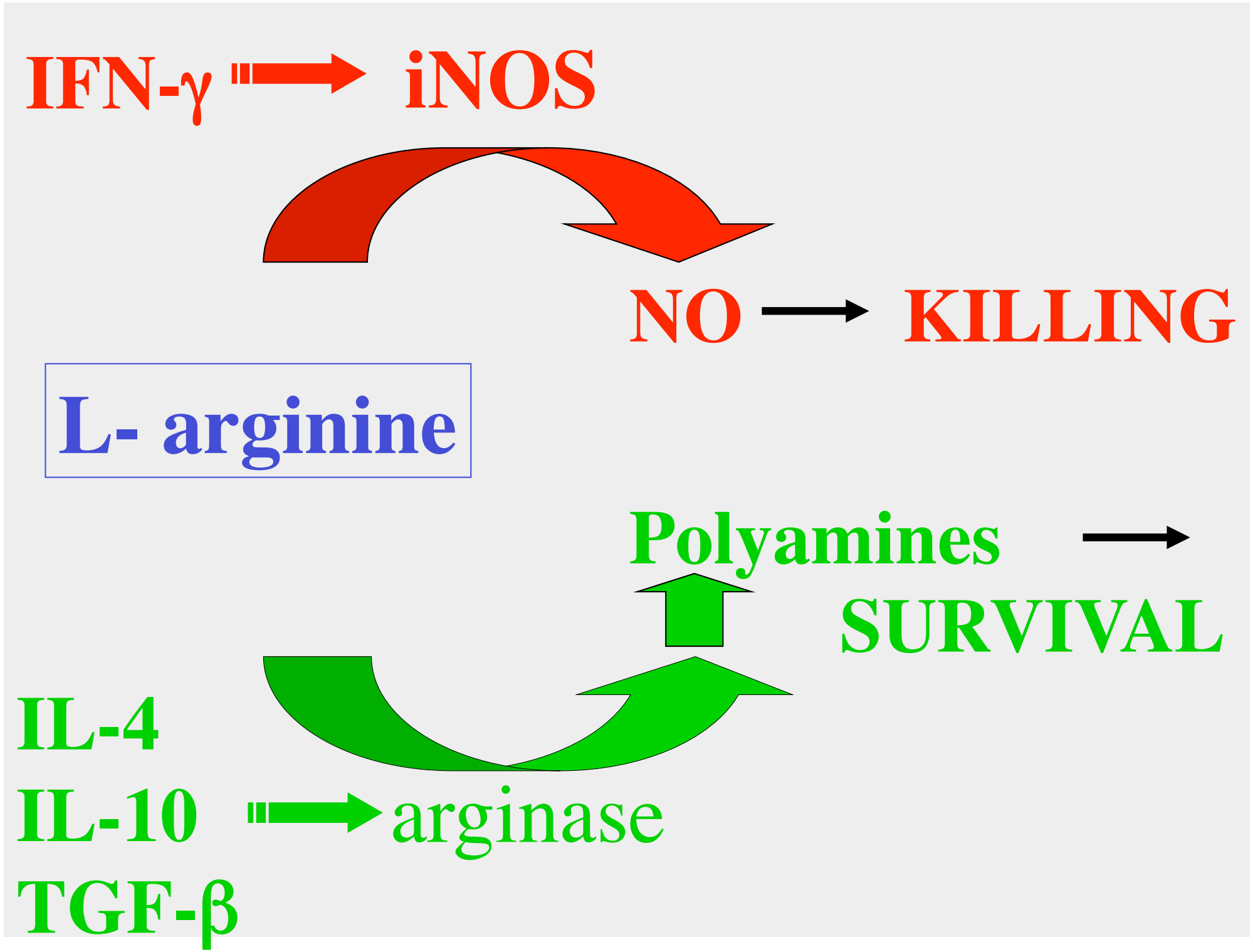
Arginase I (Liver-type arginase)

Cytosolic
Hepatocytes, RBC

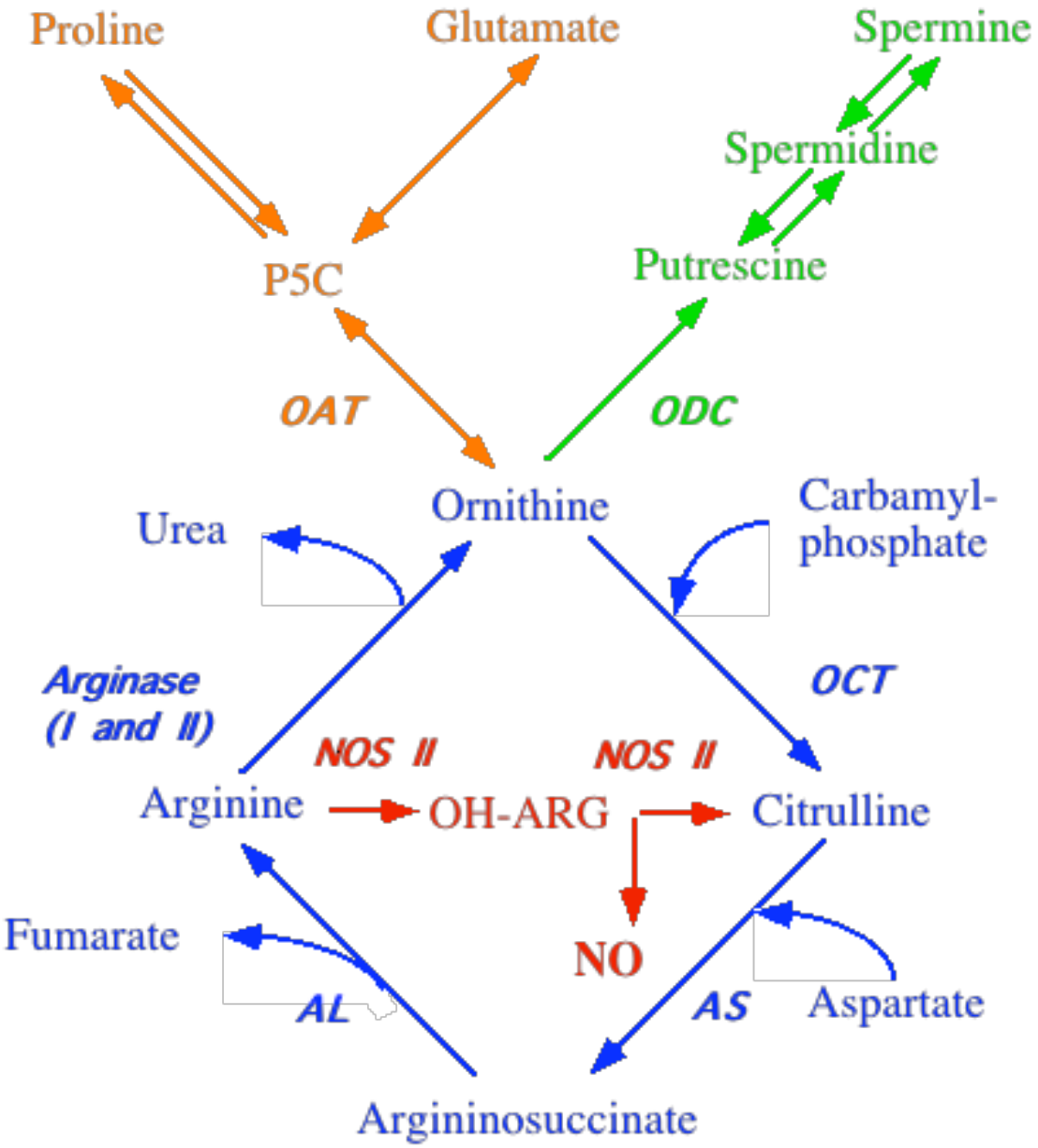
Inducible in **- macrophages**
- dendritic cells

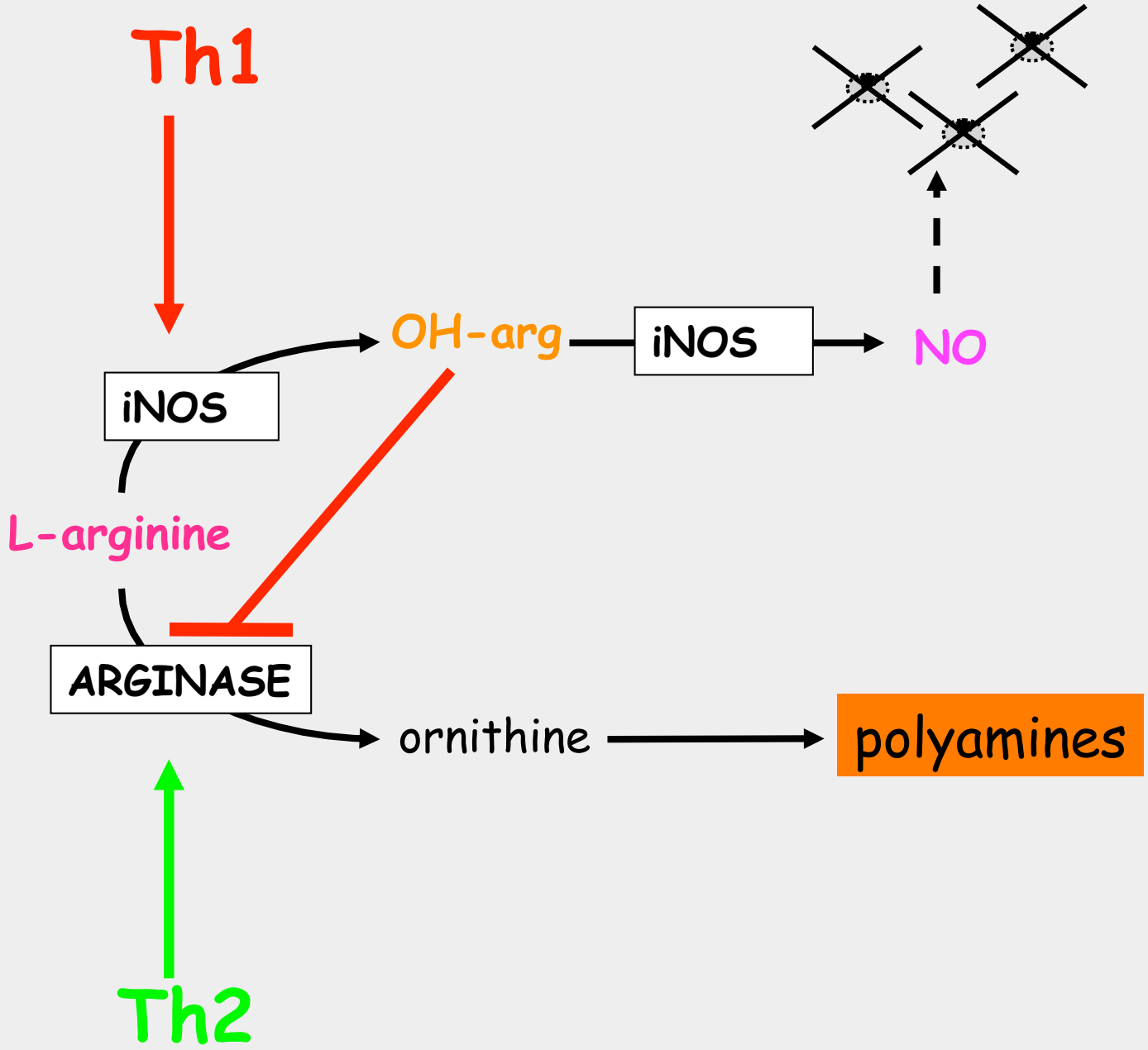
Arginase I -/- mice: not viable
Human arginase 1 deficiency
(Progressive dementia, spasticity, short stature)




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

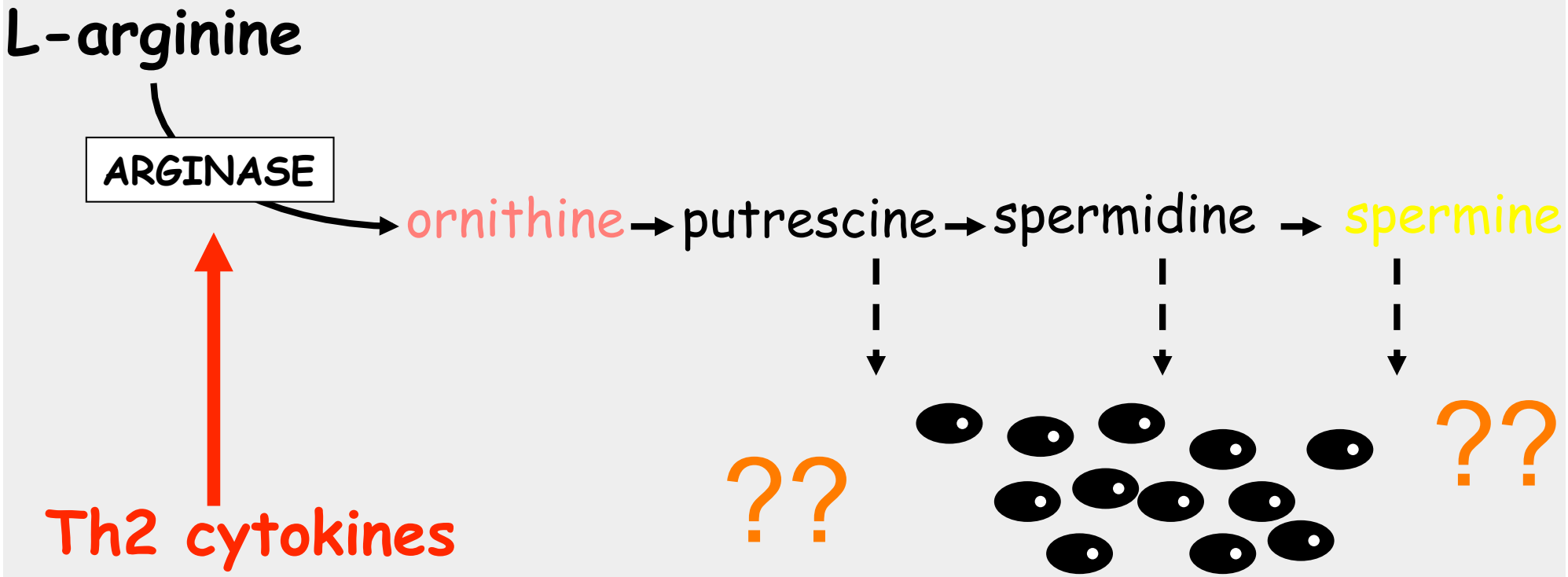
Metabolism of arginine in murine macrophages




Th1
OH-arg
iNOS
NO
iNOS
L-arginine
ARGINASE
ornithine
polyamines
Th2

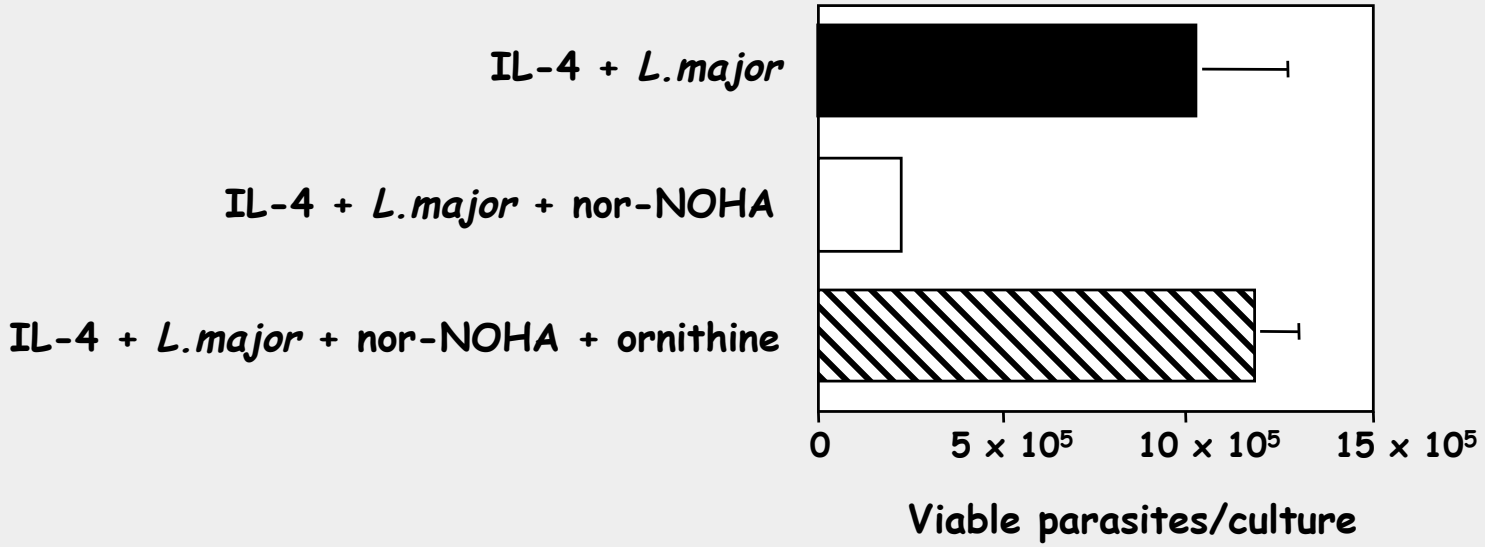
Leishmania survival mechanisms

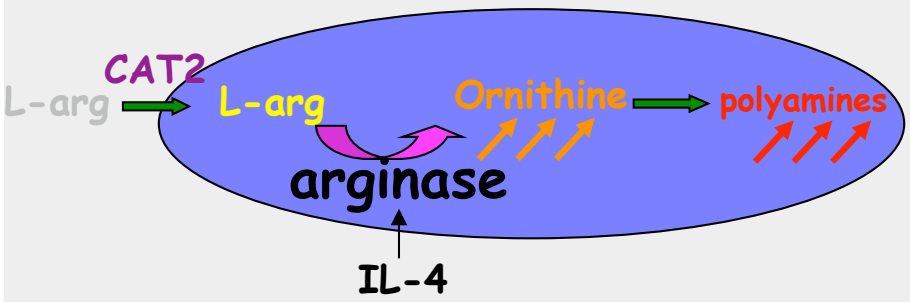
Can parasites use the host metabolism of L-arginine to their advantage?

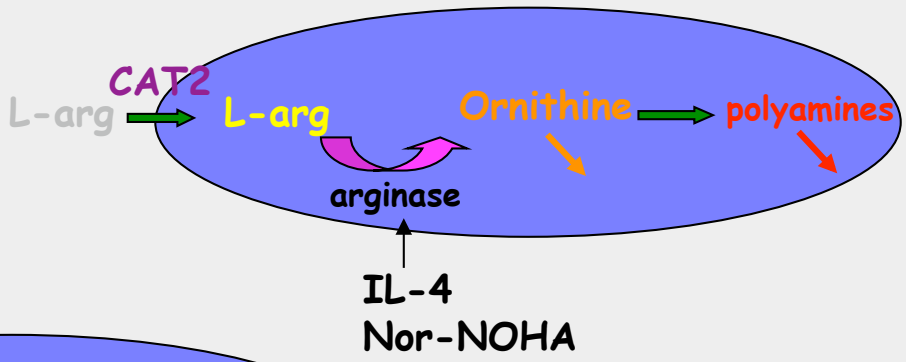


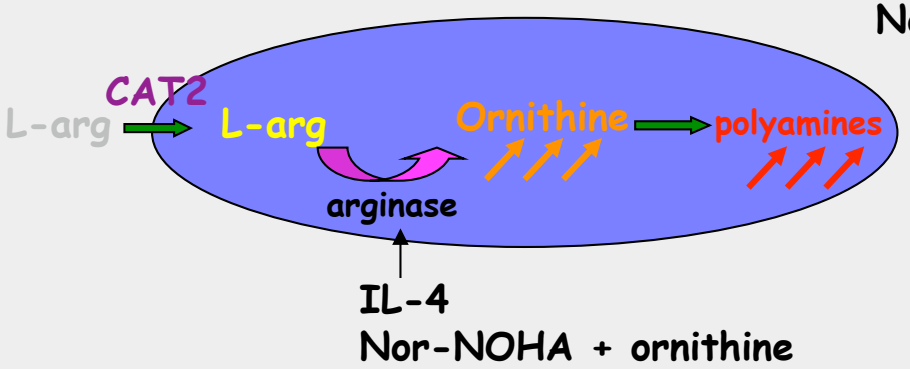
Alternative activation

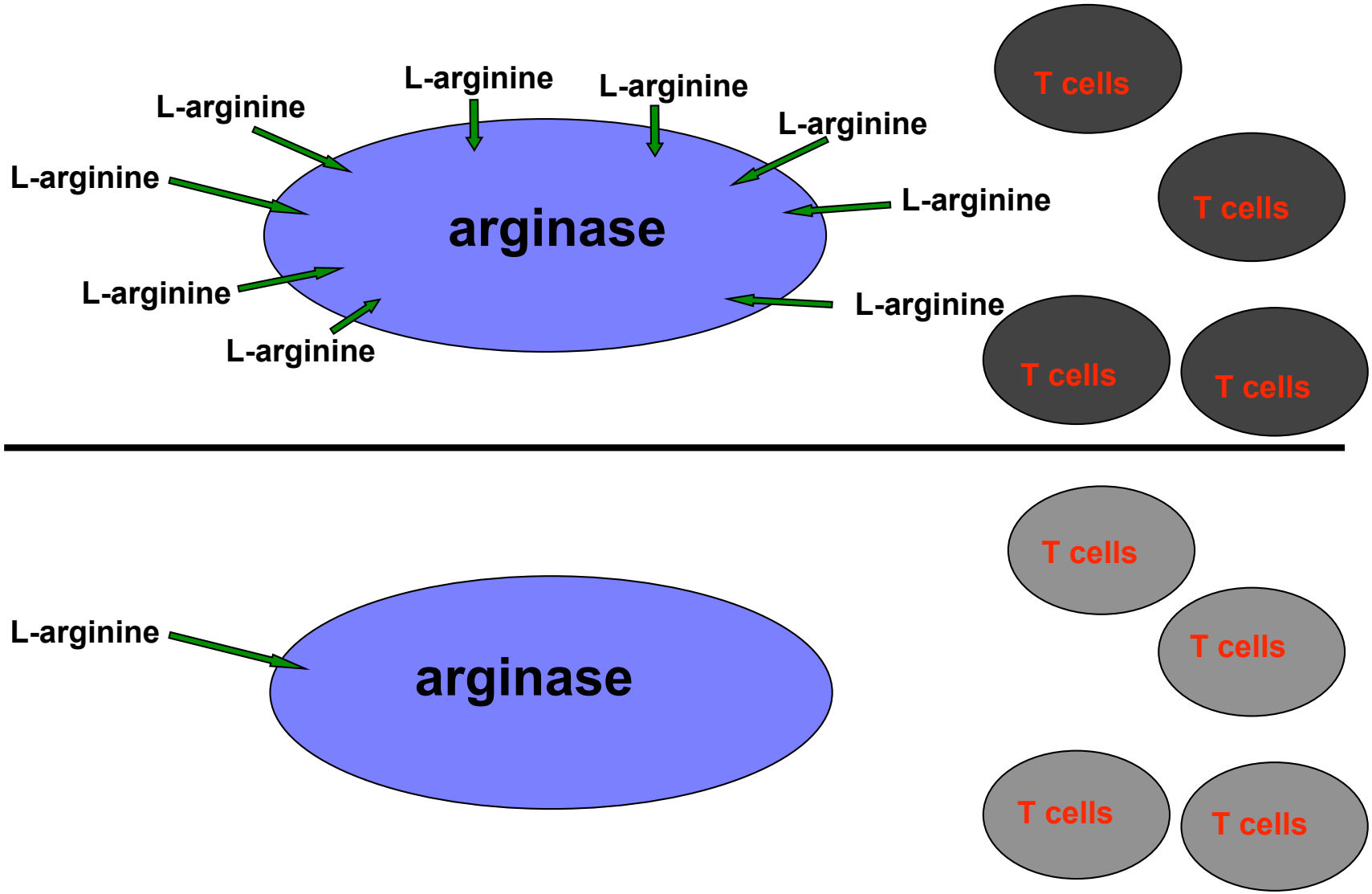
Ornithine can rescue parasite growth







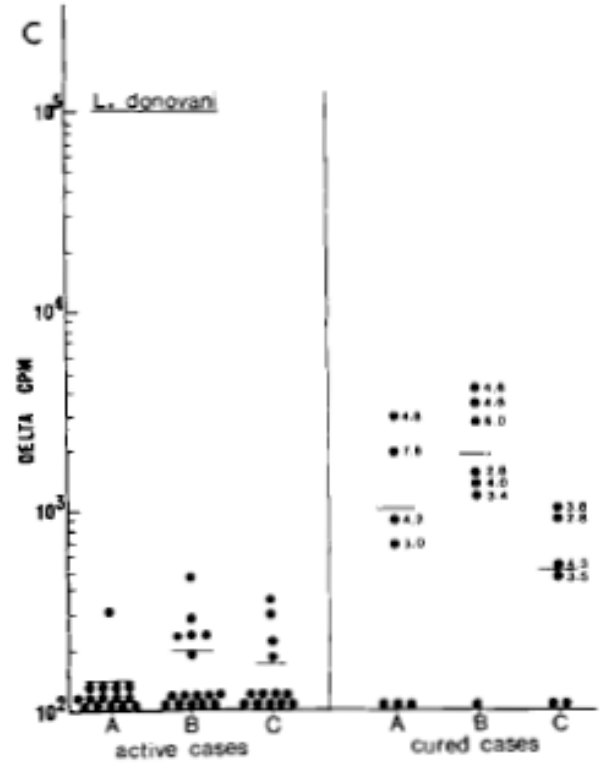




Depletion of L-arginine in the microenvironment induces T cell hyporesponsiveness

What is the relevance for leishmaniasis?

Visceral leishmaniasis



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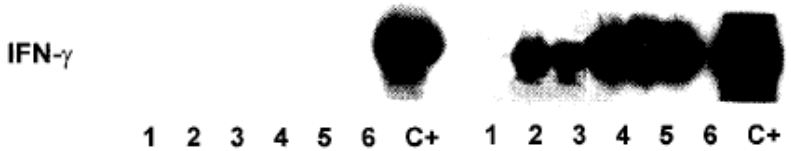
THE JOURNAL OF IMMUNOLOGY
Vol. 138, 908-913, No. 3, February 1, 1987
Printed in U.S.A.

AN ANALYSIS OF T CELL RESPONSIVENESS IN INDIAN KALA-AZAR

DAVID L. SACKS,[1]* SUMAN LATA LAL,† S. N. SHRIVASTAVA,† JENEFER BLACKWELL,[2]* AND FRANKLIN A. NEVA*

Diffuse cutaneous leishmaniasis

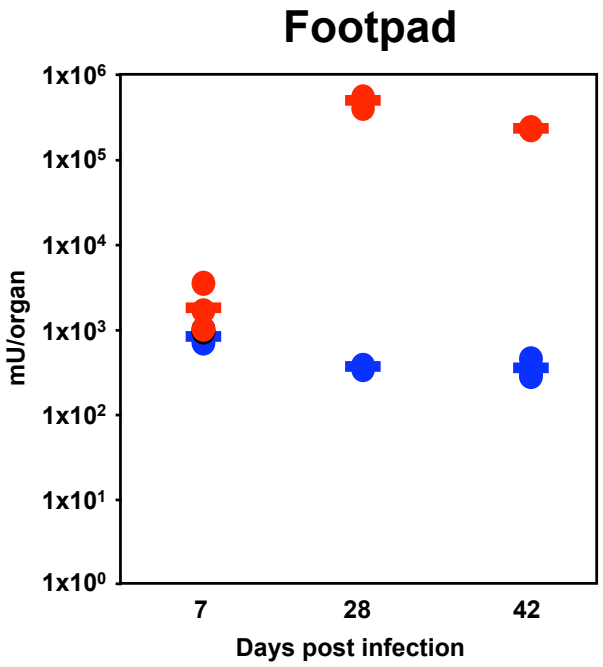


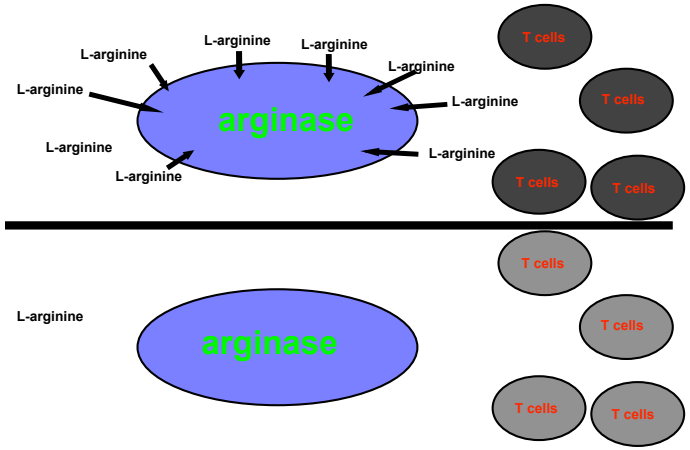
Variation of Cytokine Patterns Related to Therapeutic Response in Diffuse Cutaneous Leishmaniasis

GLÓRIA BOMFIM, CRISTIANE NASCIMENTO,* JACKSON COSTA,† EDGAR M. CARVALHO, MANOEL BARRAL-NETTO,* AND ALDINA BARRAL[1]

=> pronounced immunosuppression

Arginase-induced L-arginine depletion at the site of pathology => local T cell hyporesponsiveness?

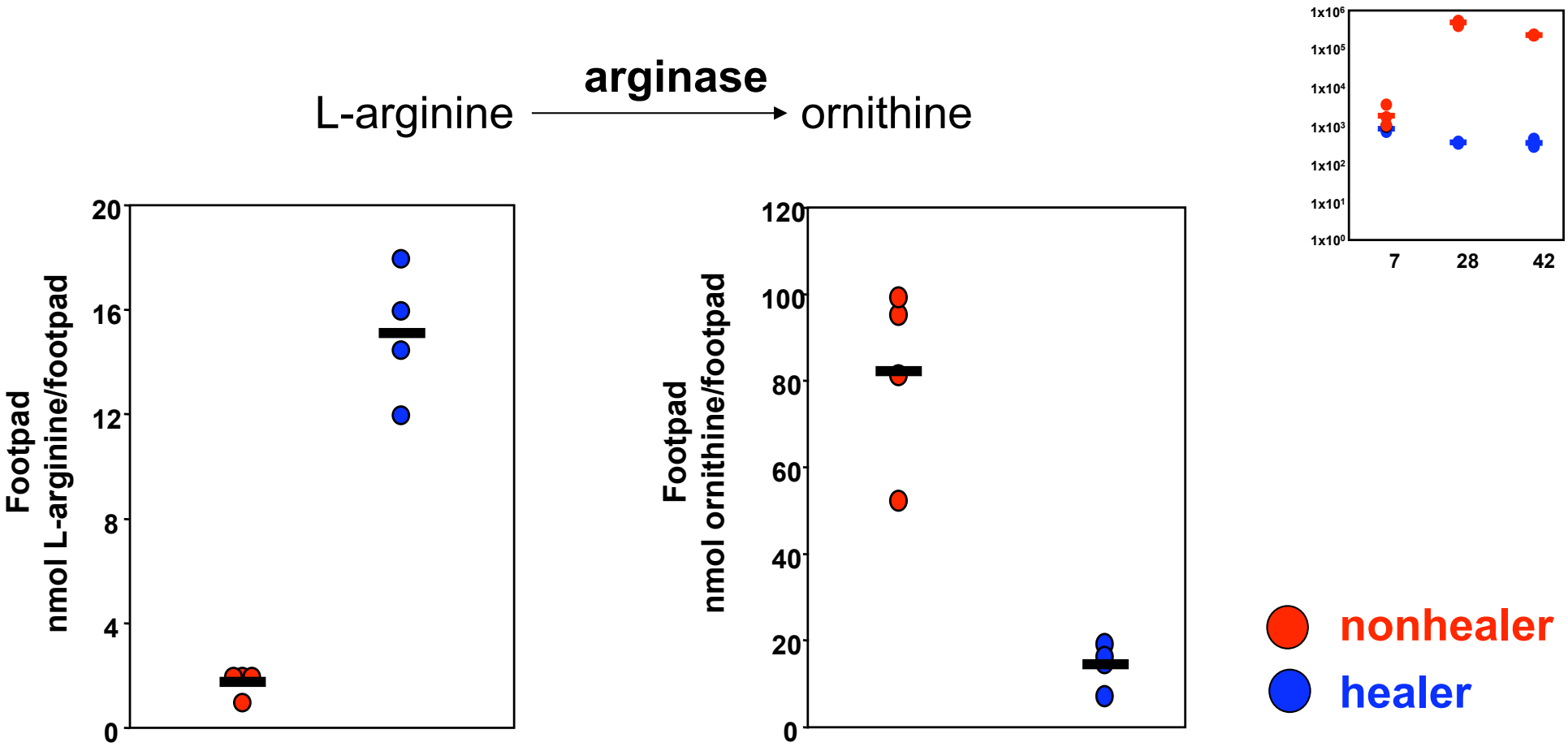




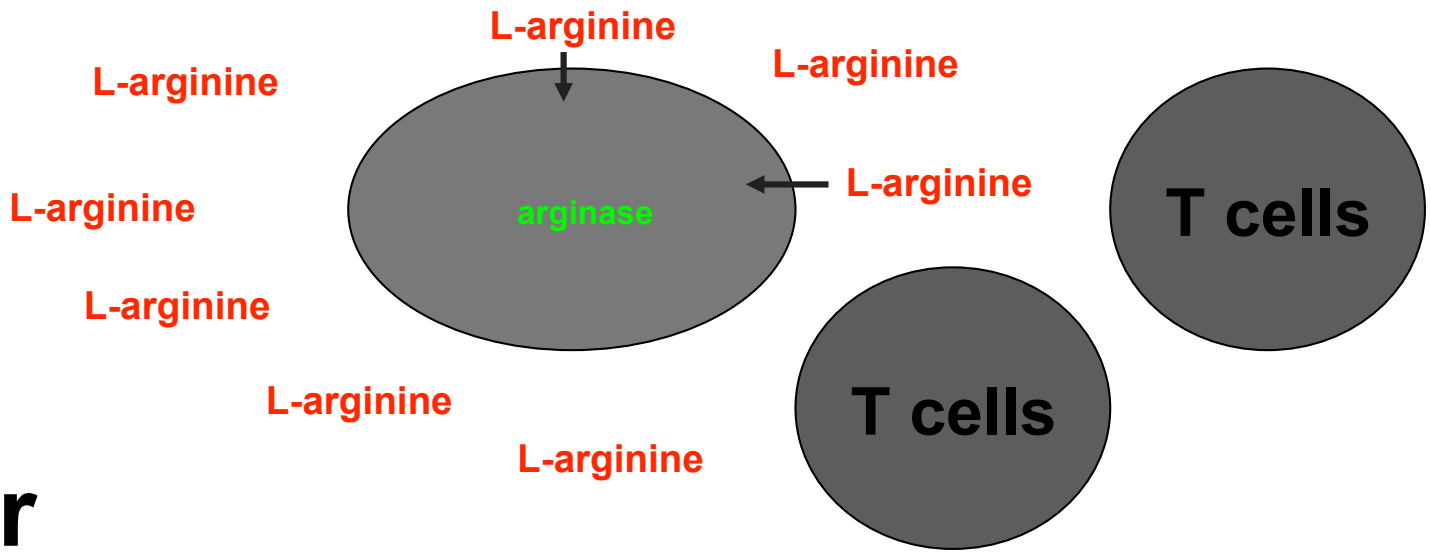
nonhealer

healer

Catabolism of L-arginine in the lesions of healer and nonhealer mice



High arginase activity at the local site of pathology induces depletion of L-arginine in the microenvironment



healer

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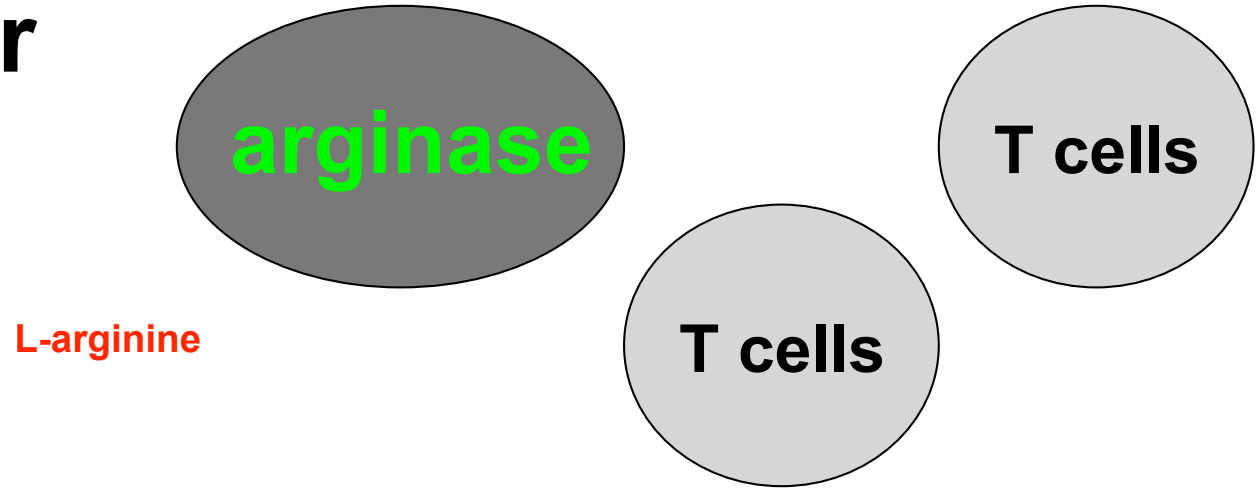
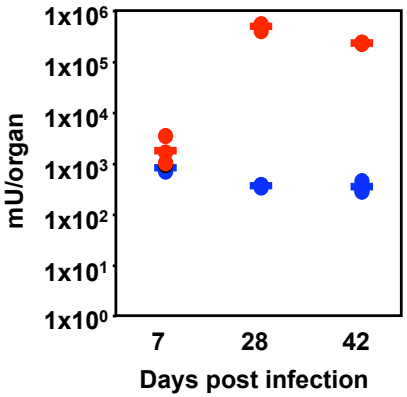
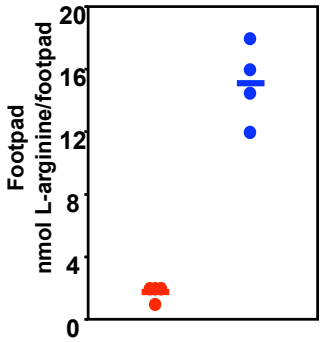
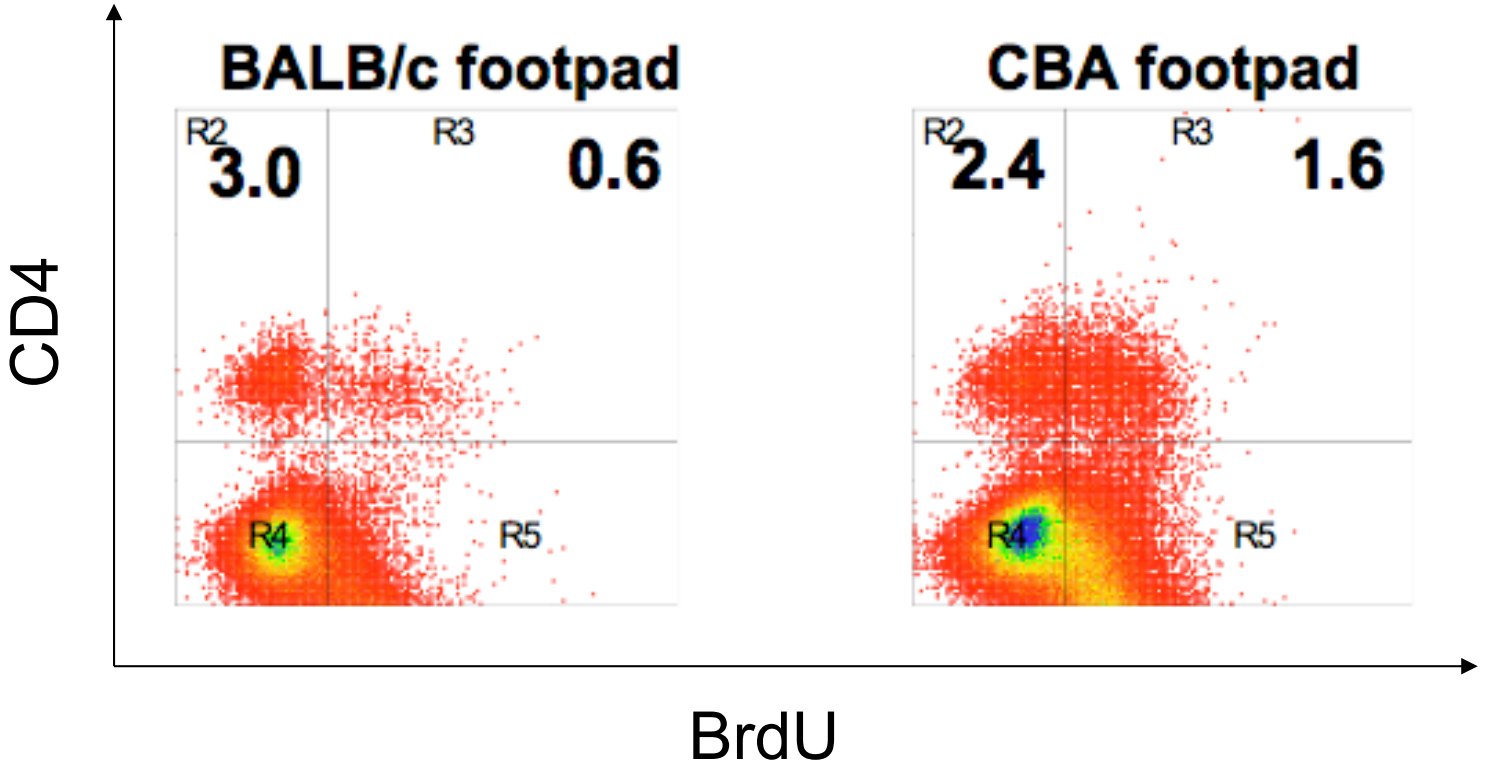
nonhealer



In vivo proliferation of CD4⁺ T cells isolated from the lesions

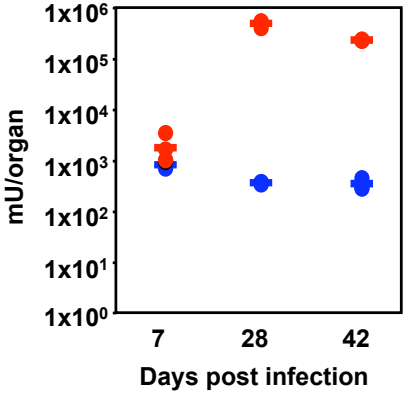


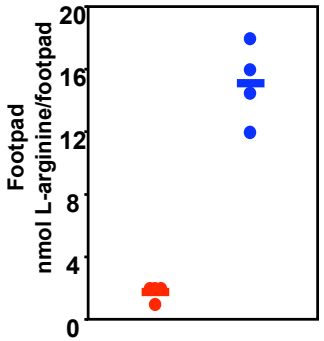


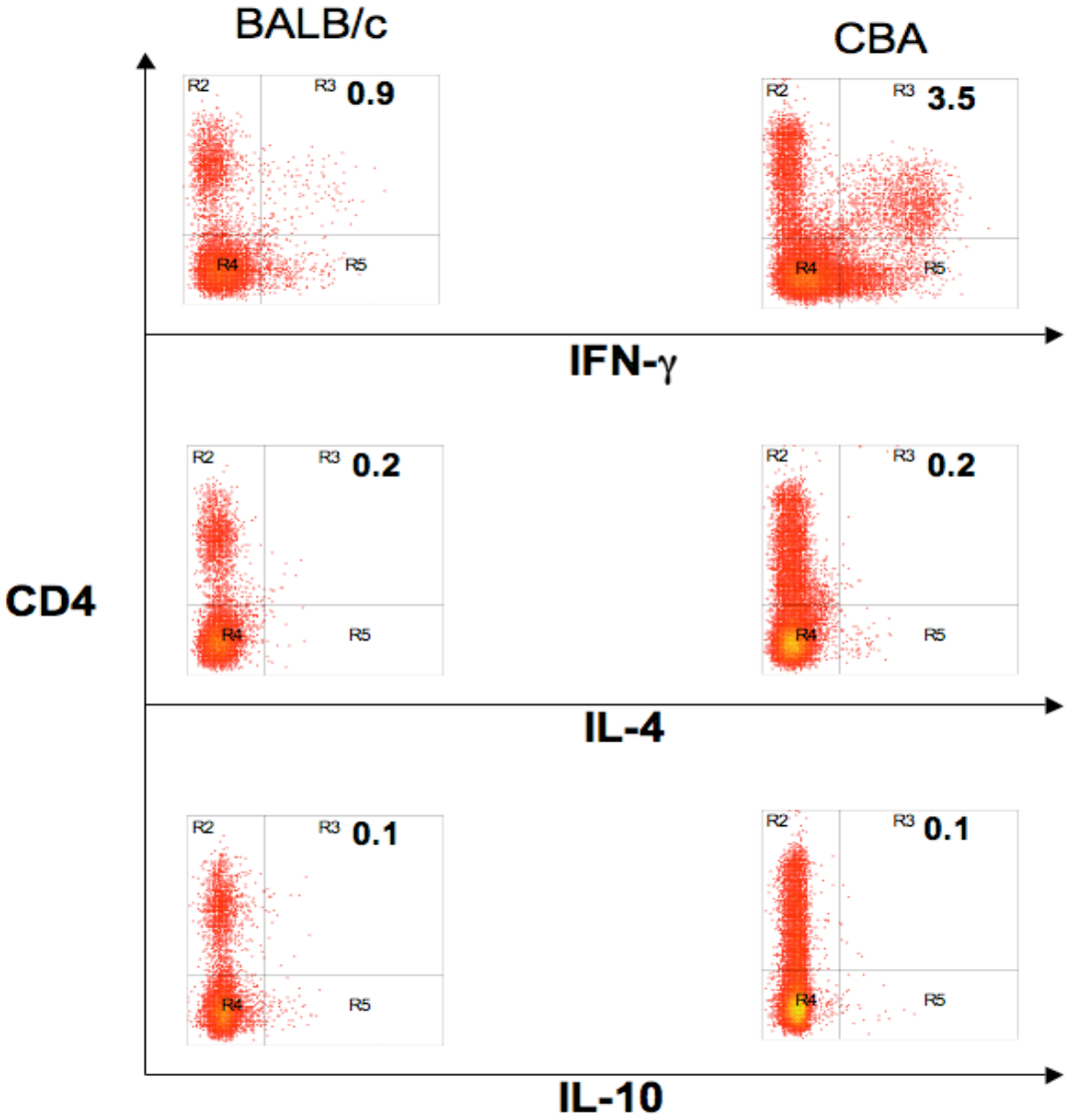


=> impaired proliferation of CD4⁺ T cells at the site of pathology

Cytokine production by CD4+ T cells isolated from the lesions







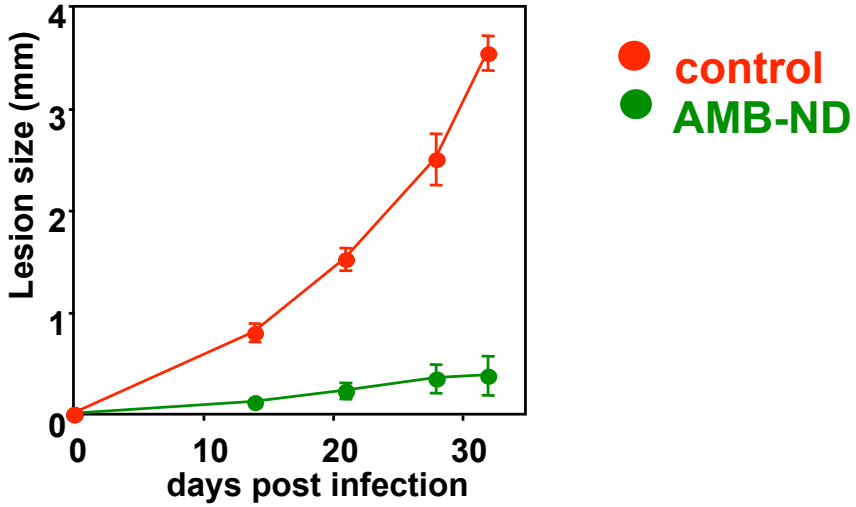
=> impaired IFN-γ production by CD4+ T cells at the site of pathology

Nonhealing leishmaniasis

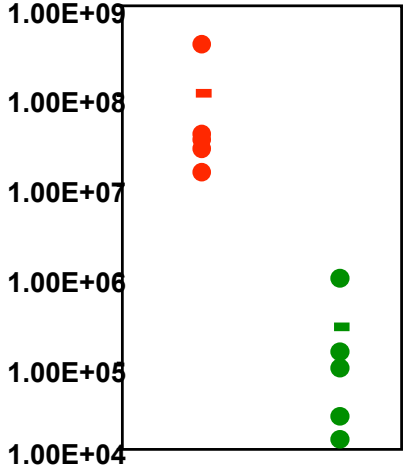
- **High arginase activity at the site of pathology locally depletes L-arginine from the microenvironment**

- **Depletion of L-arginine at the site of pathology coincide with impaired effector functions of antigen-specific $CD4^+$ T cells *in vivo***

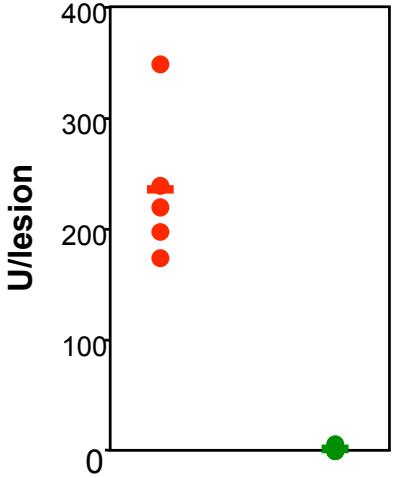
Course of lesion development in BALB/c mice treated with apolipoprotein harboring amphotericin B (AMB-ND)



Parasite load

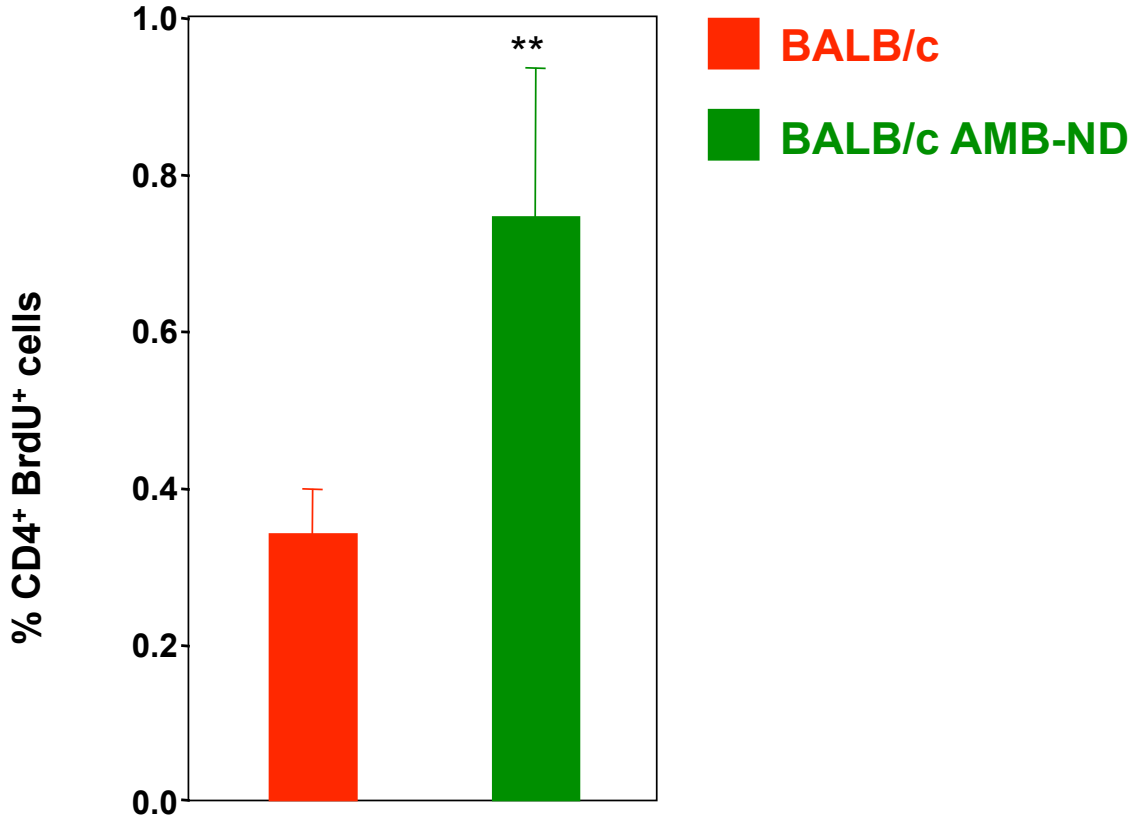


Arginase activity



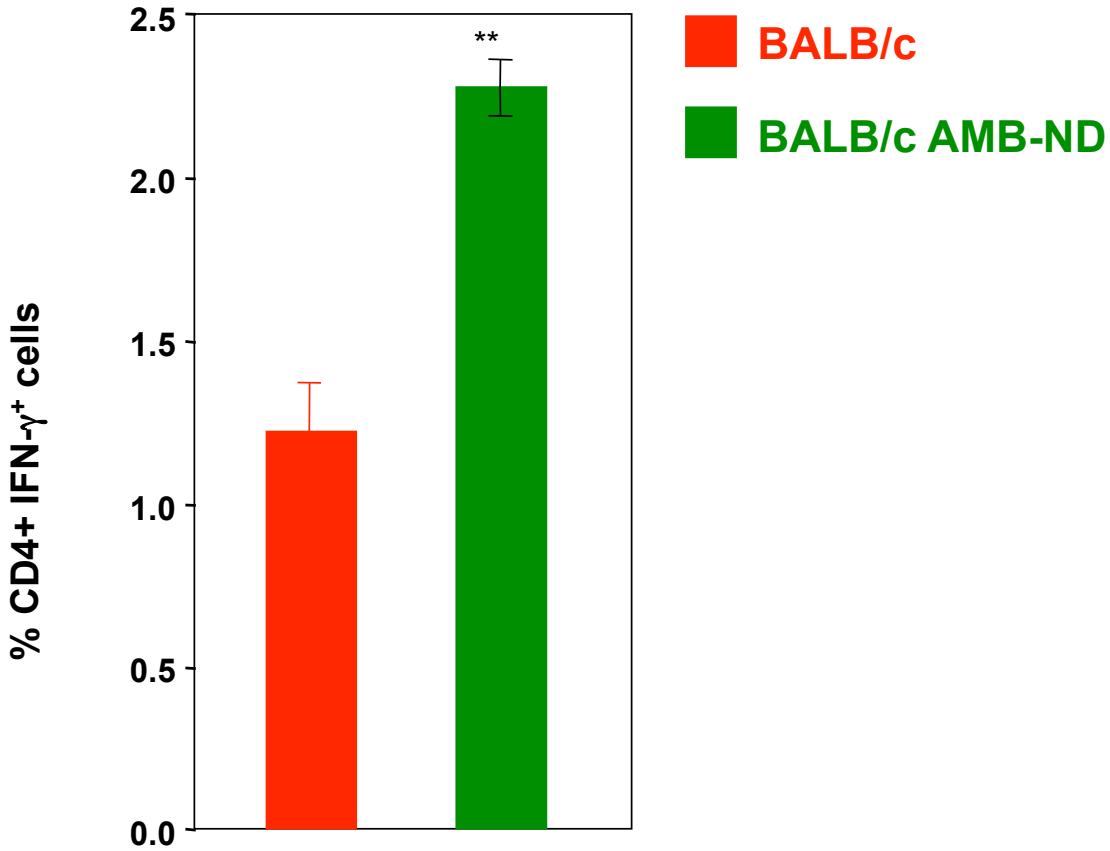
Healing of BALB/c mice is accompanied by
- reduced parasite load
- reduced arginase activity

Site of pathology



The proliferation of $CD4^+$ T cells is significantly improved in BALB/c mice treated with AMB-ND

Site of pathology

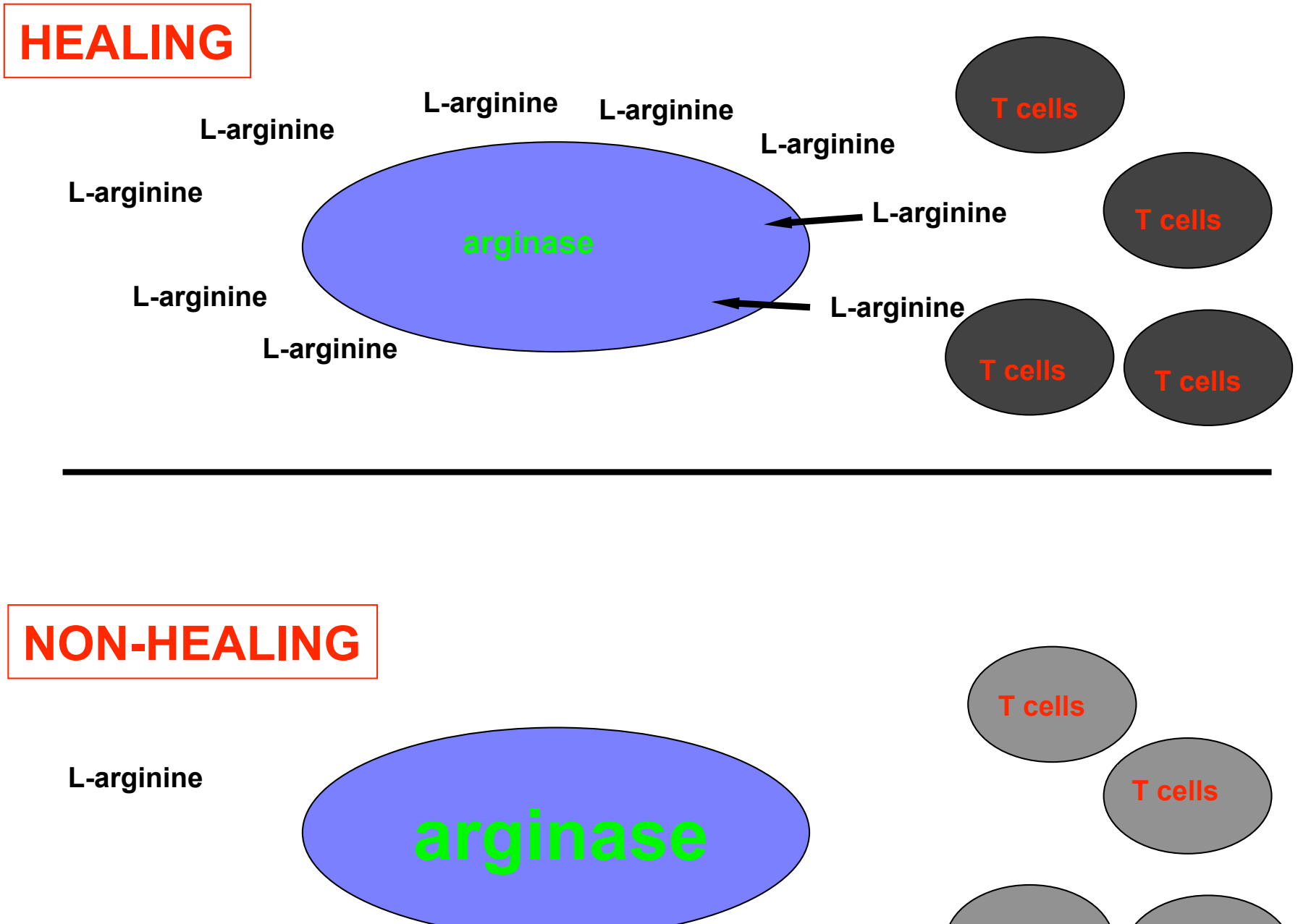


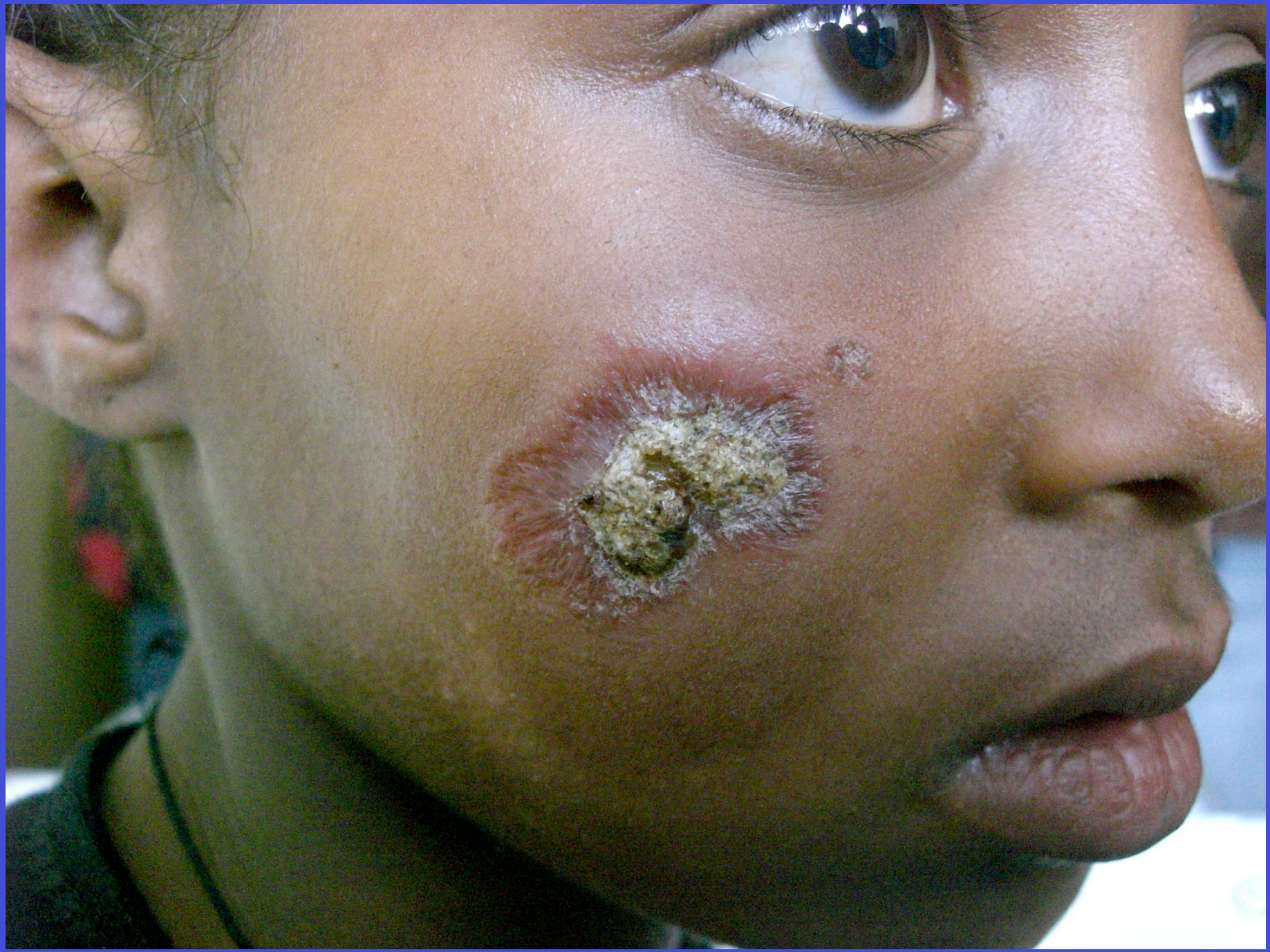
The capacity of CD4 + T cells to produce IFN-γ is significantly improved in BALB/c mice treated with AMB-ND

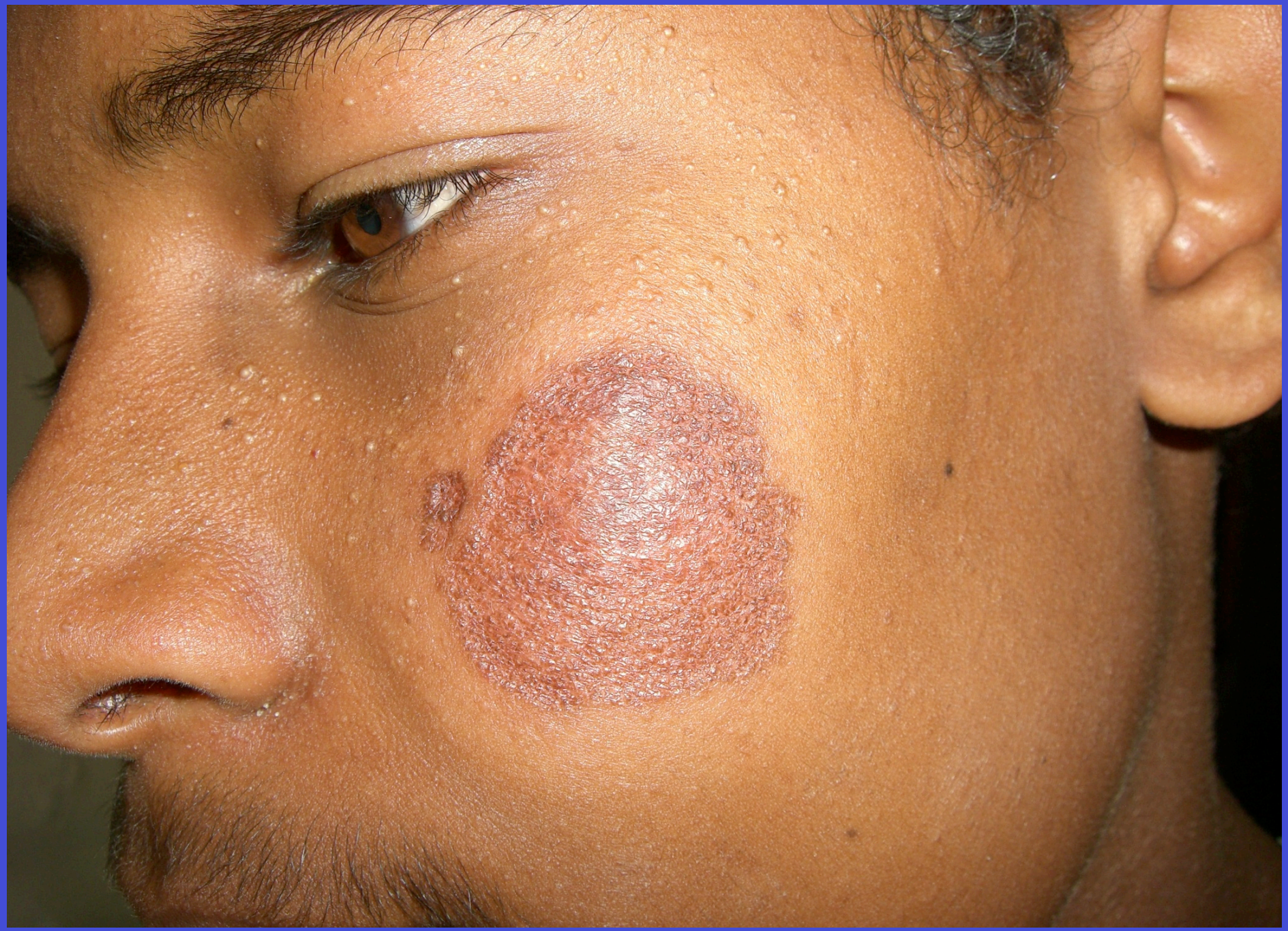
Cure of nonhealing leishmaniasis coincides with:

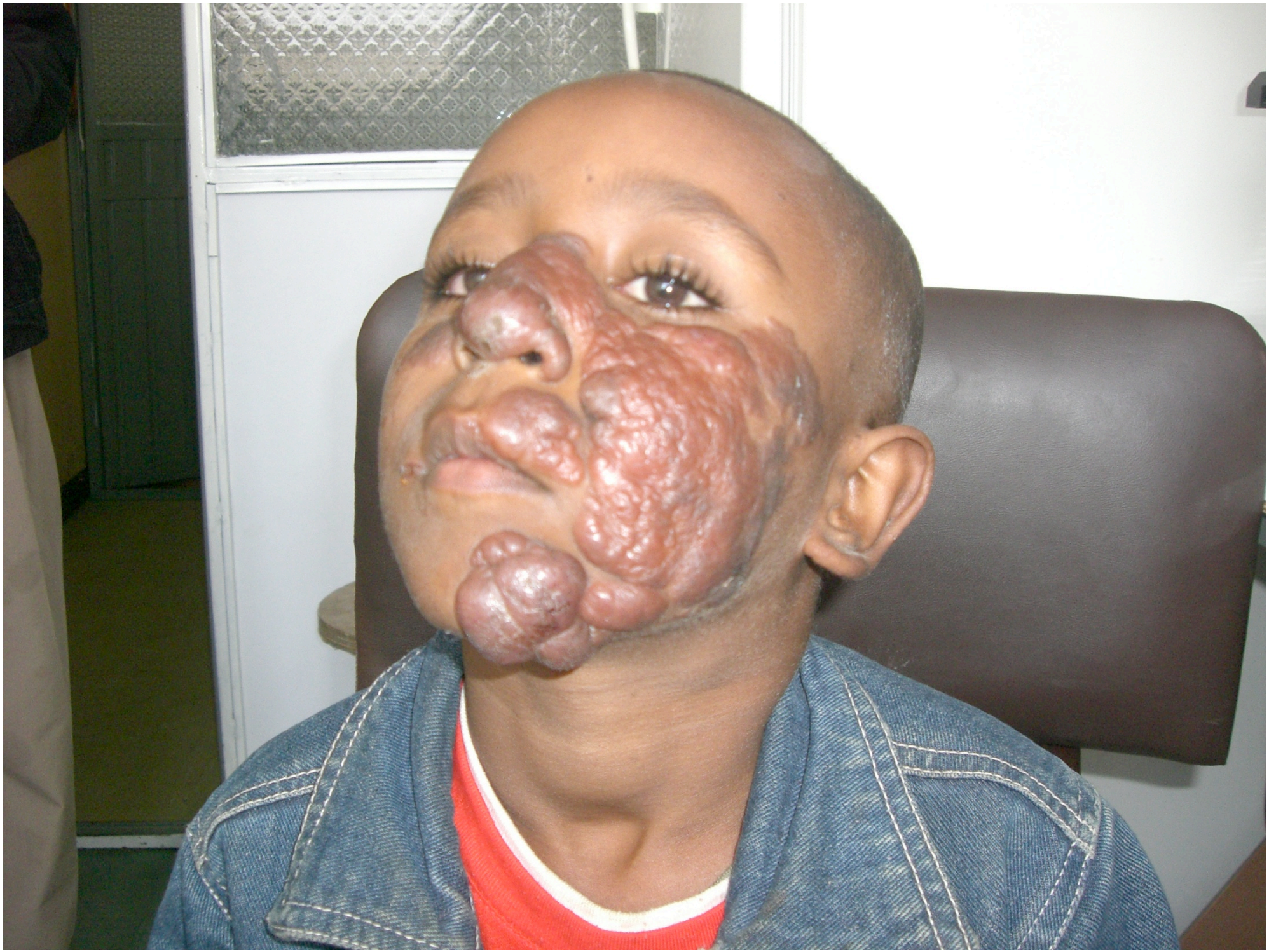
- **a downregulation of arginase activity**

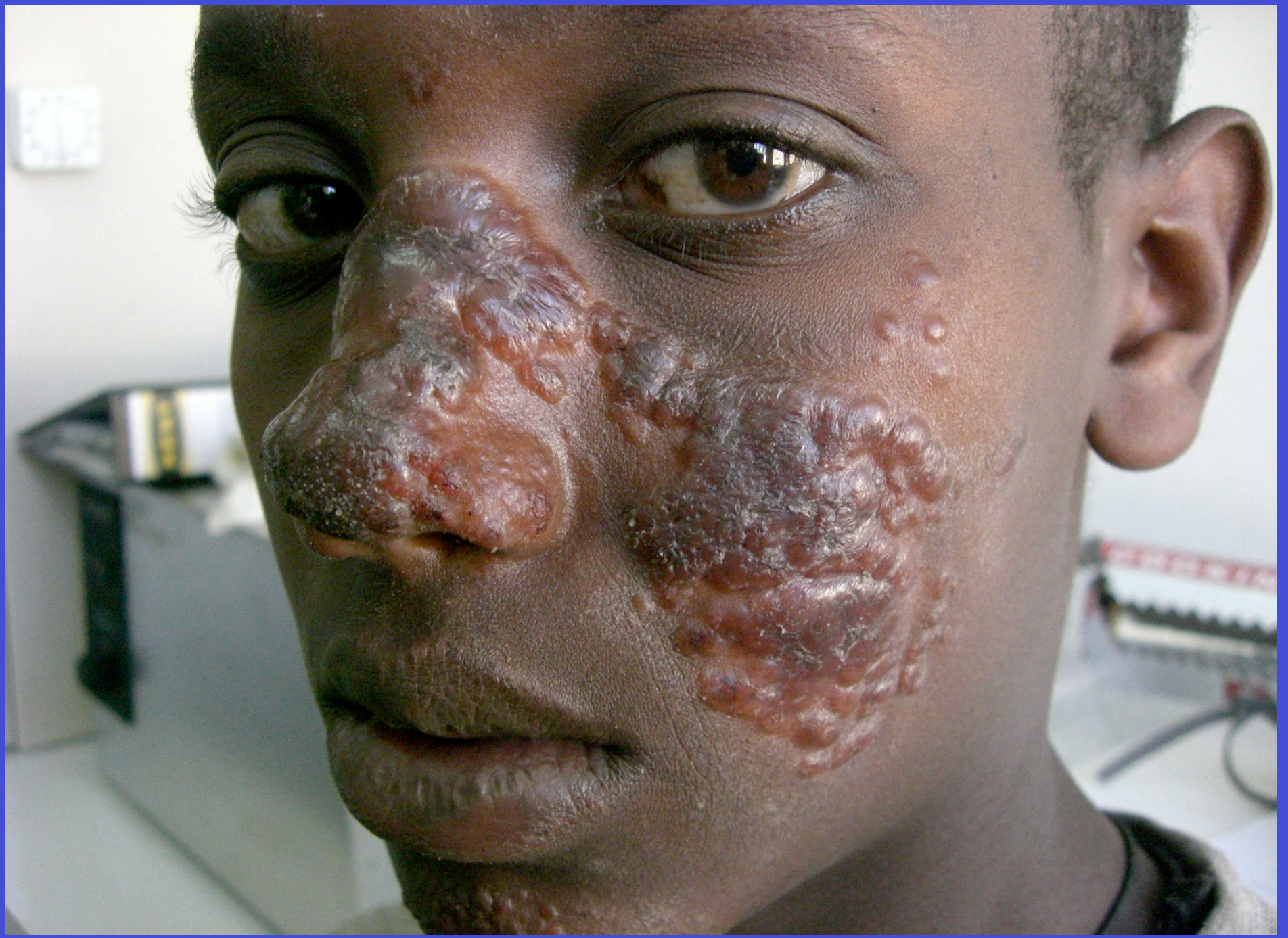
- **the restoration of $CD4^+$ T cells effector functions**

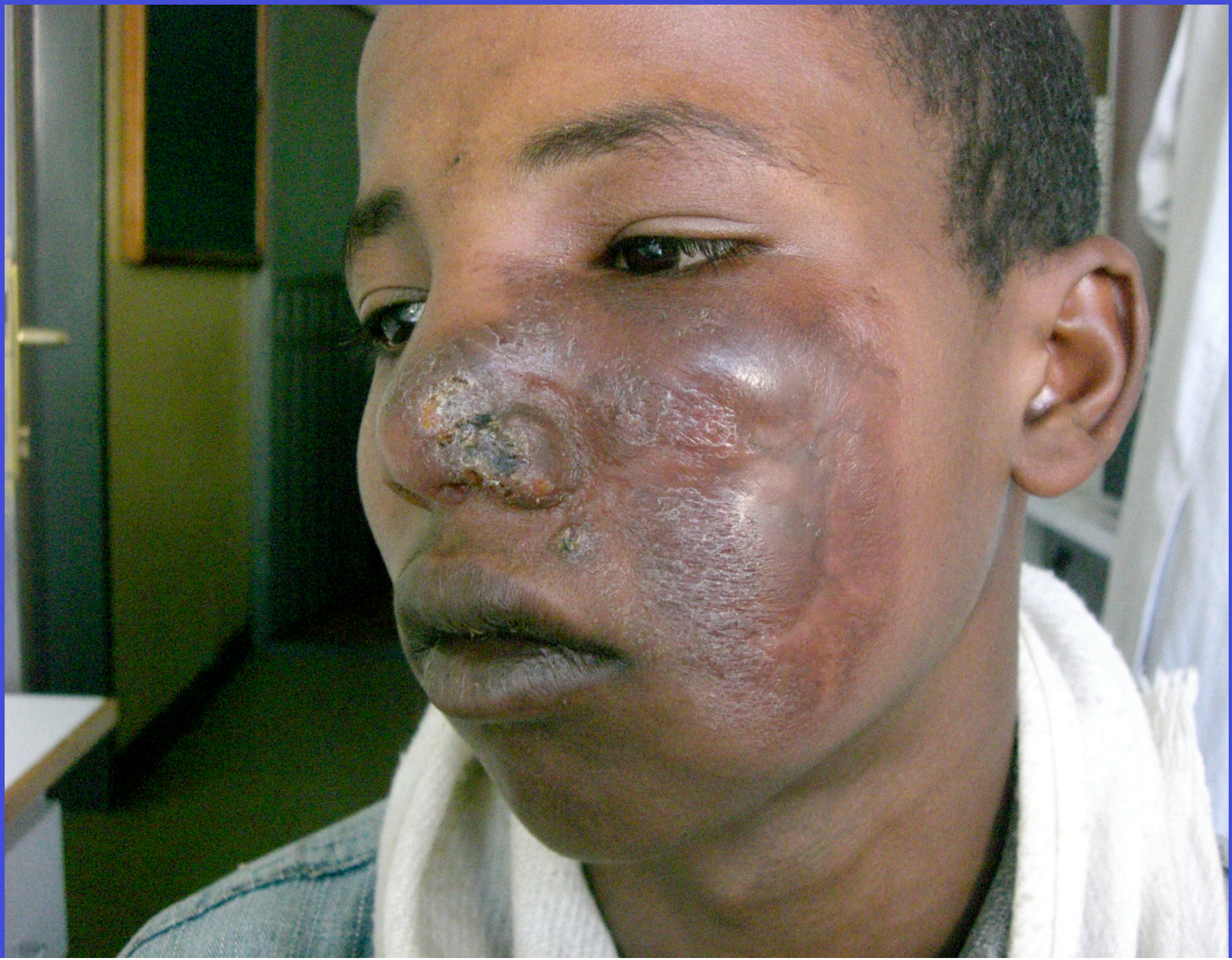

HEALING
L-arginine
L-arginine
L-arginine
L-arginine
L-arginine
L-arginine
L-arginine
L-arginine
arginase
T cells
T cells
T cells
T cells
NON-HEALING
L-arginine
arginase
T cells
T cells
T cells
T cells

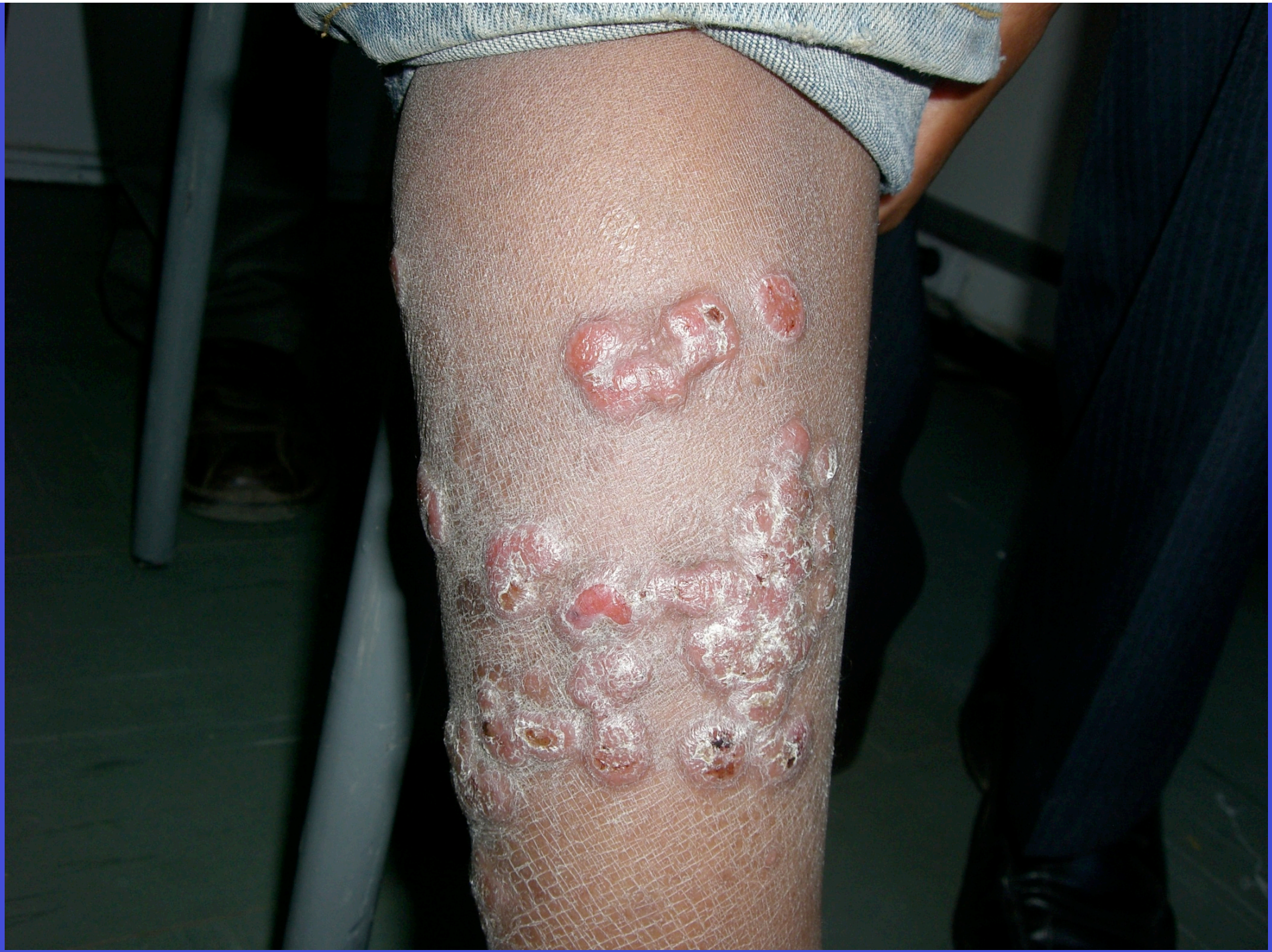


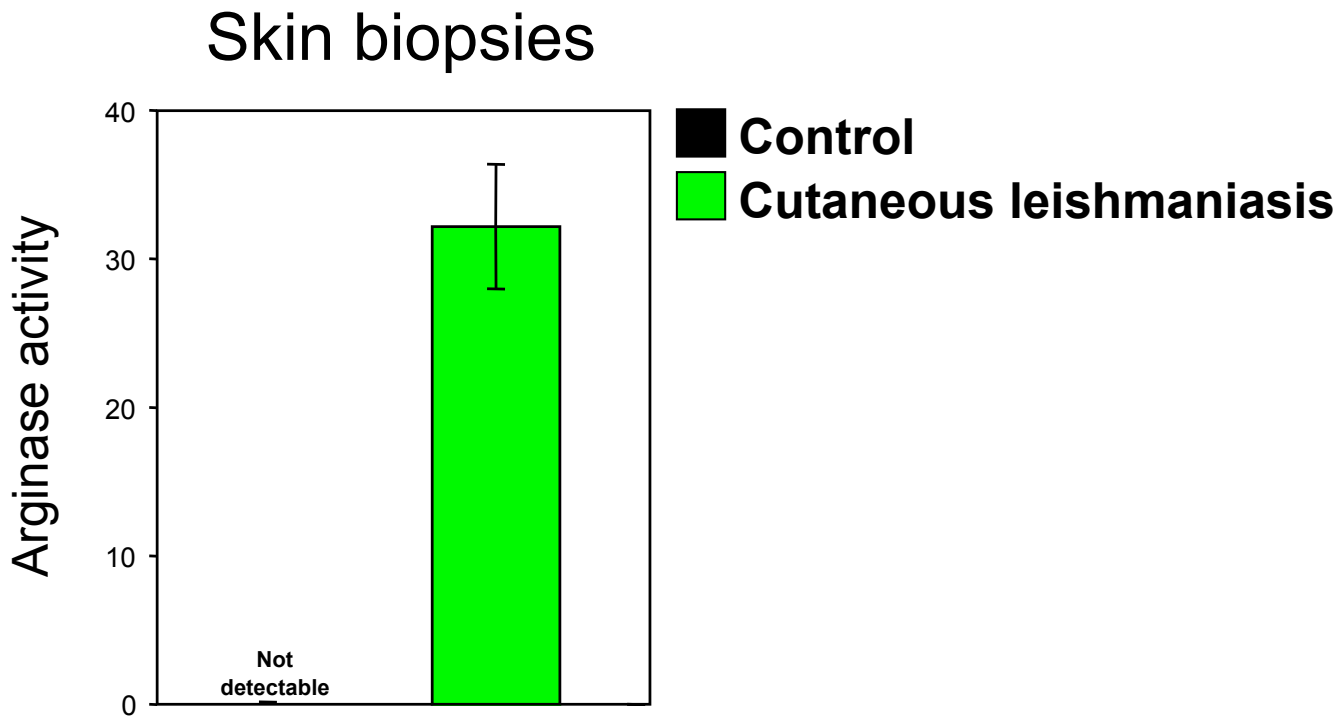




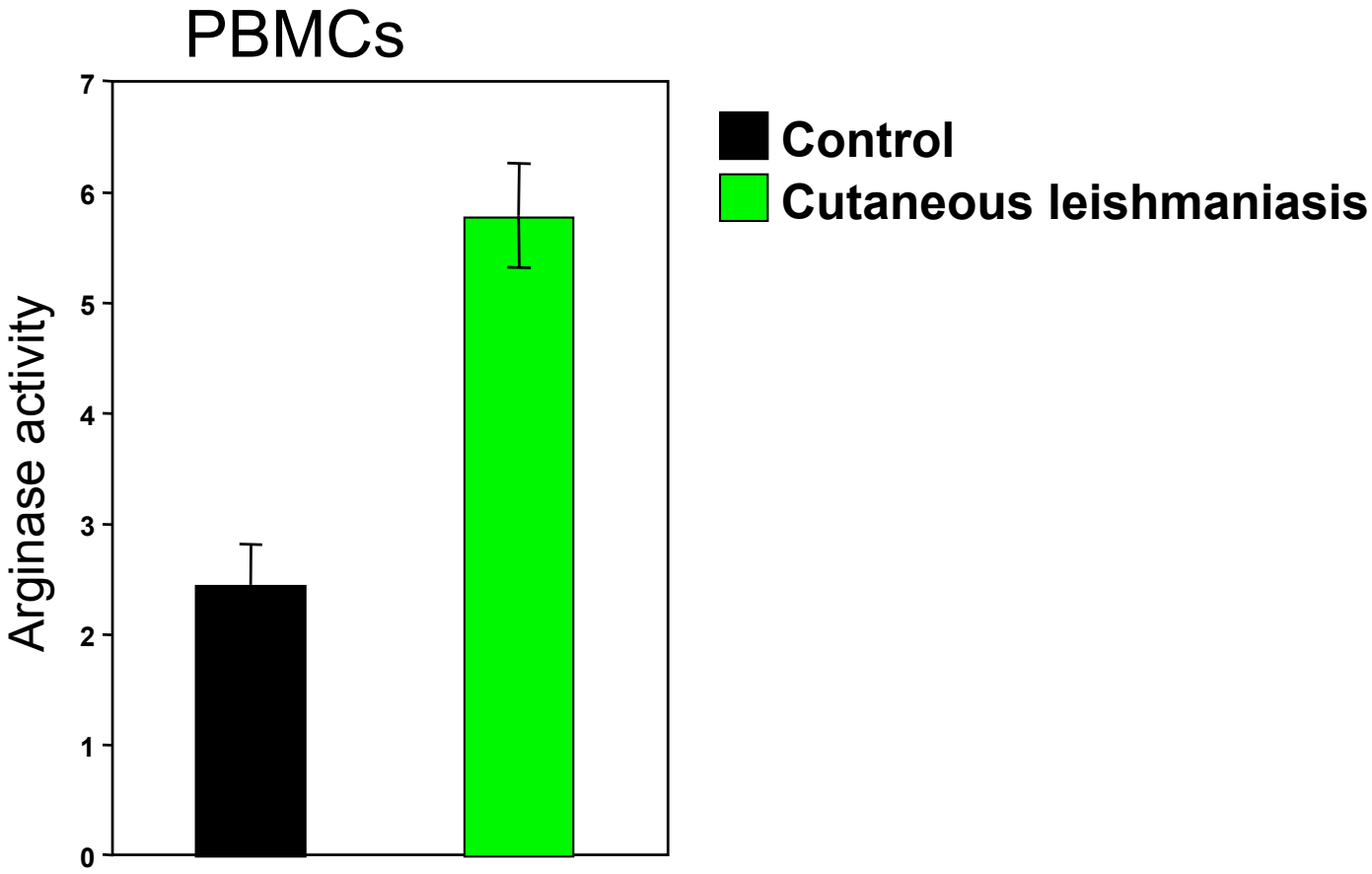




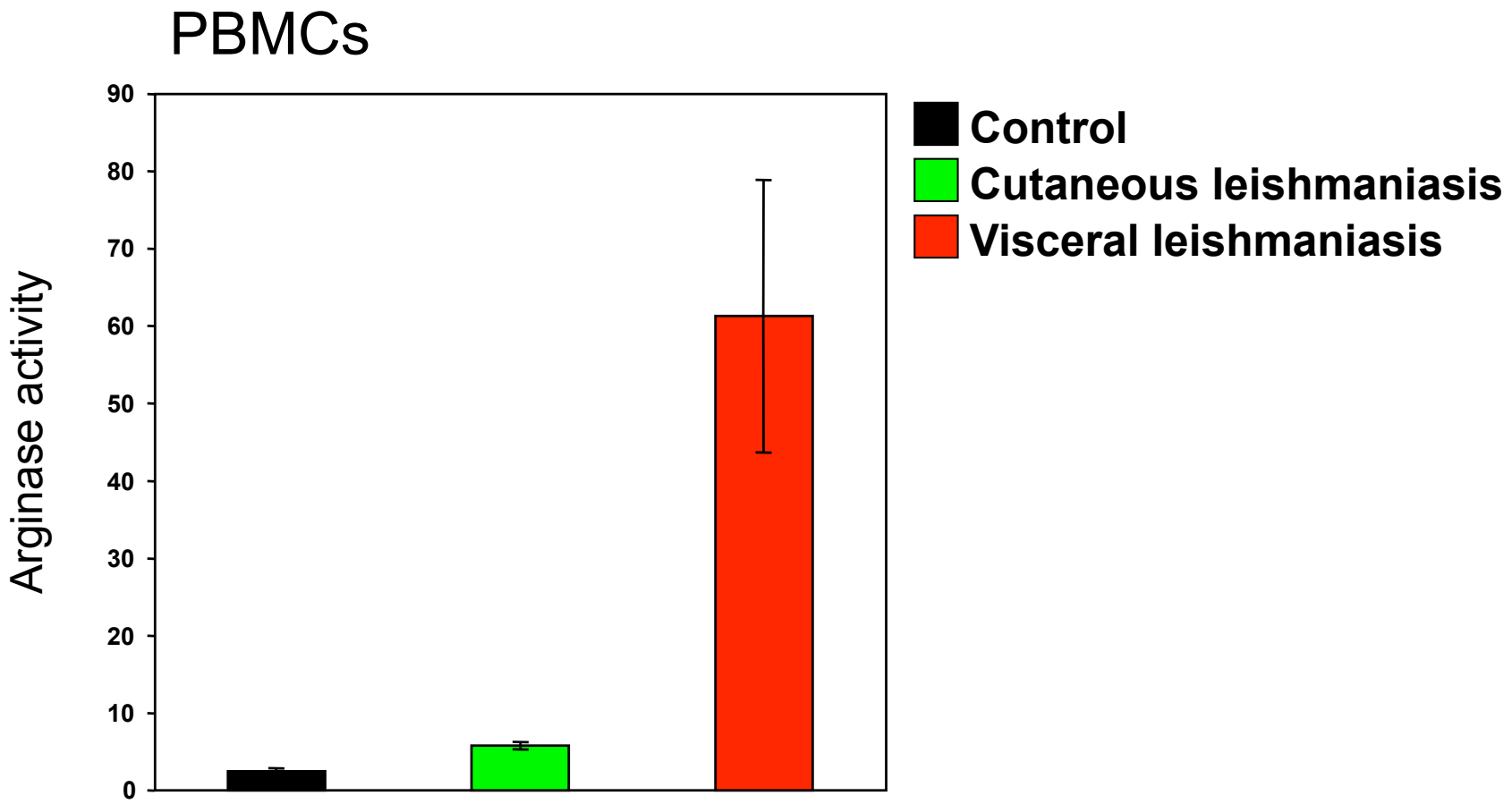




High levels of arginase are expressed in the lesions of cutaneous leishmaniasis patients



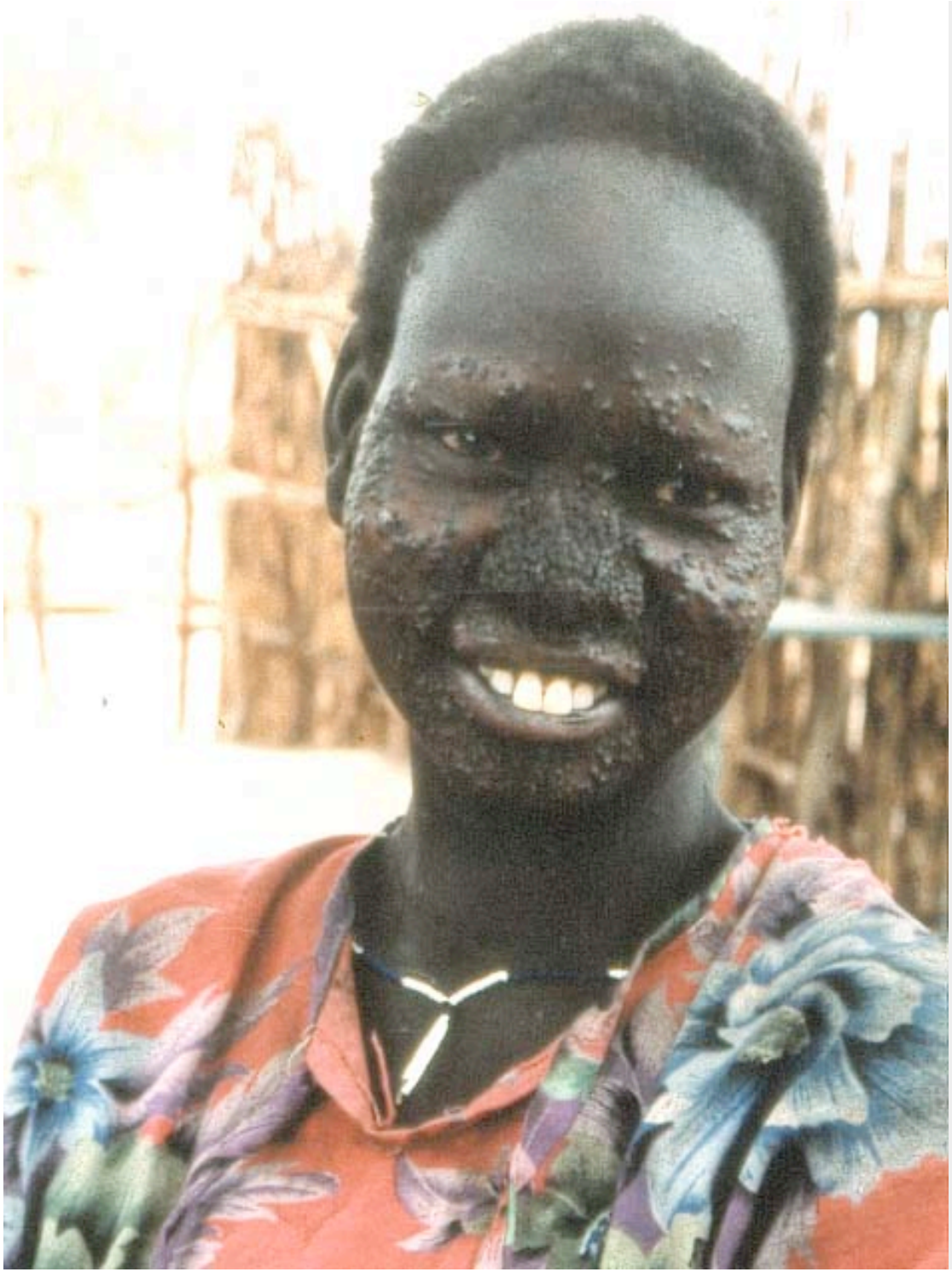
Higher levels of arginase in the PBMCs from cutaneous leishmaniasis patients



High levels of arginase are expressed in the PBMCs from visceral leishmaniasis patients

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.



Grade 2 PKDL

Polymorphisms at IFNGR1 linked to PKDL:

failure to respond to IFN-γ in post-treatment phase could lead to parasite growth in remote skin sites

Leishmania (VL)/HIV coinfection

- an “emerging disease”
- Southern Europe, Africa, India, Brazil
- 20-75% of adult VL cases are related to HIV infection
- 1.5 - 9.5% of AIDS cases suffer from new or reactivated VL