

# **Effective immunological mechanisms of resistance against *Leishmania***

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# Correlates of immune protection

- A fundamental question for vaccine development is to define immunological mechanisms of resistance against infection.
- This will give valuable information for the particular type of immune response, which may be cytotoxic T cells (CTL) response, antibody responses, or particular class of T helper (Th) responses or antibody isotypes.
- This is of vital importance since effective protection against different pathogens requires distinct types of immune responses.
- Understanding the immunological mechanisms that mediate vaccine efficacy will give valuable information for the **design** of candidate vaccines and their **evaluation**.

# For a given pathology (infection), we have to distinguish between:

- The types (mechanisms) of immune responses during infection (chronic)
- The mechanisms of immune control during infection (those involved in the healing process)
- The eventual mechanisms of vaccine-induced protective immunity

**Theses mechanisms could be completely different**

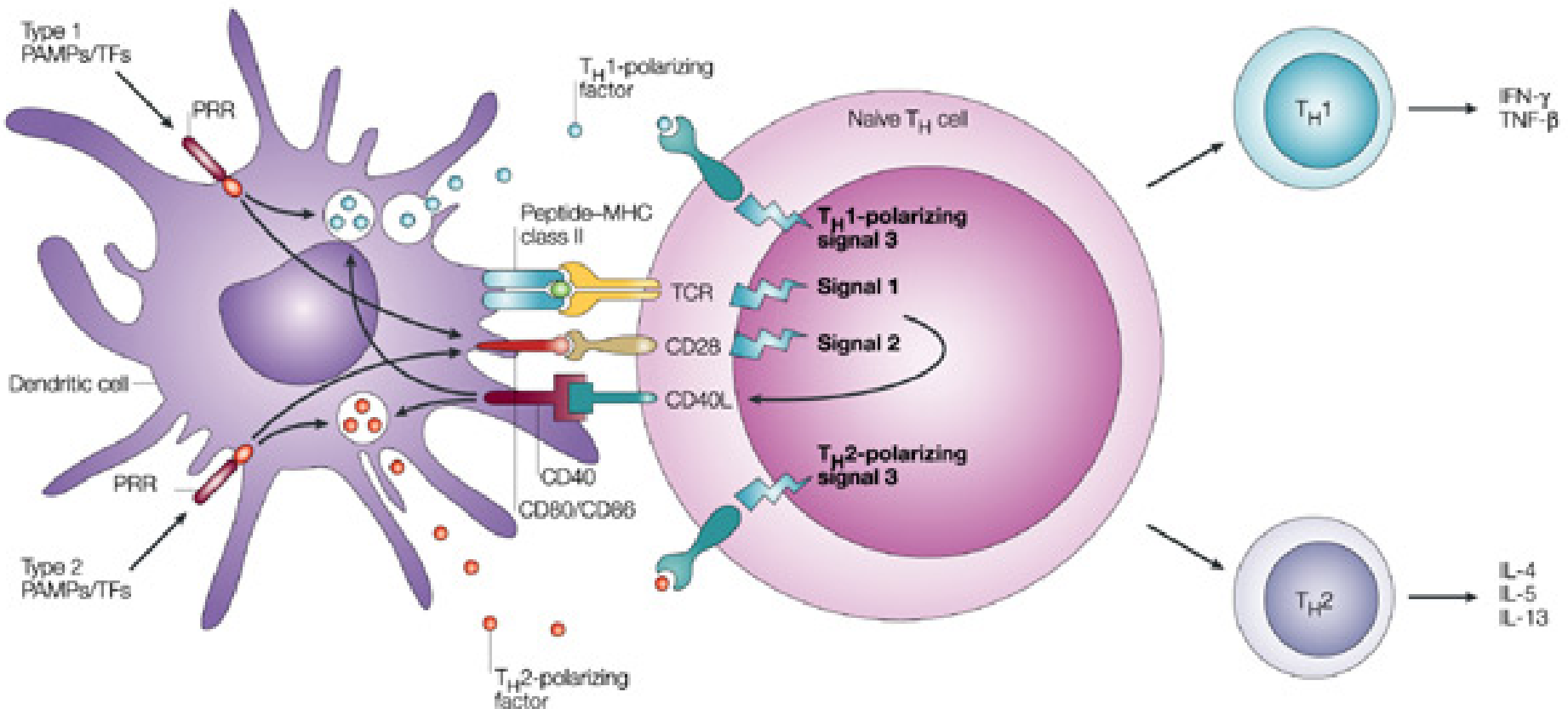
# Anti-*Leishmania* responses

*The whole mechanisms of the innate and adaptive immune responses developed during the human infection with Leishmania parasites*

## Role

- In the **pathogenesis** (visceral and cutaneous leishmaniasis)
- In the **elimination** and/or the **control** of the parasite multiplication
- In the **resistance** to re infection (crucial for vaccine development)

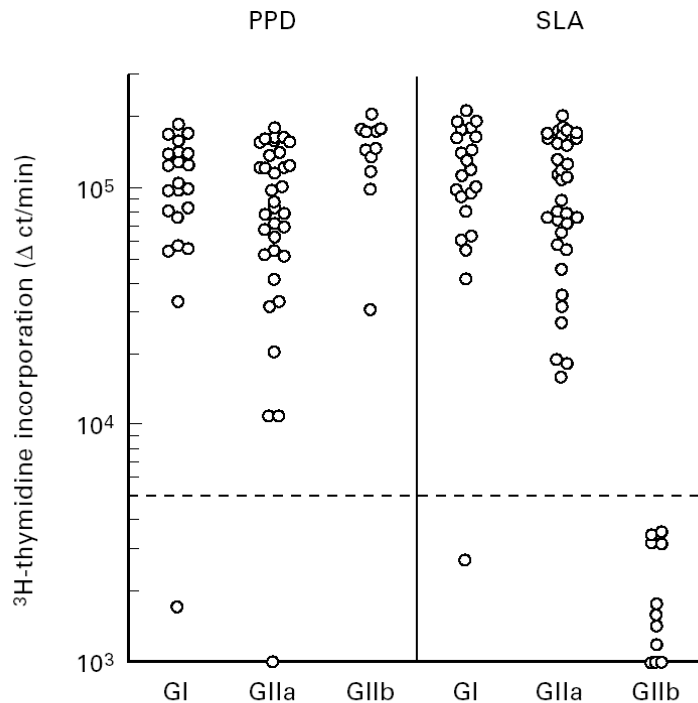
# T cell functional polarization: obnubilation by the murine model



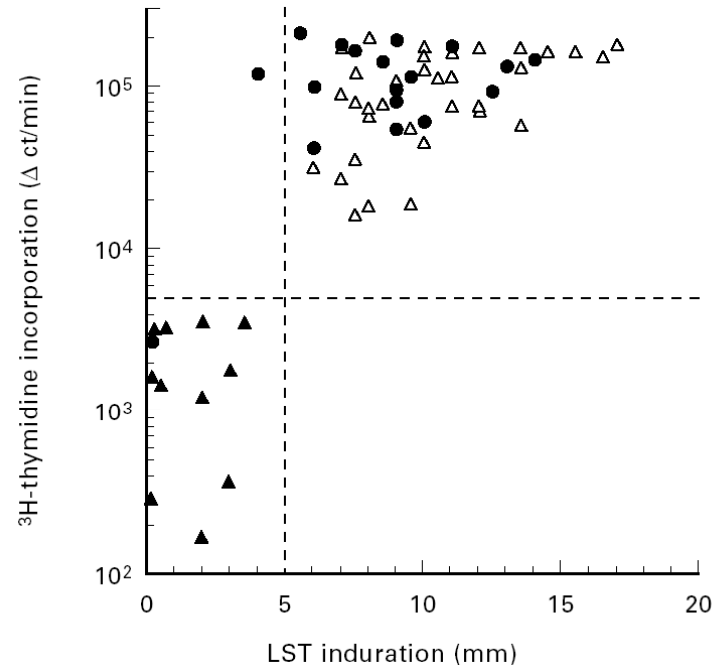
# Leishmanin Skin Test as correlate for protection?

- This test measures the parasite-specific delayed-type hypersensitivity reaction.
- It is commonly employed in epidemiological studies for the detection of current or prior *Leishmania* infection.
- LST reactivity classically reflects a CD4<sup>+</sup> Th1 cell-mediated immune response against the parasite.
- The LST reactivity is classically associated with resistance to *Leishmania* parasite.

# Concordance LST reactivity/SLA-specific lymphoproliferative response/SLA-specific IFN- $\gamma$ production



**Fig. 1.** *In vitro* lymphoproliferative responses to purified protein derivative (PPD) or soluble *Leishmania major* antigens (SLA) in individuals with healed localized cutaneous leishmaniasis (LCL) (group I) and from leishmanin skin test (LST)<sup>+</sup> (group IIa) and LST<sup>-</sup> (group IIb) individuals without a history of LCL. Each point represents an individual proliferative response expressed as  $\Delta$ ct/min (mean count of antigen-stimulated triplicate culture – mean count of control triplicate culture).



**Fig. 2.** Correlation between leishmanin skin test (LST) expressed as diameter of induration (mm) and lymphocyte proliferation in response to soluble *Leishmania major* antigens (SLA) ( $\Delta$ ct/min). Groups are defined as in Fig. 1. ●, Group I;  $\Delta$ , group IIa;  $\blacktriangle$ , group IIb.

diameter of skin induration and the  $\Delta$ ct/min of SLA-stimulated cultures (Spearman rank correlation coefficient  $r=0.6$ ,  $P<$

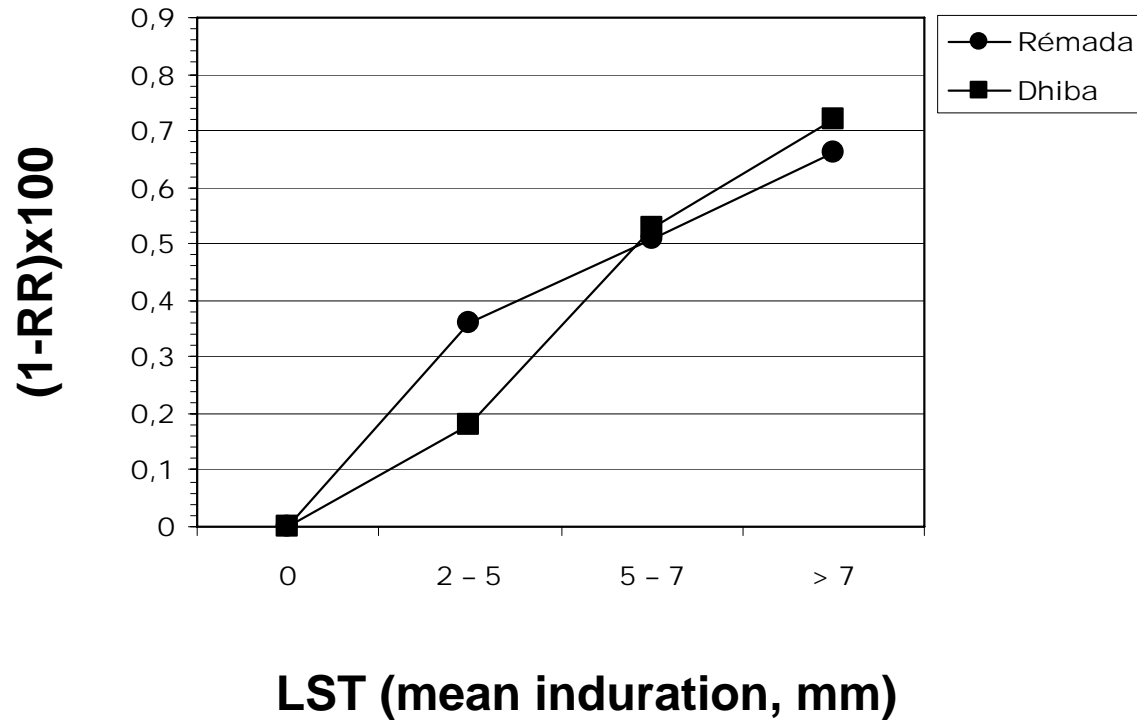
**Table 2. Relative risk (RR) and preventive fraction (PF) of zoonotic cutaneous leishmaniasis lesions according to intensity of leishmanin skin test (LST) reaction.**

LST reaction size, mm	Remada				Dhiba			
	Participants with ZCL ( <i>n</i> = 155)	Participants without ZCL ( <i>n</i> = 117)	RR (95% CI)	PF, %	Participants with ZCL ( <i>n</i> = 25)	Participants without ZCL ( <i>n</i> = 157)	RR (95% CI)	PF, %
0	102	31	Reference	...	18	70	Reference	...
0–5	26	27	0.64 (0.48–0.85)	36	1	3	0.82 (0.14–4.69)	18
5–7	15	25	0.49 (0.32–0.74)	51	2	19	0.47 (0.12–1.85)	53
>7	12	34	0.34 (0.21–0.56)	66	4	65	0.28 (0.10–0.80)	72

**NOTE.** CI, confidence interval; PF = 1 – RR.



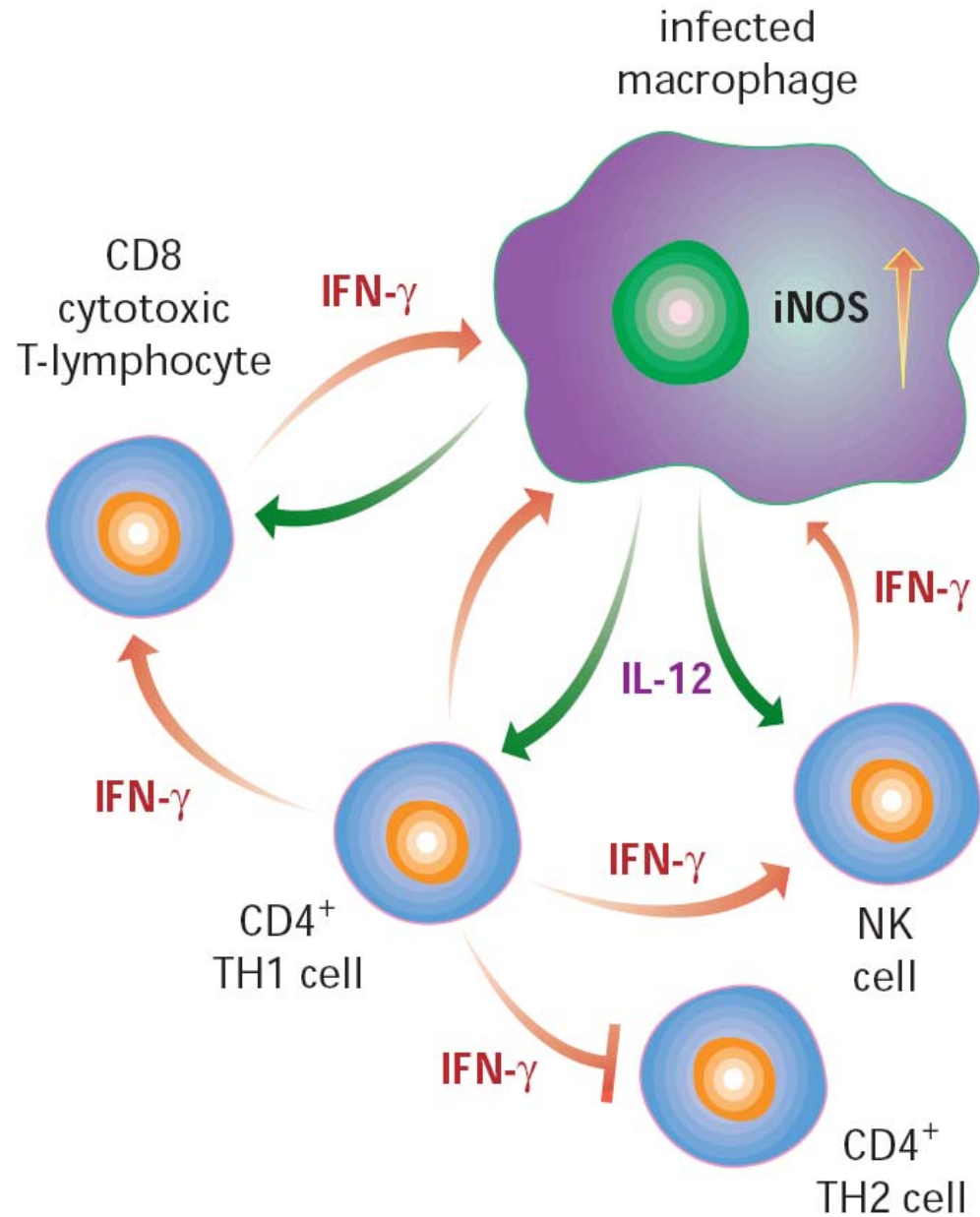
# Protection fraction of LST reactivity



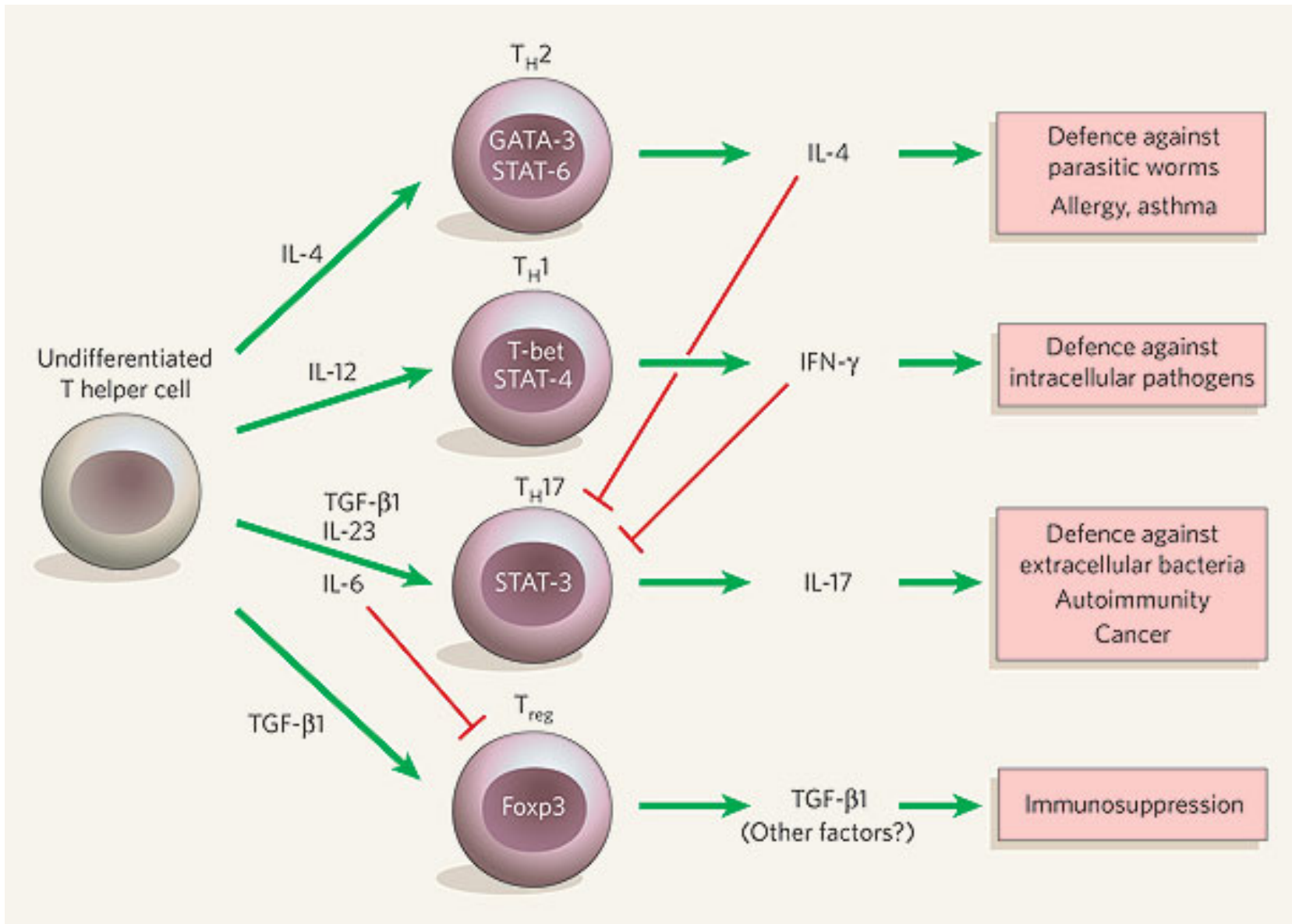
# Based on this correlate of protection

- With the support of UNDP/WHO/TDR, several vaccines (dead parasites +/- BCG) has been evaluated for their immunogenicity and efficacy in:
  - Latin America : [ Castes, 1994; Armijos, 1998; De Luca, 1999; Velez, 2000; De Luca, 2001; Follador, 2002; Armijos, 2003; De Luca, 2003; Velez, 2005]
  - Sudan: [ Khalil, 2000; Satti, 2001; Kamil, 2003]
  - Iran: [ Sharifi, 1998; Momeni, 1999; Khalil, 2000; Mahmoodi, 2003]
- In all these studies, indicators of Th1 response (**LST reactivity and/or PBMC proliferation and/or IFN- $\gamma$  production**) have been used for the selection of the naïve individuals and as a correlate of protection.
- Although tested vaccines were safe and immunogenic (i.e. in terms of LST conversion and/or increase of specific-IFN-g production by PBMC), **significant, long-lasting protection could not be demonstrated.**

# What are the other potential effectors?



# Cytokines & the T helper cell lineages



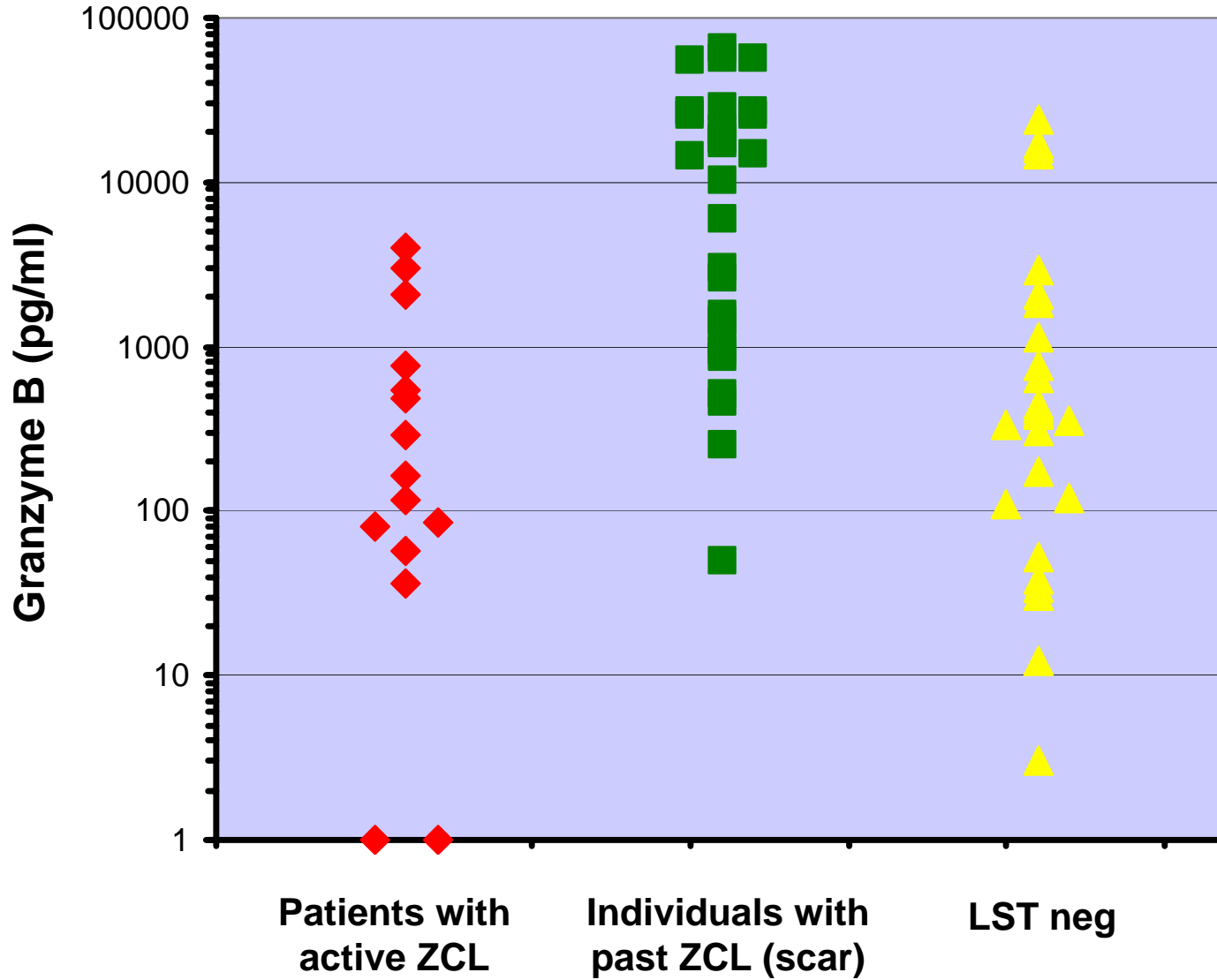
# How to study such immunological mechanism in the protection against human leishmaniasis? The difficulties emerge:

- **Limits of the experimental models (mouse) for vaccine evaluation:** *The experimental models are more predictive for the validation of vaccines when the effector mechanism requires an antibody response. For those that need a cellular (or mixed) response, things are much more complicated.*
- **Difficulties of studying of human leishmaniasis:**
  - (i) Heterogeneity of the human populations, parasitic isolates and the transmitting vectors, (ii) Impossibility of making experimental studies (with a well defined isolate by injecting a precise number of parasites), (ii) difficulties to access to the biological material of human origin*

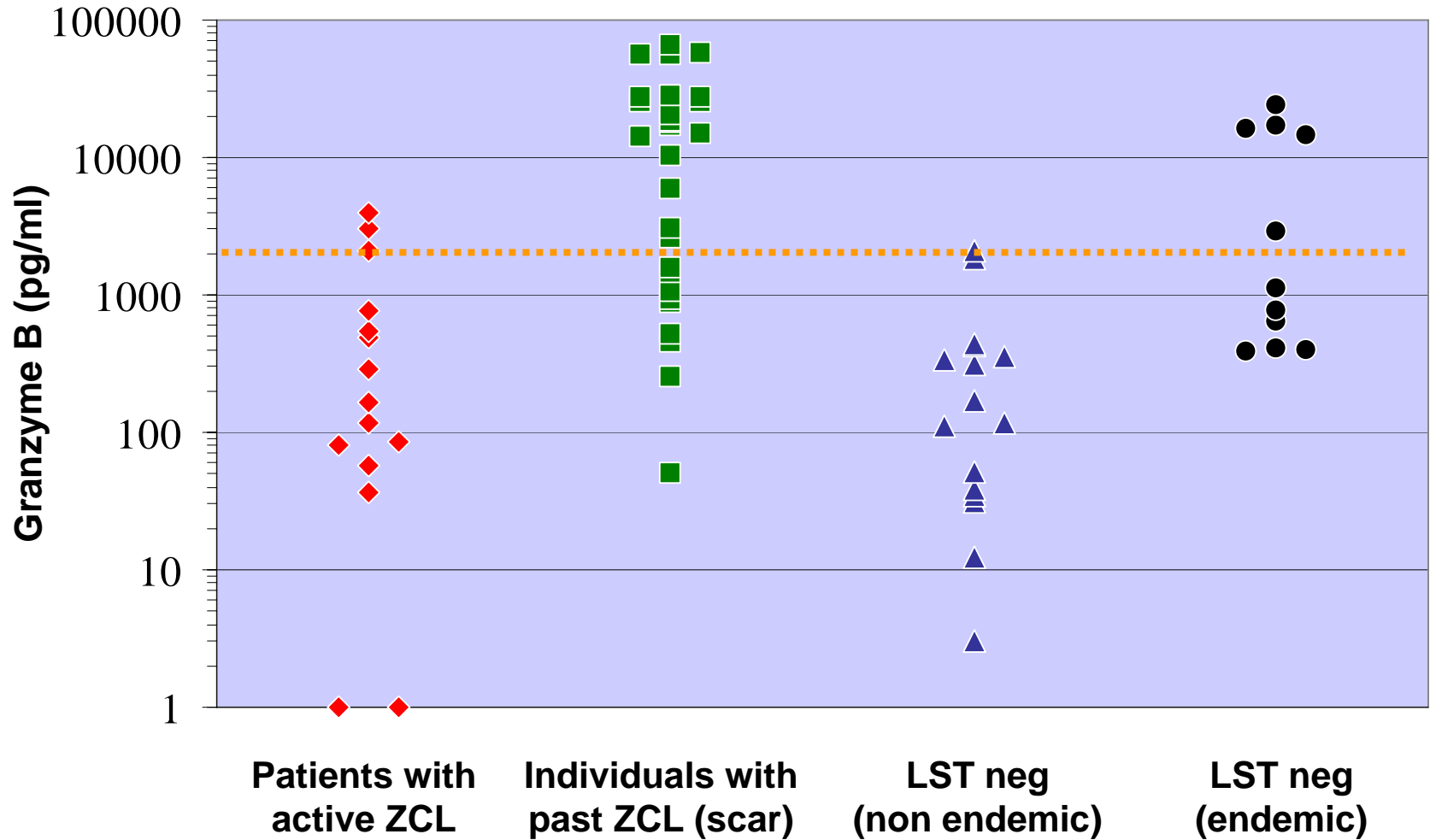
# For *Leishmania*

- The effector immune mechanisms that are associated with resistance against the parasite involves the cellular arm of the immune system.
- In nature the disease (or the simple asymptomatic infection) is "immunizing".
- The study of the naturally exposed individuals gives us the opportunity "of trying" to analyze these factors.
- That requires longitudinal follow-up studies of exposed individuals.

# Granzyme B production

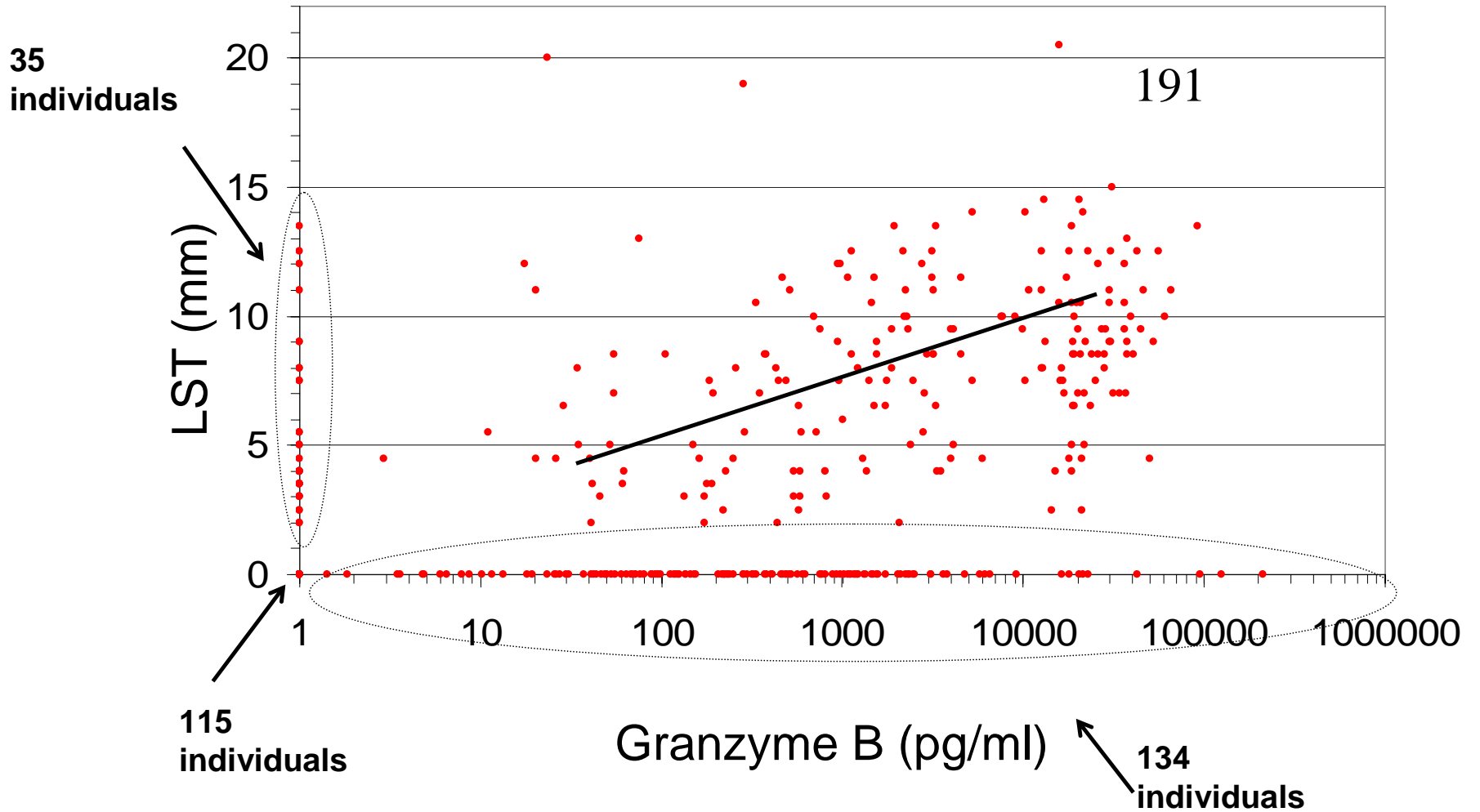


# Granzyme B production

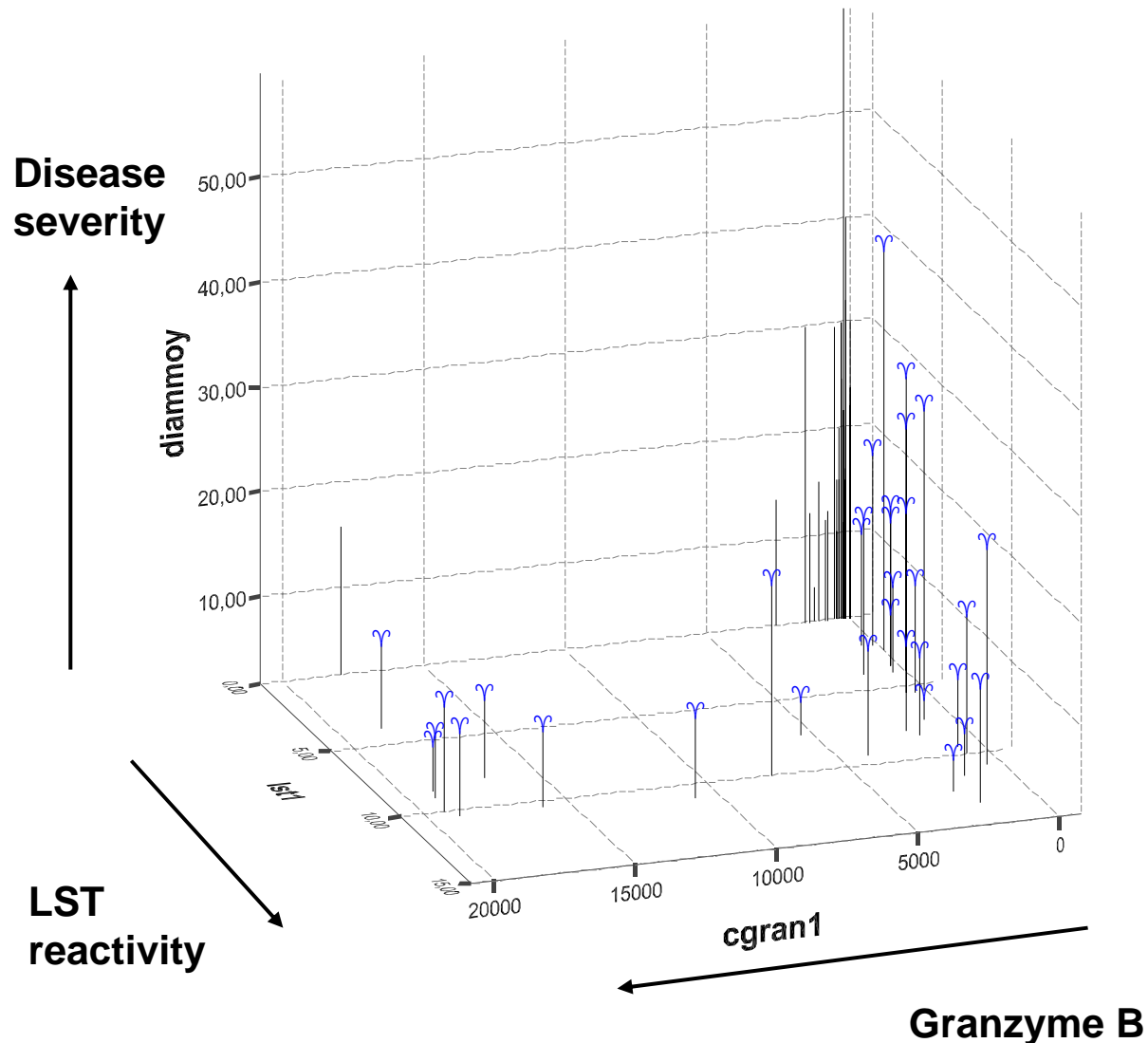




# Granzyme B/LST in individuals living in *L. major* transmission area



# ZCL disease severity according to LST reactivity and granzyme B production (at baseline)



# During the follow-up of 453 individuals (for whom we have the values of granzyme B and LST reactivity),

- 89 (out of 453) developed one or more lesions of ZCL.
- The severity of the disease was quantified by using two criteria:
  - - The lesion size (with its max, threshold: 600mm<sup>2</sup>)
  - - Total duration of the disease (threshold: 4 months)
- On the 89 patients (65 are regarded as non severe and 23 severe)
- **The presence of granzyme B (> 2000pg/ml) has a very significant protective effect against the development of the severe forms of ZCL (85% according to the size and 75% according to the duration of evolution).**



**non-immune**



**disease**



**immune**

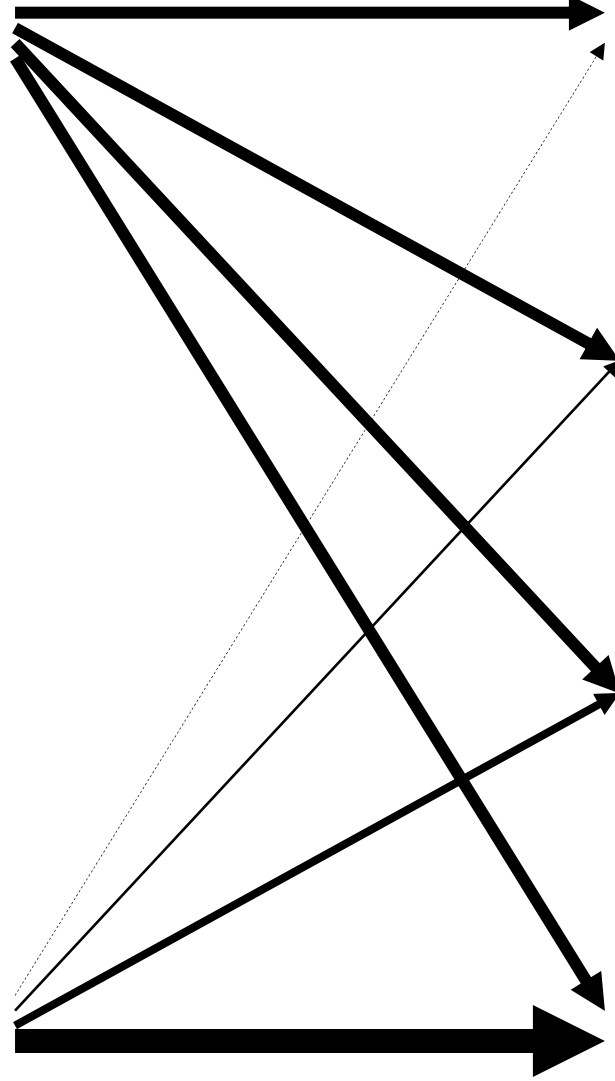


**healthy**

Increasing immunity



Low force of infection

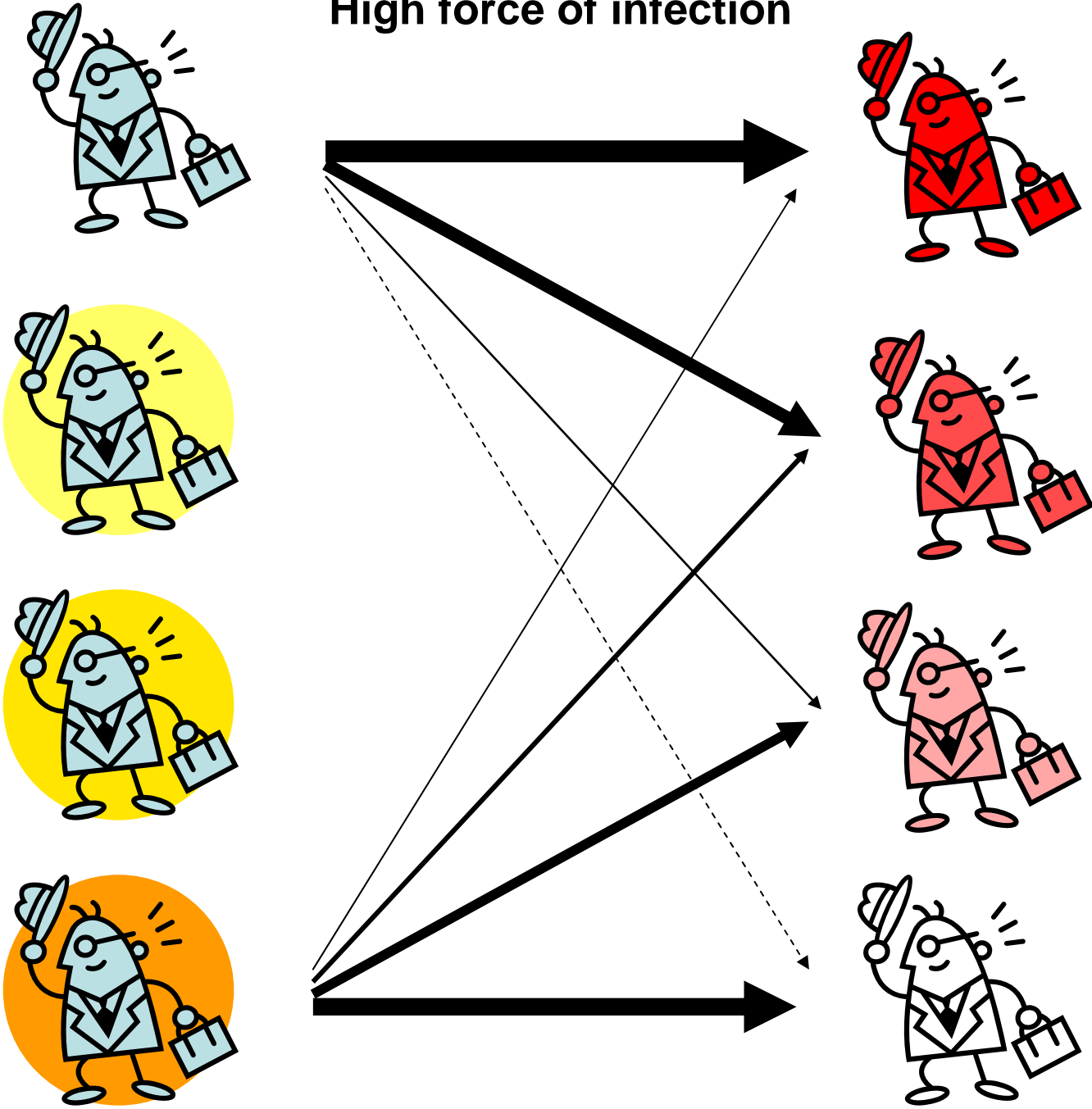


Increasing severity

High force of infection

Increasing immunity

Increasing severity



# Postulates and assumptions I

- The evolution of an infection by *L major* depends on the:
  - Immune status of the host
  - Transmission pressure of the parasite (number of infecting bites)
  - Intrinsic virulence of the parasite (isolate)
- The development of active disease or only the asymptomatic infection confers some resistance to a subsequent clinical infection . However, this protection is not absolute, with limited duration.
- The site of an infecting bite can remain asymptomatic or can evolve to the development of a more or less severe lesion (size, duration).
- ZCL lesion is the consequence of both: **parasitic multiplication and the intensity of the cellular immune response** of the host.
- The most severe lesions are associated with the highest levels of IFN- $\gamma$  producing cells.

# Postulates and assumptions II

- The development of ZCL lesion is the resultant of the time delay between parasitic multiplication and the arrival of the effector cells.
- The severity of a lesion is a function of the time between the injection of the parasites and the recruitment of the effector cells.
- High parasite load needs more effector cells (for parasite control), the lesion will be more severe.

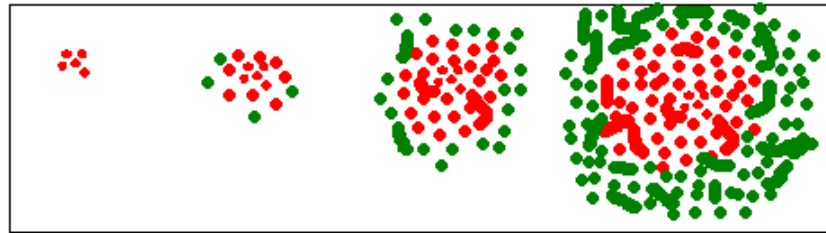


# Postulates and assumptions III

- Innate immunity can be sufficient for control to a low number of infecting bites (there would be a threshold of infecting bites for lesion development, this threshold can depend on genetic factors of the host)
- If innate immunity is overflowed, naive individuals will develop one or more lesions.
- “Immune” individuals has a pool of effector lymphocytes, this pool can control a certain number of additional infecting bites.
- However, even with highly “immune” individuals, in the presence of high transmission pressure, new lesion(s) could develop.
- Protection against leishmaniasis depends on both the **quality** and the **intensity** of the immune response.
- In an area of parasite transmission, the intensity of the immune response is function of the total number of previous infecting bites (with or without disease development)
- However, the development of a clinical lesion confers an immunity higher than that conferred by an asymptomatic infection.

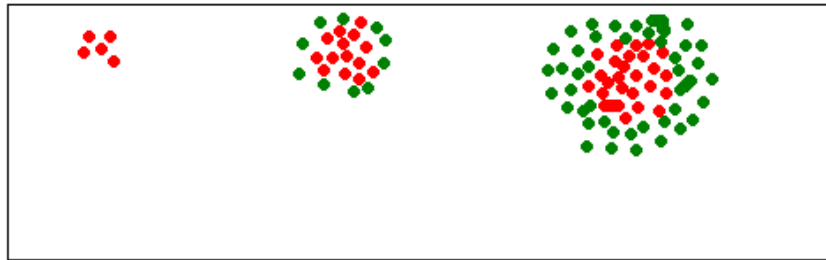
# Different scenarios according to the initial immune status

Naive individual



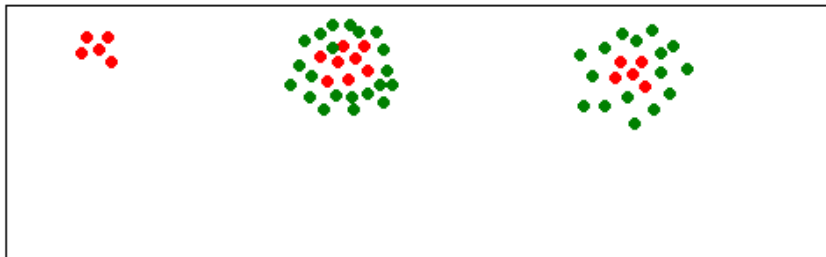
Severe lesion

Immunity: level 1



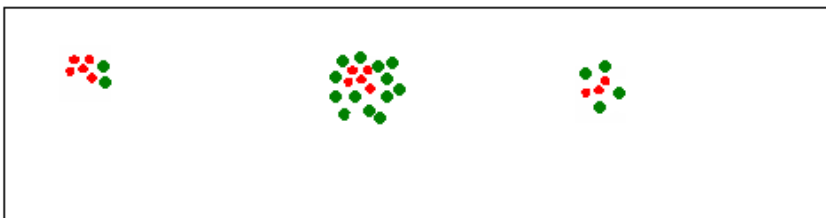
Intermediate lesion

Immunity: level 2



Induration

Immunity: level 3

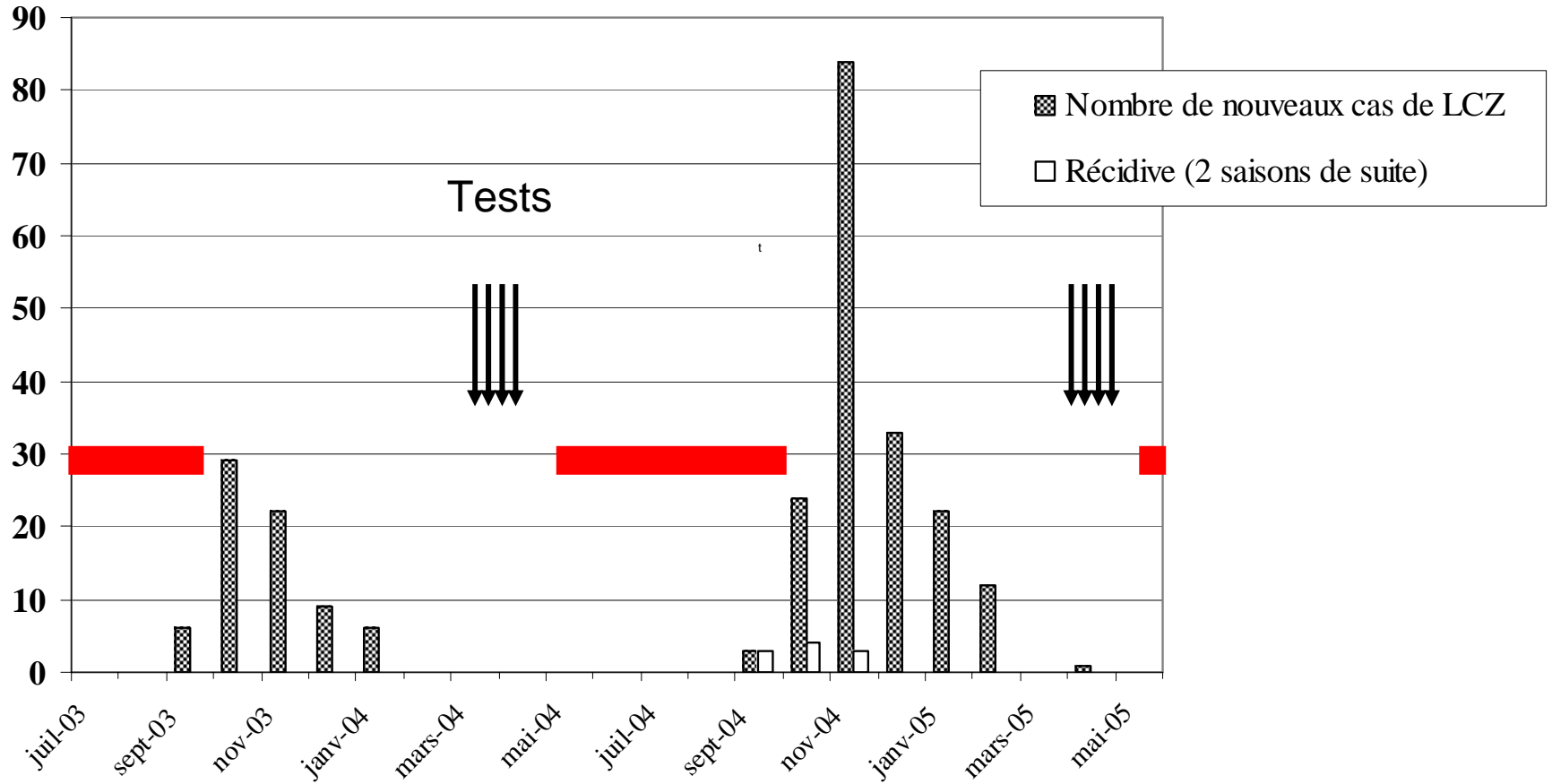


No lesion

# Correlates of protection

- Indicators for the **nature** and the **intensity** of immune response
- For their analysis, we have to analyze disease **severity**
- The use of longitudinal **follow-up** studies of **naturally** exposed individuals

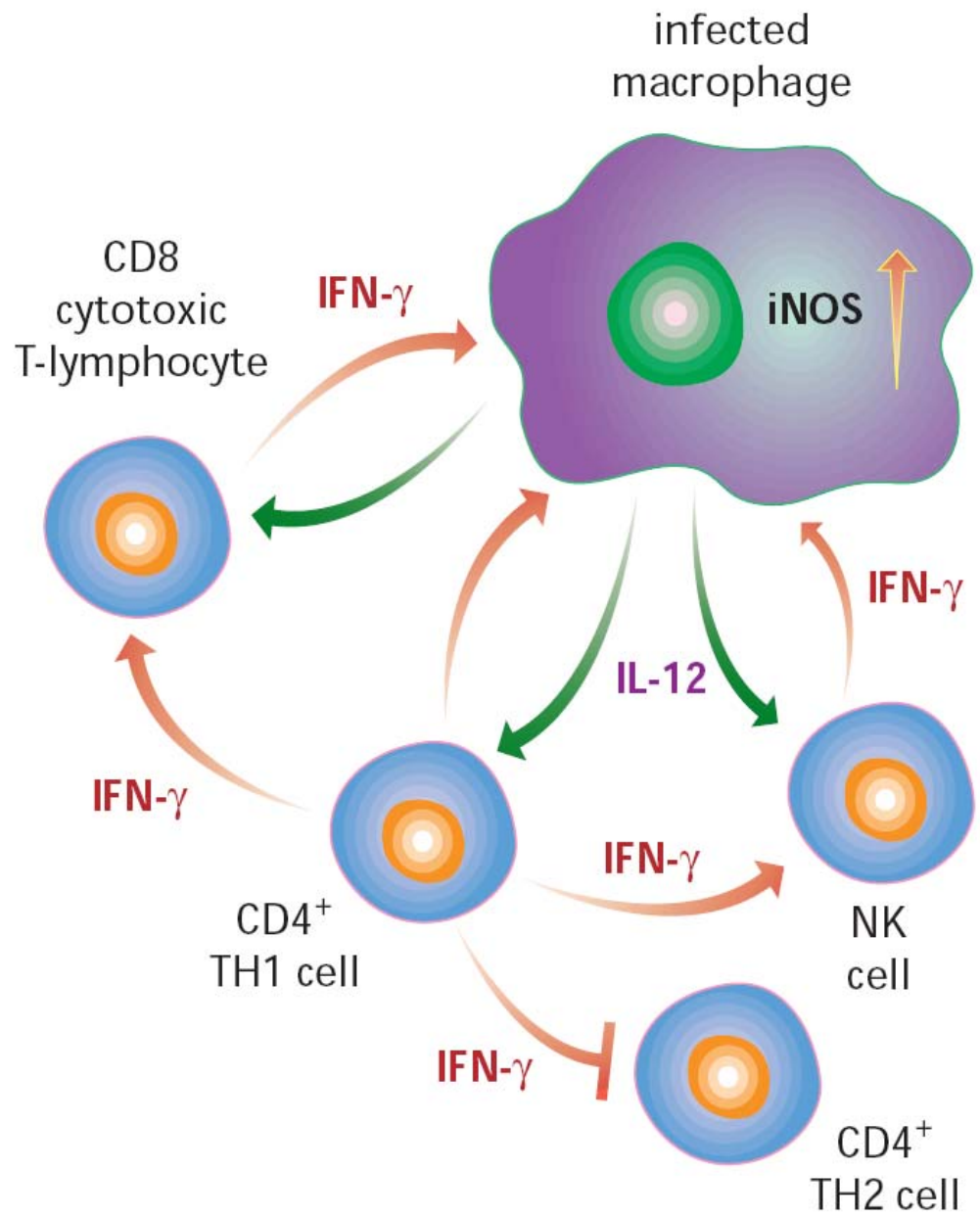
## Nombre de cas de LCZ se présentant au Centre de soin de Mnara (Juin 03-Mai 05)



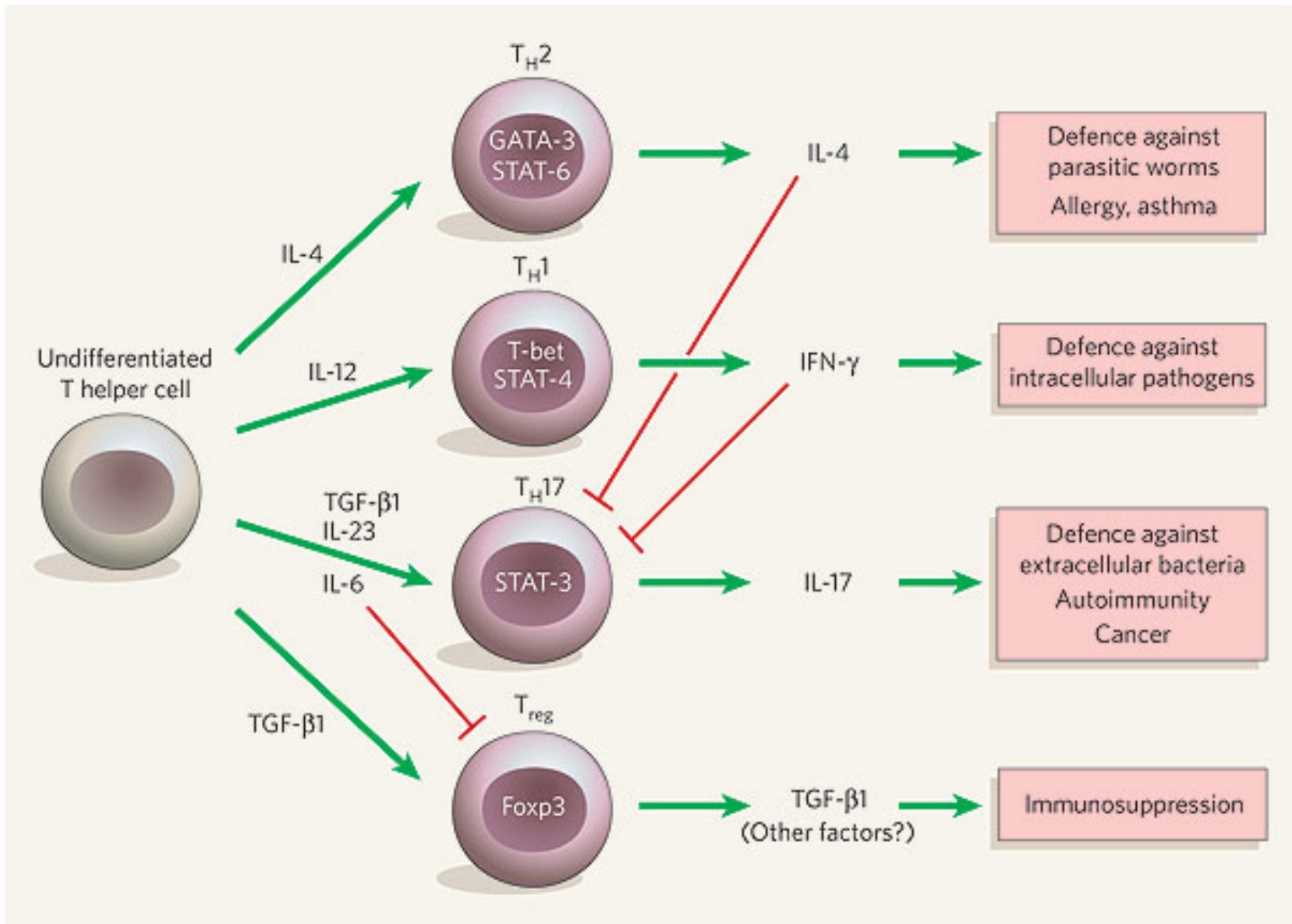
█ : Transmission season

# Concretely, which are the tests which we will use and evaluate as potential correlates?

- It is necessary to take account of the limits of experimental medicine
- **Some suggestions:** We propose to study certain indicators of the involvement of the various actors of the cellular immune responses (innate and adaptive).
- One of the best techniques for assessing multiple functions of T cells simultaneously is multiparameter flow cytometry, by assessing different combinations of phenotypic markers and cytokine or other effector molecules (IFN- $\gamma$ , TNF, IL-2 etc.) at the single-cell level, one can define the quality of the CD4<sup>+</sup> and CD8<sup>+</sup> T-cell cytokine response.

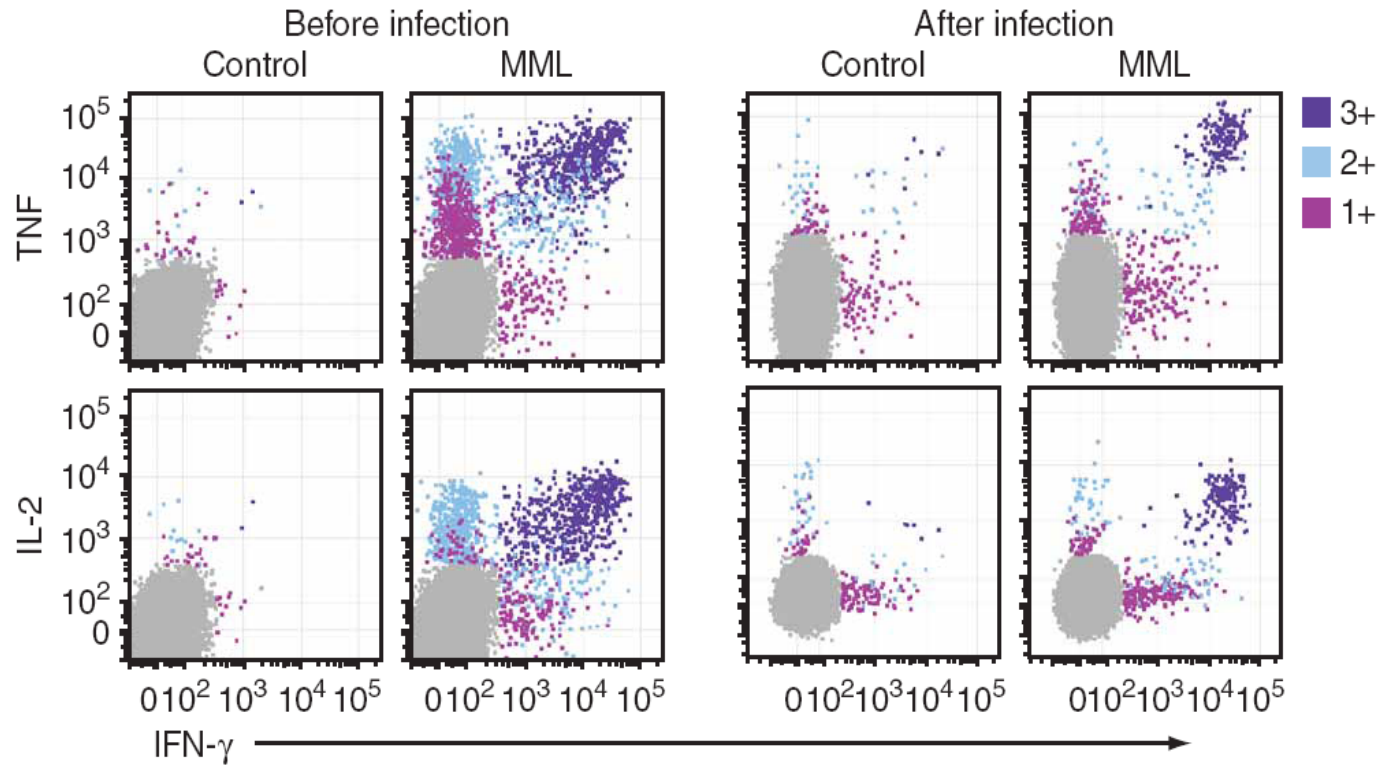


# Cytokines & the T helper cell lineages



# Prediction of protection in vaccinated mice against *L. major*

(From Darrah et al, 2007)



This study showed that there are distinct differences in the potency of effector cells demarcated by whether they secrete multiple cytokines and that single-positive CD4+ IFN-g-producing cells would be far less efficient as effector cells.



# In conclusion

- We still need to better understand the mechanisms of the natural resistance against the infection to be able to develop the suitable vaccines; because one cannot perhaps make better than nature.
- Understanding the immunological mechanisms that mediate vaccine efficacy will give valuable information for the **design** of candidate vaccines and their **evaluation**.