Effective immunological mechanisms of resistance against *Leishmania*

Hechmi Louzir
Institut Pasteur de Tunis
hechmi.louzir@pasteur.rns.tn

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

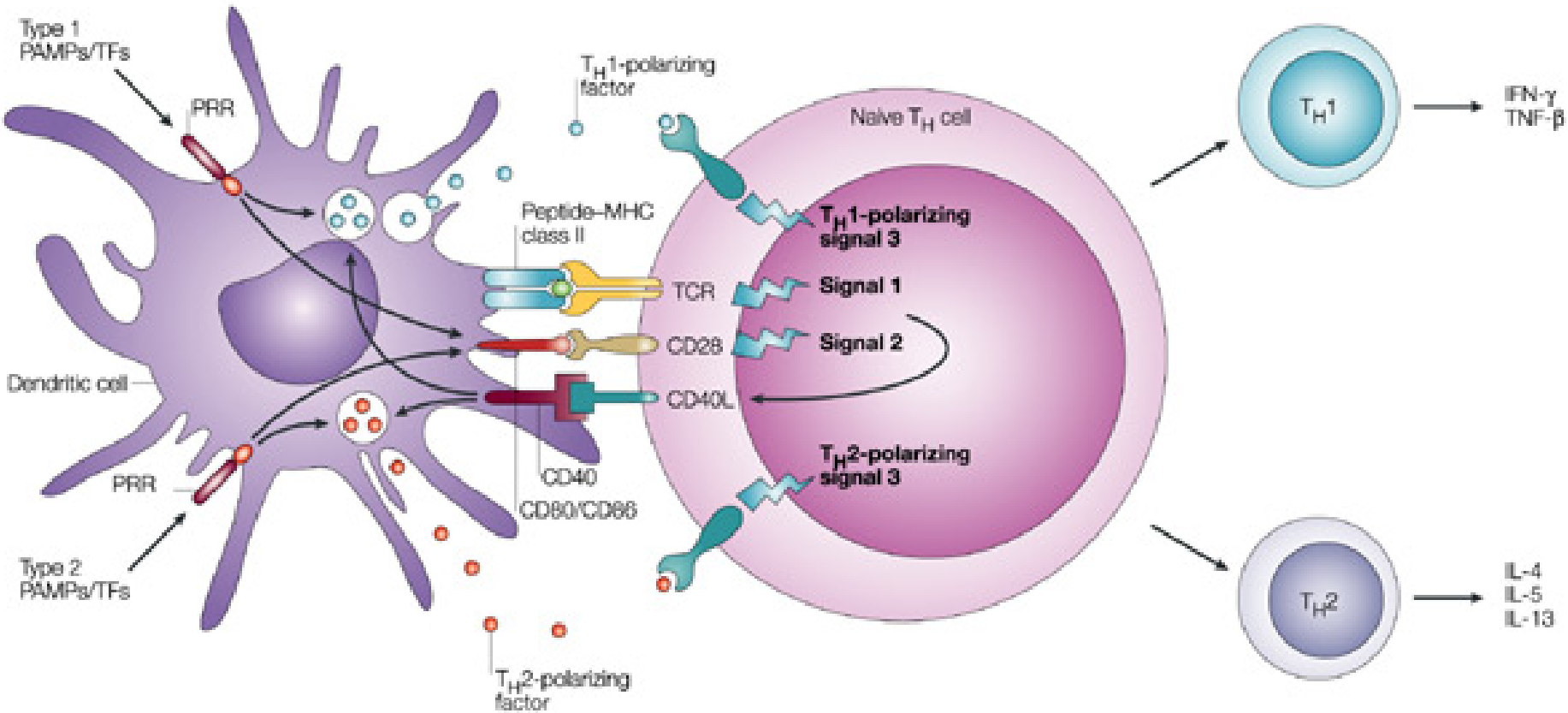
Anti-*Leishmania* responses

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 $CD4^+$ Th1 cell-mediated immune response against the parasite.
- The LST reactivity is classically associated with resistance to *Leishmania* parasite.

Concordance LST reactivity/SLA-specific lympho-proliferative response/SLA-specific IFN-γ production

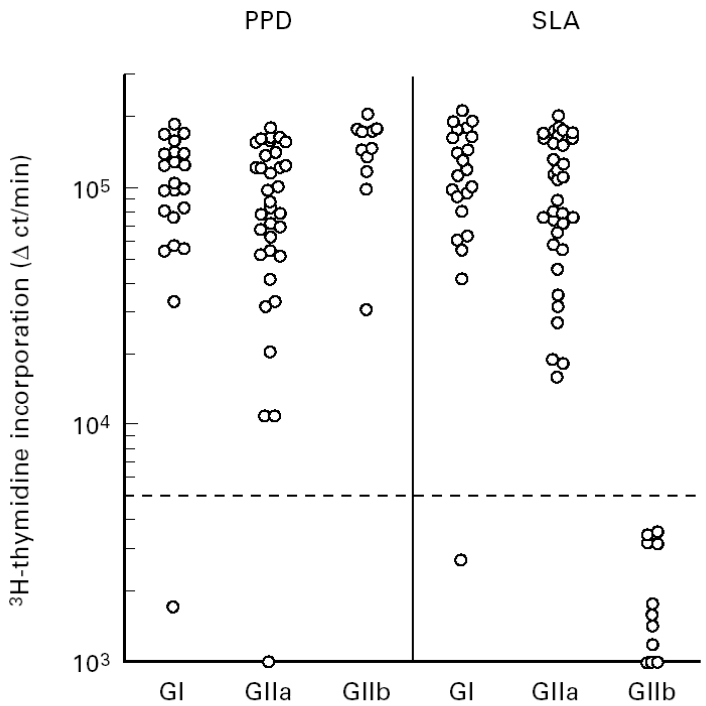


Fig. 1. *In vitro* lymphoproliferative responses to purified protein derivative (PPD) or soluble *Leishmania major* antigens (SLA) in individuals with healed localized cutaneous leishmaniasis (LCL) (group I) and from leishmanin skin test (LST)$^+$ (group IIa) and LST$^-$ (group IIb) individuals without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

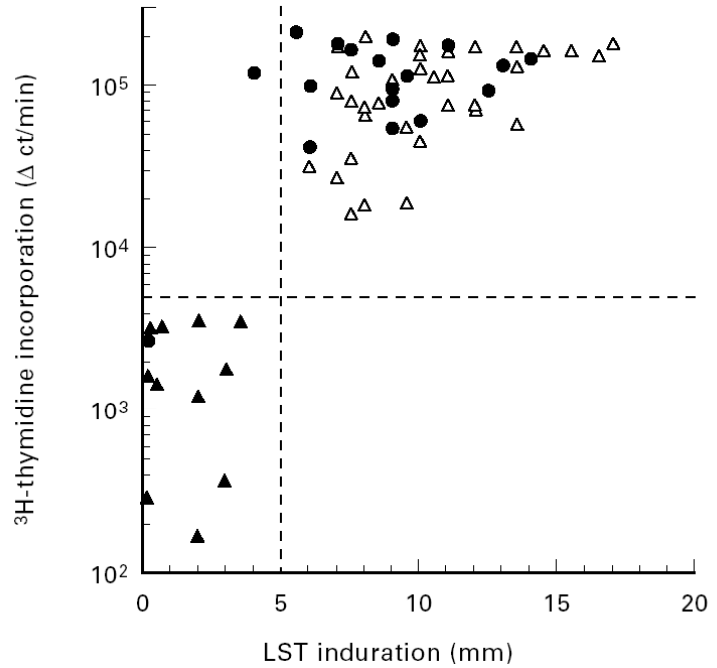


Fig. 2. Correlation between leishmanin skin test (LST) expressed as diameter of induration (mm) and lymphocyte proliferation in response to soluble *Leishmania major* antigens (SLA) (Δct/min). Groups are defined as in Fig. 1. ●, Group I; Δ, group IIa; ▲, group IIb.

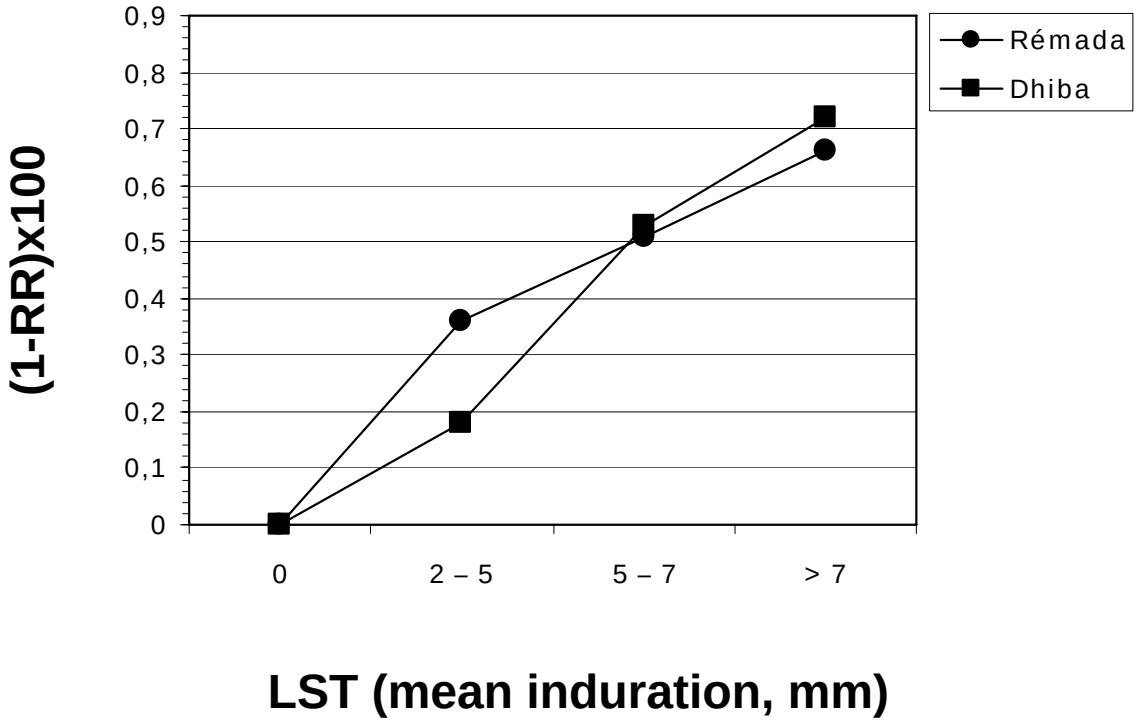
diameter of skin induration and the Δct/min of SLA-stimulated cultures (Spearman rank correlation coefficient $r = 0{\cdot}6$, $P <$

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

	Remada				Dhiba			
LST reaction size, mm	Participants with ZCL (*n* = 155)	Participants without ZCL (*n* = 117)	RR (95% CI)	PF, %	Participants with ZCL (*n* = 25)	Participants without ZCL (*n* = 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

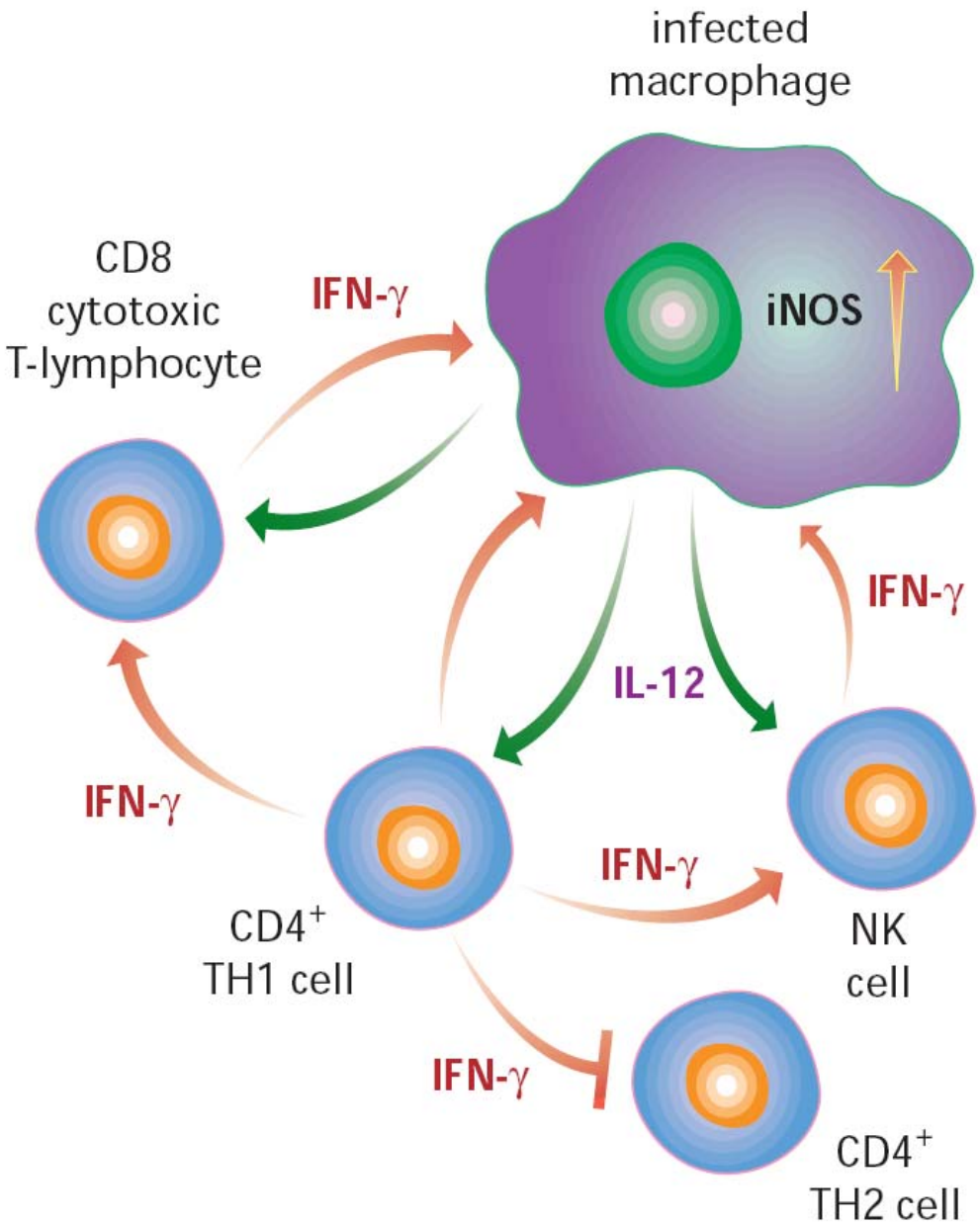
- With the support of UNDP/WHO/TDR, several vaccines (dead parasites +/- BCG) has been evaluated for their immunogenicity and efficacy in:

  - Latin America : [Castes, 1994; Armijos, 1998; De Luca, 1999; Velez, 2000; De Luca, 2001; Follador, 2002; Armijos, 2003; De Luca, 2003; Velez, 2005]

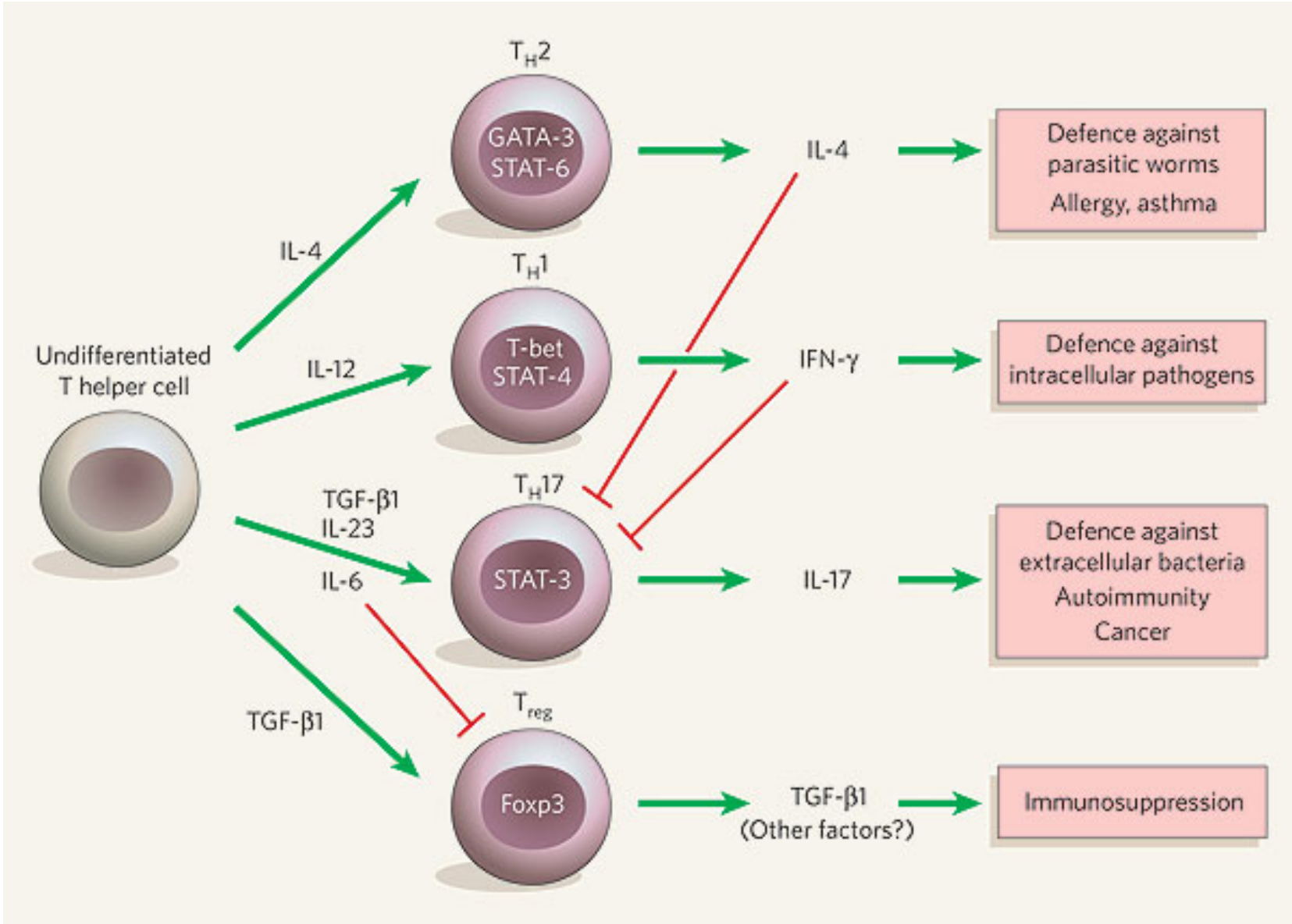
  - Sudan: [Khalil, 2000; Satti, 2001; Kamil, 2003]

  - 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-γ 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



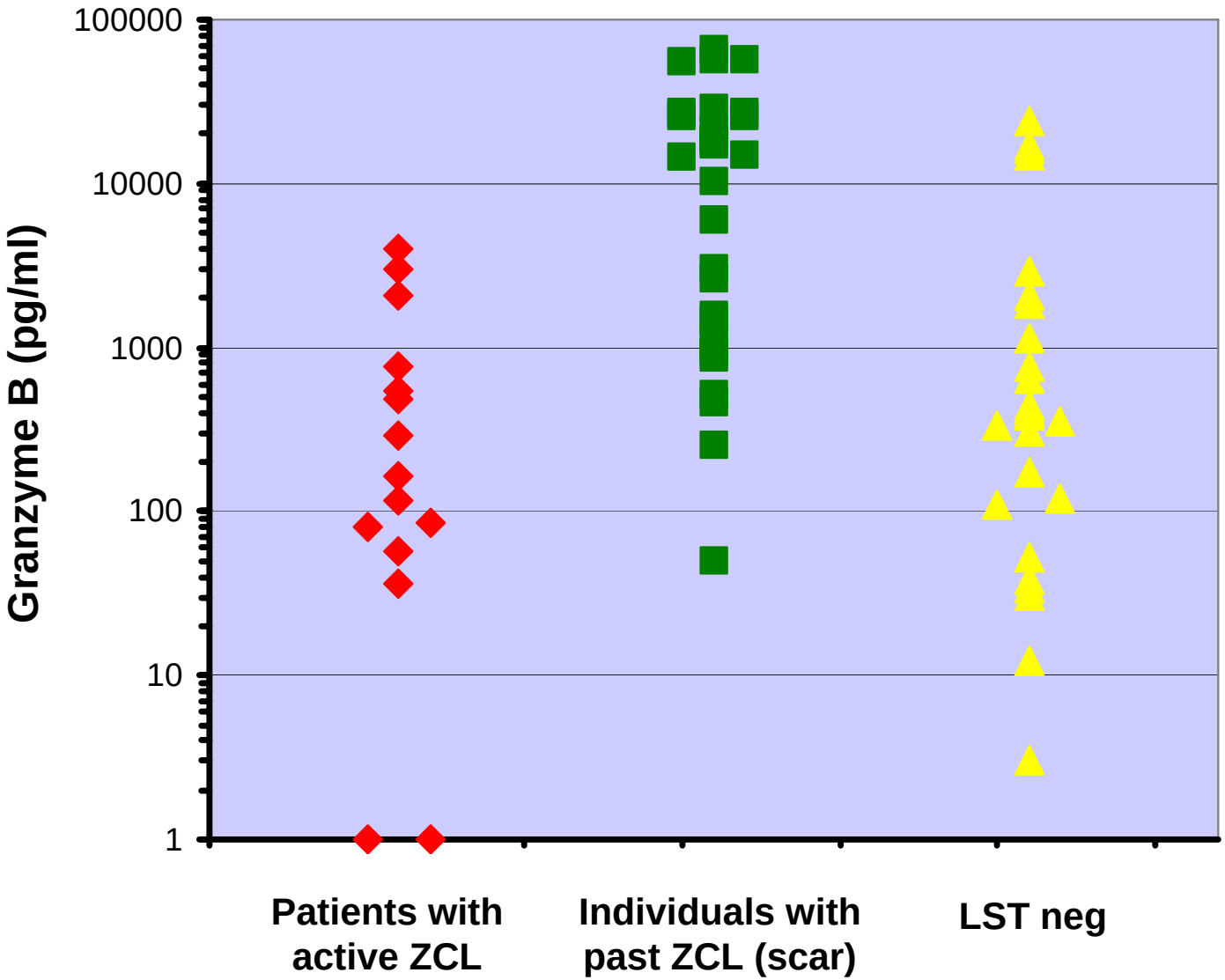
How to study such immunological mechanism in the protection against human leishmaniasis? The difficulties emerge:

- **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

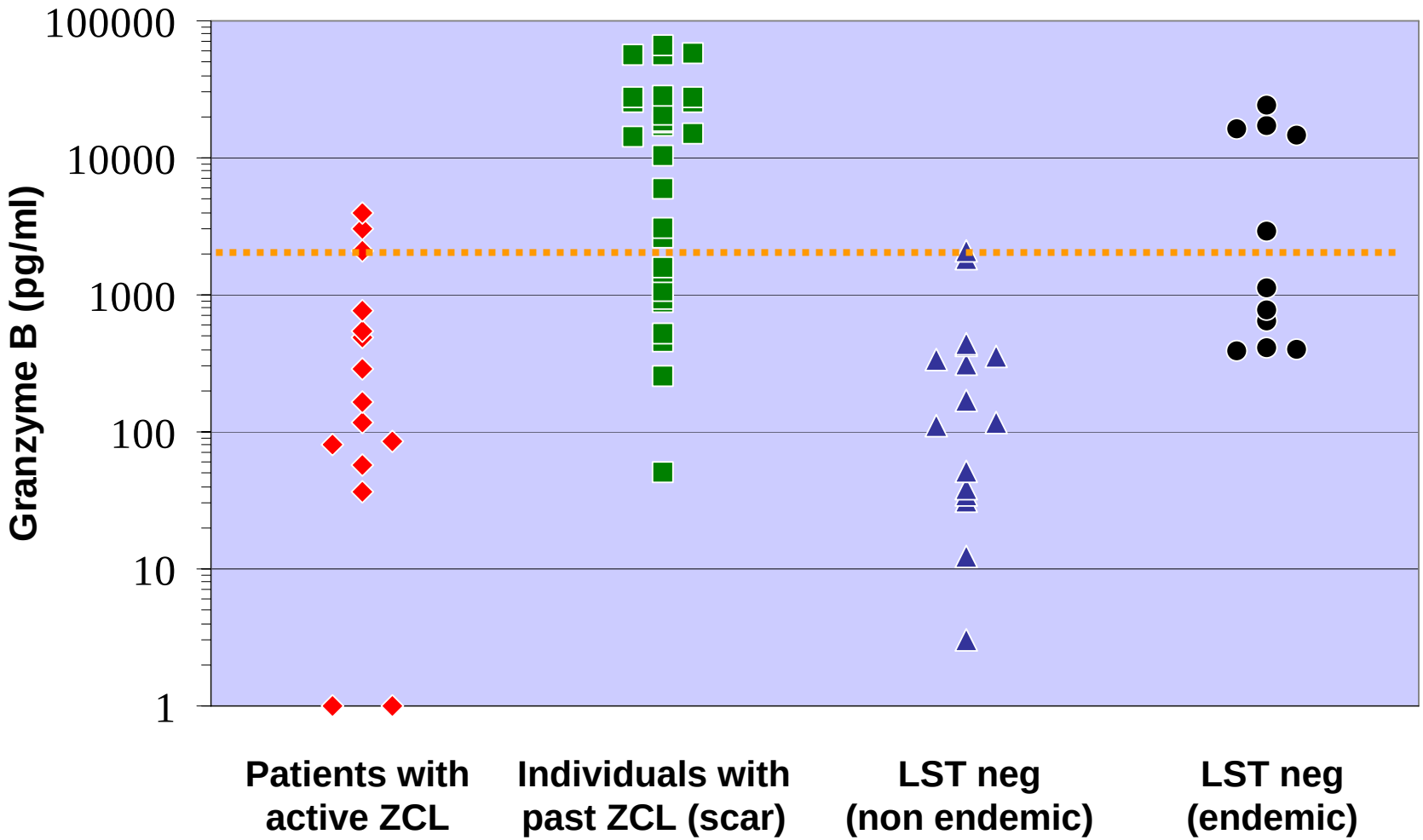
For *Leishmania*

- 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.

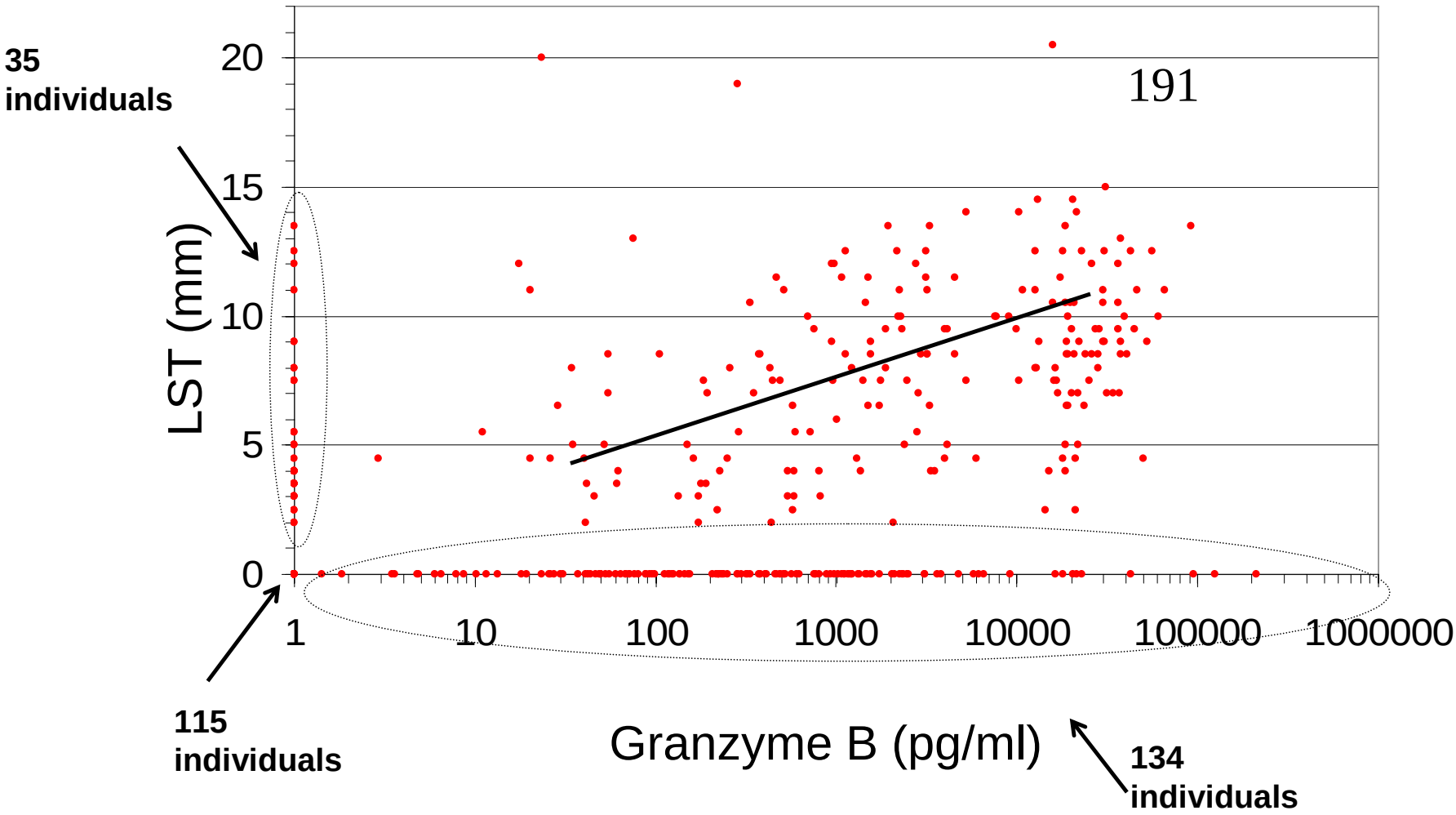
Granzyme B production



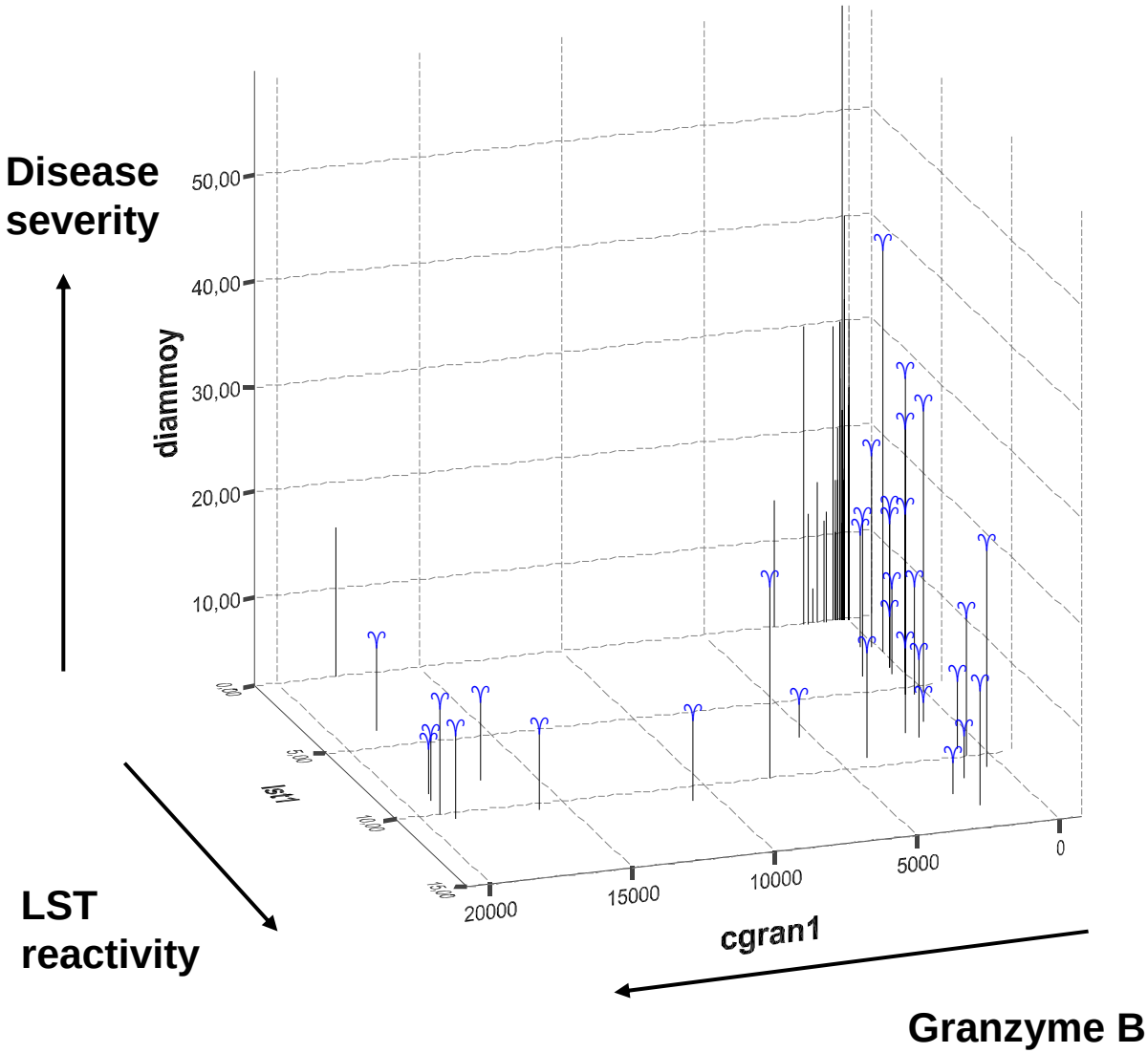
Granzyme B production



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 $_2$)
- - 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).**

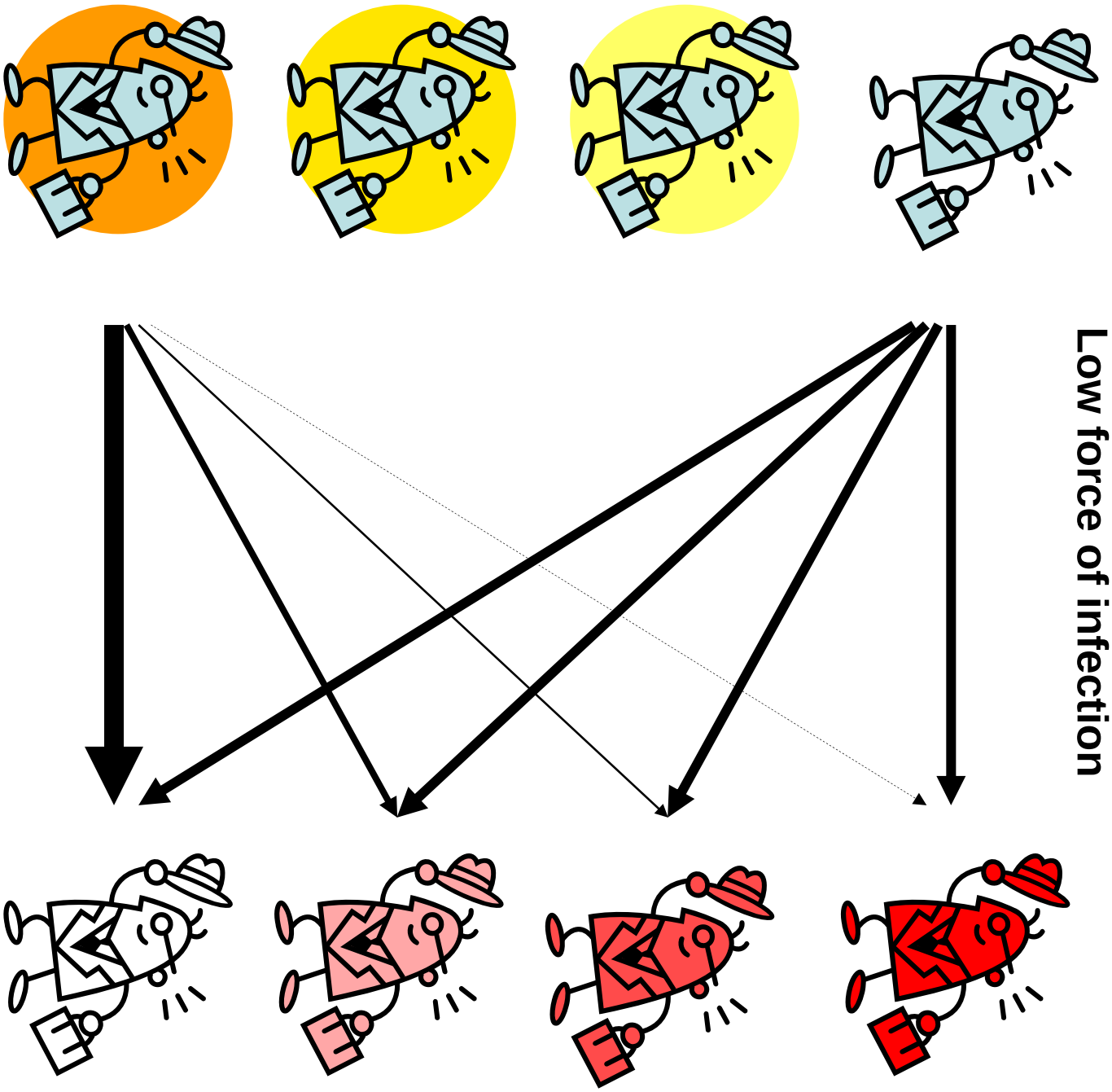
non-immune

disease

immune

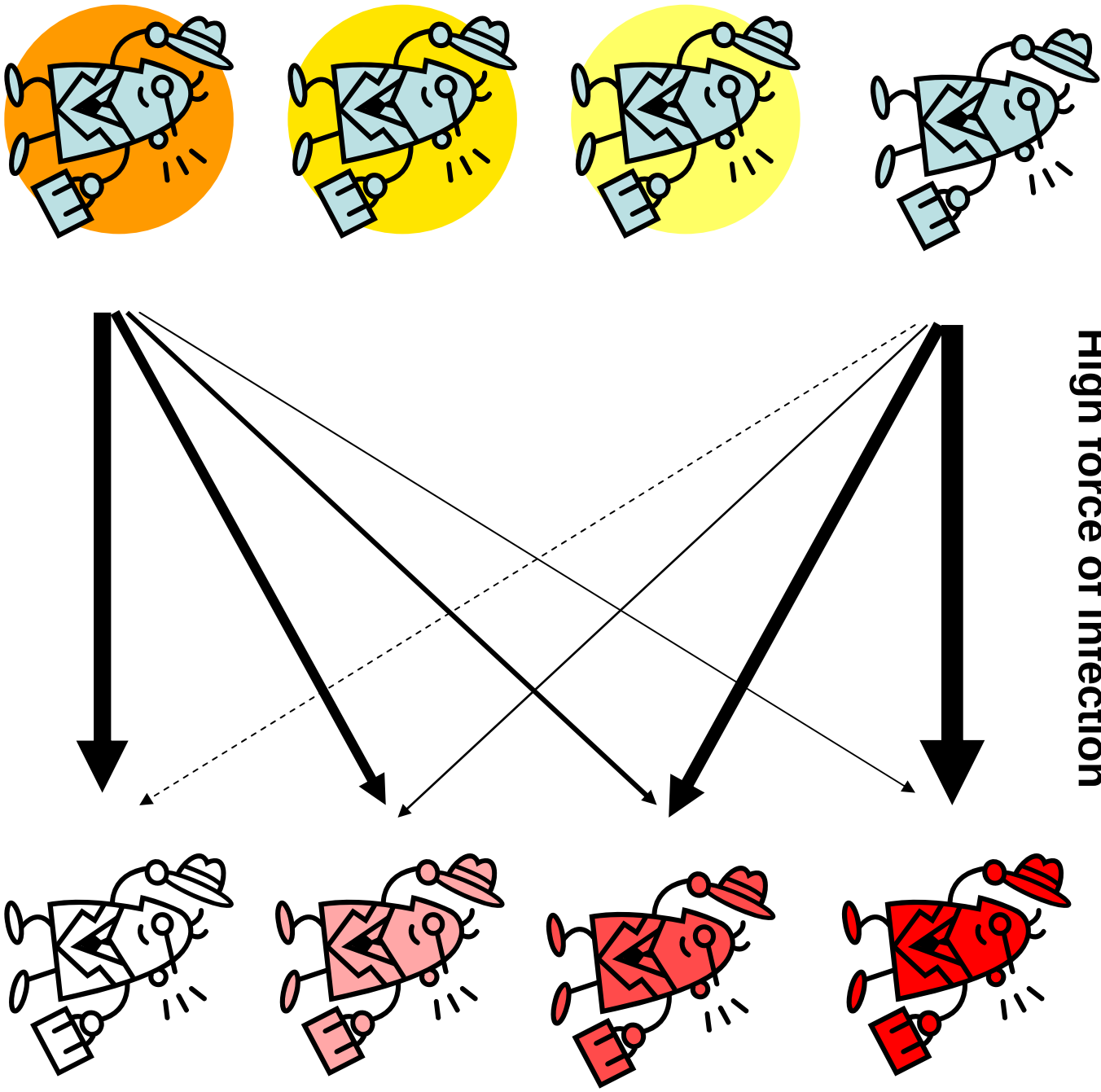
healthy

Increasing immunity



Increasing severity

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-γ 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.

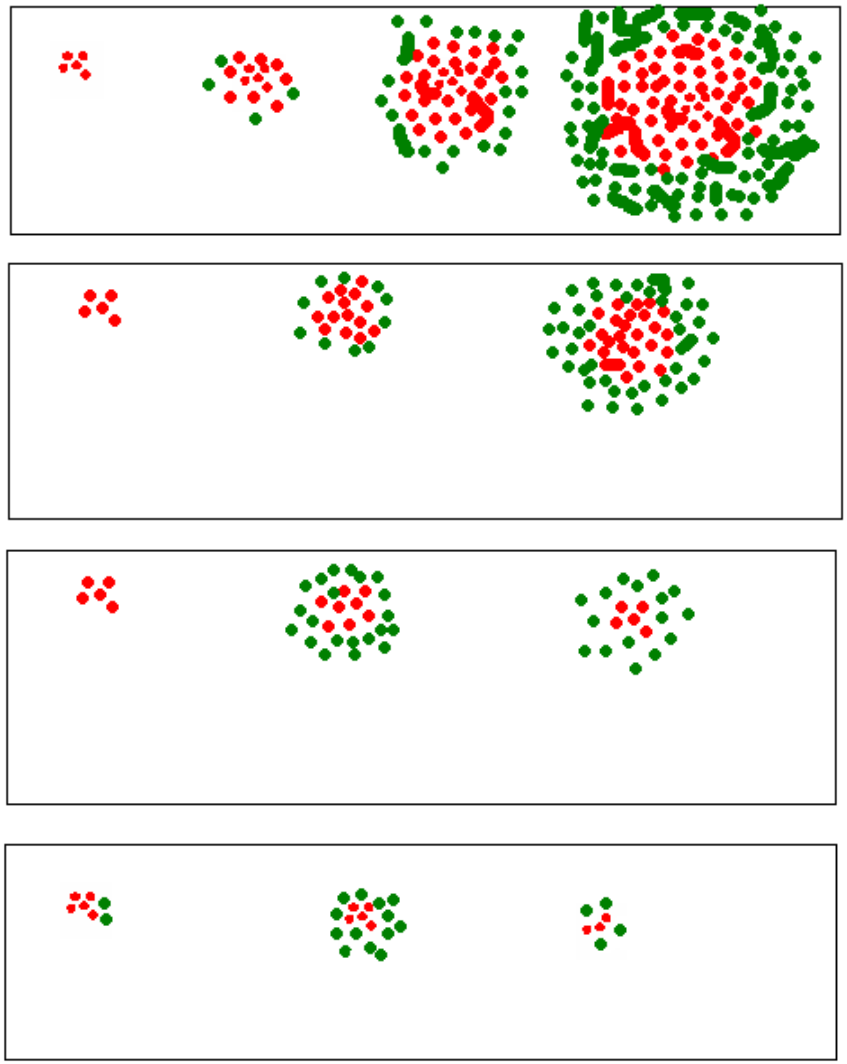
Different scenarios according to the initial immune status

Naive individual

Immunity: level 1

Immunity: level 2

Immunity: level 3



Severe lesion

Intermediate lesion

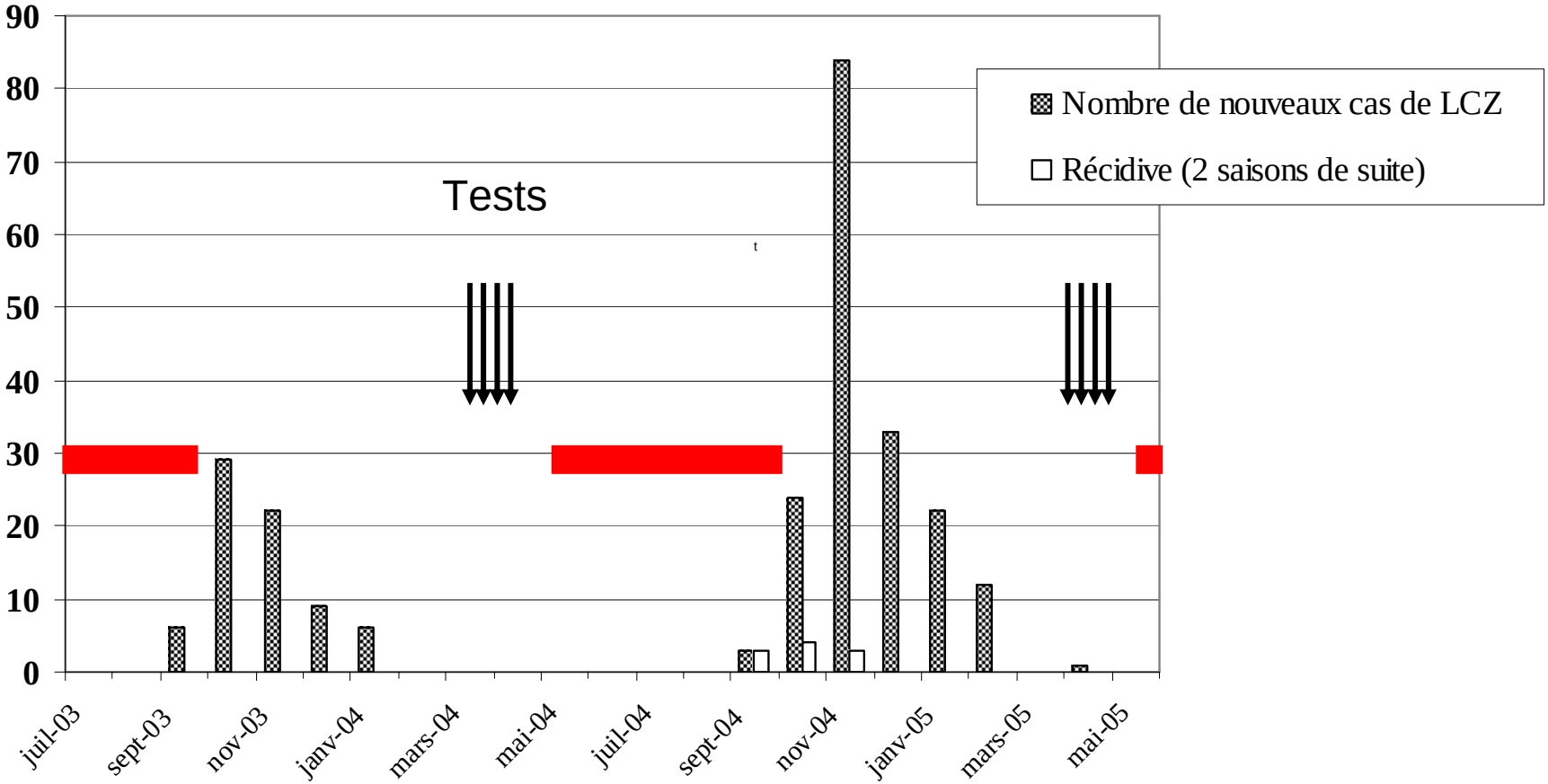
Induration

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

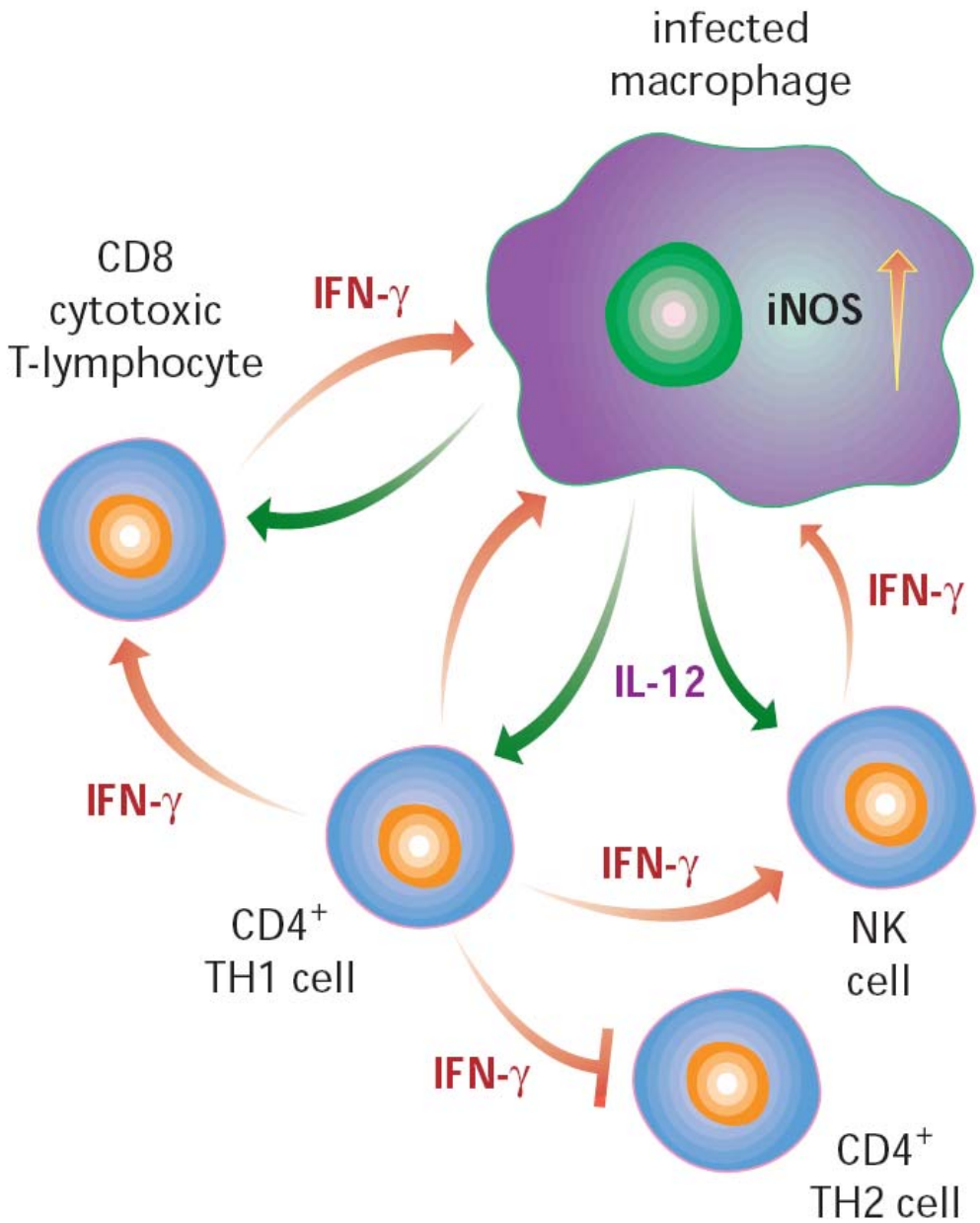
Nombre de cas de LCZ se présentant au Centre de soin de Mnara (Juin 03-Mai 05)



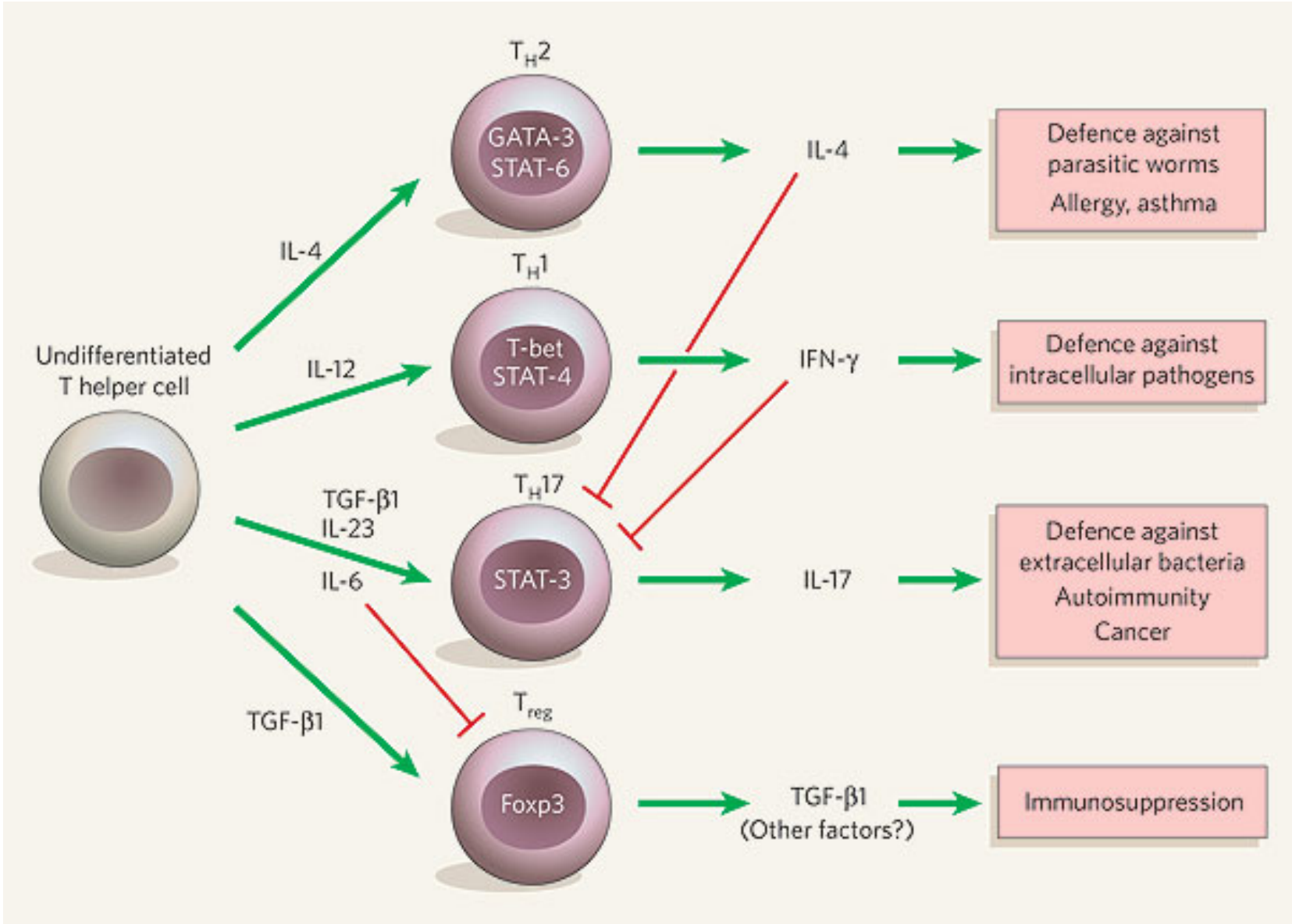
: Transmission season

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 he 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-γ, TNF, IL-2 etc.) at the single-cell level, one can define the quality of the $CD4^+$ and $CD8^+$ T-cell cytokine response.

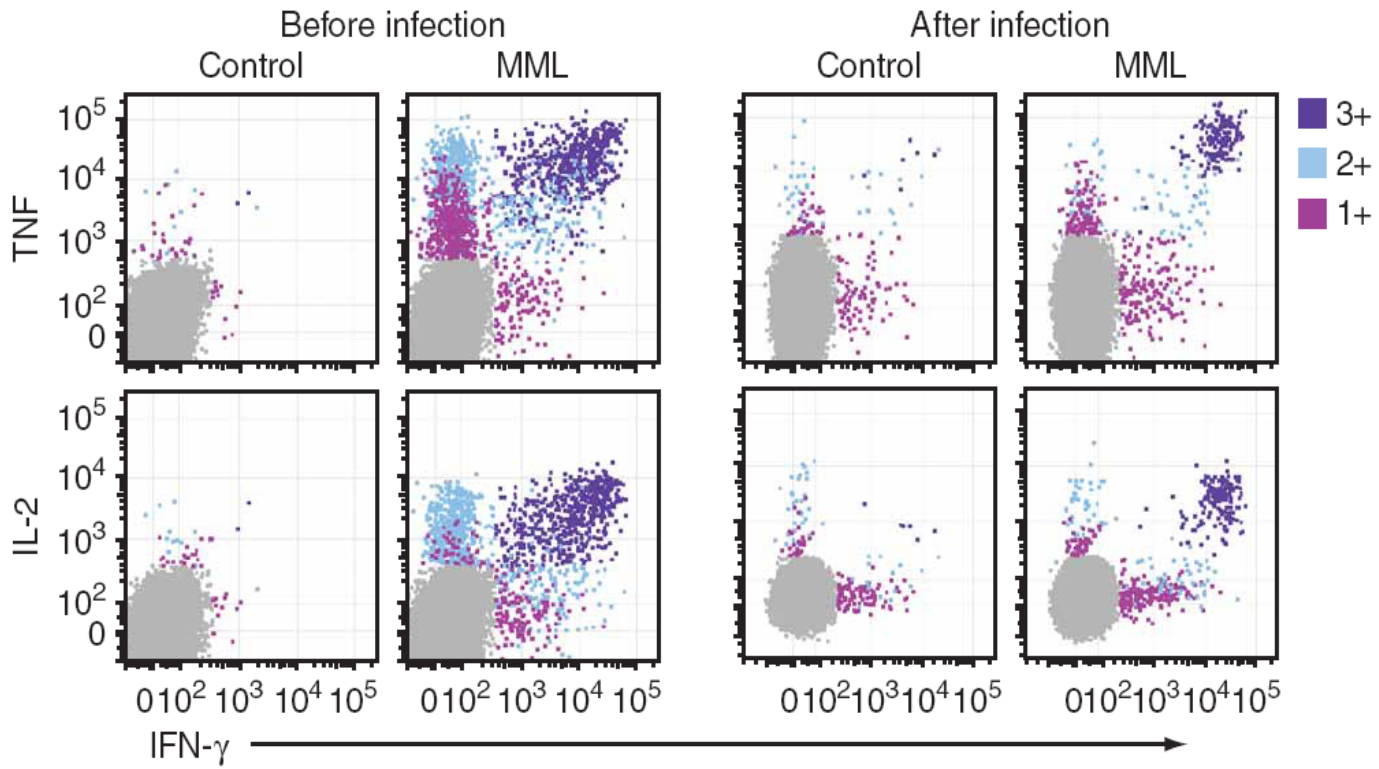

infected
macrophage
iNOS
CD8
cytotoxic
T-lymphocyte
IFN-γ
IFN-γ
IL-12
IFN-γ
IFN-γ
IFN-γ
CD4+
TH1 cell
NK
cell
CD4+
TH2 cell

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.