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+ 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-γ production

Cellular immune response to L. major

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 = 0.6,  𝑃<
Table 2. Relative risk (RR) and preventive fraction (PF) of zoonotic cutaneous leishmaniasis lesions according to intensity of leishmanin skin test (LST) reaction.

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

**NOTE.** CI, confidence interval; PF = 1 – RR.
Protection fraction of LST reactivity

(1-RR) x 100

LST (mean induration, mm)
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; Mahmoudi, 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

- Patients with active ZCL
- Individuals with past ZCL (scar)
- LST neg

Granzyme B (pg/ml)
Granzyme B production

Patients with active ZCL

Individuals with past ZCL (scar)

LST neg (non endemic)

LST neg (endemic)
Granzyme B/LST in individuals living in *L. major* transmission area

- 35 individuals
- 115 individuals
- 134 individuals
- 191 individuals

Granzyme B (pg/ml)

LST (mm)
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

Low force of infection

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
  - 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)

Tests

Nombre de nouveaux cas de LCZ

Récidive (2 saisons de suite)

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-γ, TNF, IL-2 etc.) at the single-cell level, one can define the quality of the CD4⁺ and CD8⁺ T-cell cytokine response.
Cytokines & the T helper cell lineages
Prediction of protection in vaccinated mice against \textit{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.