Inter individual variability of blood donor's Macrophages to get infected by *Leishmania donovani* promastigotes

LeishRisk

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Introduction and rationale

The main goal of the RAPSODI project is to develop a human-compatible vaccine targeting most, if not all, *Leishmania* species that cause the different clinical manifestations of Leishmaniasis, and all the associated procedures and methods required for the subsequent clinical trials.

The co-evolution of pathogens as *Leishmania* and their hosts for long periods reaching thousands or even millions of years, often leads to the emergence of adaptive phenomena of resistance to infection. The complexity of human genetic study highlights the need to develop, beside global complex approaches, imperfect but more useful experimental models for evidencing human interindividual functional variability (immunocellular phenotype) associated with the risk of developing leishmaniasis. The model proposed by RAPSODI will focus on permissiveness variability in the human host macrophage certainly offer a simplified view of reality compared to the complexity of a mammalian organism. However these models are relatively easy to implement and can be used to test scenarios and multiple hypotheses that are difficult to achieve *in vivo.*

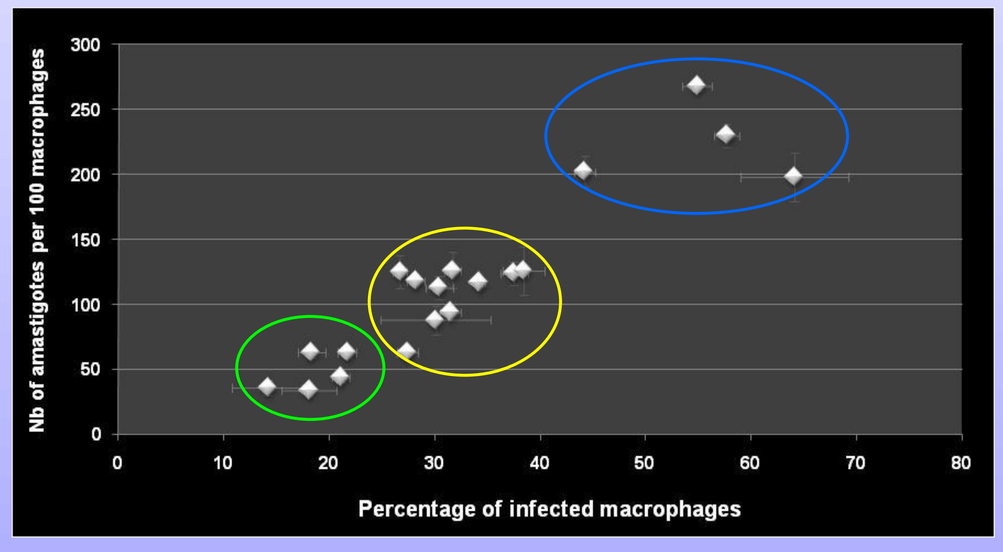


Figure2: Graphic representation of the inter-individual variability of human macrophage permissiveness in 19 non exposed blood donors: evidence of three groups of individuals

Objectives

To reach this goal, several scientific objectives were defined:

- **To provide proof of concept of the new experimental approach by evaluating the inter-individual variability of human macrophages to get infected by promastigotes of *L. donovani* using a robust but fastidious macrophage infection macro-assay, only adapted to large volume of fresh blood samples, in non exposed individuals.**
- **To furnish additional proof of feasibility in the field by finalizing a useful and robust *in vitro* macrophage infection microassay adapted to limited volume of field blood samples**
- **To define common and standardised cell biological procedures within the consortium.**
- **To identify and evaluate new immunogenetic biomarkers of natural resistance or susceptibility towards Leishmaniasis .**

Materials and methods

PBMCs were enriched via centrifugation through Ficoll gradient. Monocytes will be isolated from PBMC by their adherence properties.

Different PBMCs or monocyte's isolations protocols were tested and optimized to choose which retain the best cell,s viability, yield and properties.
Adherent macrophages will be let to differentiate for 7 days in 6-well plastic culture plates (macro assay) or in 8-well glass chambers microplates (micro assay. On day 7, they were infected by stationary phase promastigotes of *L. donovani* in a ratio of 10 parasites/1 macrophage. Non internalised parasites were washed out at t=4h post infection.
AS infections are not performed in a single experiment, to avoid experimental bias, THP1 monocytic cell line was used as Gold standard of infection level. 48h duplicate macrophage infected monolayers will be fixed with methanol and stained with Giemsa for microscopic determination of the the number of intracellular parasites and the percentage of infected macrophages to calculate parasitic index.

Deliverables

Validation of the macrophage infection macro assay

The results showed:
- a relative good repeatability in the adherent cell number by well for a same individual after 7-day differentiation. By contrast, a significant variability was observed between individuals. Adherent macrophages were systematically counted before *in vitro* infection to ensure a same parasite/macrophage ratio (10:1) and so avoid uncertain results;
- The reproducibility in macrophage permissiveness when experiments were performed at different times (1, 2 and 3 months) with the same individual;
- the validity of the Gold standard of infection level by the demonstration that infection levels were reproducible when experiments were performed at different time with the same experimental conditions.

Proof of concept of the new experimental approach

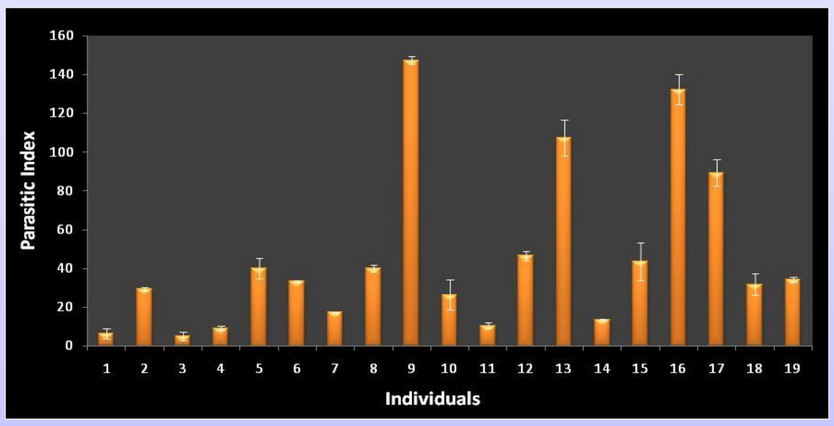


Figure1: Graphic representation of the inter-individual variability of human macrophage permissiveness in 19 non exposed blood donors: PI determination

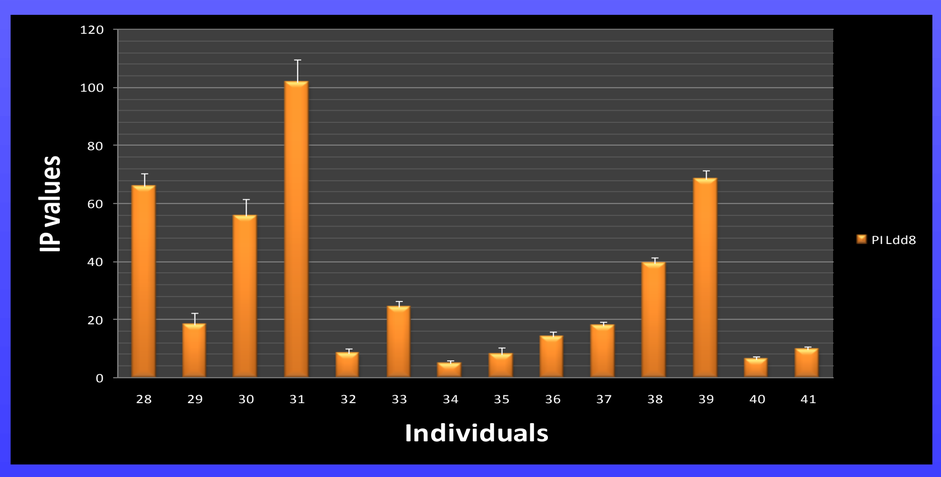
Experiments conducted on 19 samples of blood donors from a non exposed area (Bordeaux, France) revealed a significant and a high inter-individual variability in macrophage sensitivity to *L. donovani in vitro* infection in human population. PI values fluctuated from 5 to 145 (Figure 1).
We clearly evidenced 3 groups of individuals: a highly (n=5), an intermediate (n=10) and a slightly (n=4) infected group (Figure 2).

Proof of concept of feasibility in the field

Best conditions to improve and standardize a macrophage infection micro-assay applicable to limited volume of field blood sample (10 ml) are detailed in table below

PARAMETERS	BEST CONDITIONS	IMPROVEMENT
PBMC isolation	Lymphoprep TM Ficoll gradiant	Best Cell's yield
PBMC conservation	Useful and robust freezing and thawing protocols	Delayed experiment that can be performed together
Culture slides	BD Falcon glass chambers	Best conditions for macrophage's viability, yield and adherence
Culture medium	IMDM medium	
Cell density by well	2 X 10^6 PBMC/cm^2	
Macrophage's adherence	2% hSAB instead 10% hSAB	
Parasite-macrophage ratio	Addition of a new macrophage isolation step	Ratio of similar order
RNA isolation	TRIZOL extraction with Glyco-blue coprecipitant	Best RNA purity and yield

Significant *ex vivo* macrophage infectivity variability was observed with limited human samples by using micro-method in a single experiment (14 samples) (see below IP values).



Conclusion: all together, these results contribute to validate the proposed experimental approach to analyze the impact of human genetic diversity on the macrophage response upon infection.