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Proteins involved in the apoptosis of the intracellular form of *Leishmania*

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Abstract
Leishmaniases are worldwide neglected tropical vector-borne diseases due to *Leishmania* parasites, against which new therapeutic tools must be developed. While different stimuli induce *Leishmania* apoptosis, the proteins and metabolic pathways involved are poorly understood in the insect form and completely unknown in the mammal form, due to difficulties studying this intramacrophage form. In this context, this PhD project aims at understanding *Leishmania* cell death in the mammal host and its consequences on the host cell through, notably, dual transcriptomic profiling of infected macrophages after gene edition. It combines molecular biology, cell biology techniques, fluorescence microscopy, video-microscopy, motility assays, quantitative biology and bioinformatics. This project could be decisive in deciphering the interactions between *Leishmania* and host cells related to apoptosis, opening prospects for new therapeutic avenues against leishmaniases.

Keywords
host-pathogen interaction; *Leishmania*; apoptosis; dual transcriptomics; microscopy; gene edition; cell biology

Scientific question and Objectives
Leishmania are protozoan parasites transmitted by the bite of a female sand fly, and responsible for leishmaniases, that still represent a global scourge, notably due to their wide distribution and to the absence of really satisfying treatments. In this context, *Leishmania* apoptosis could be a prime target. Indeed, despite a classic pattern of apoptosis, key mammalian apoptotic proteins could not be found in *Leishmania*. Yet, recent studies have identified proteins involved in *Leishmania* apoptosis, more precisely in the promastigote form of the parasite, *ie* the flagellated extracellular form found in the digestive tract of the insect vector. No study has been done on the apoptosis of the amastigote form, found in phagocytic host cells, notably due to the difficulty of experimentation, whereas it is the more relevant form for human health. This project aims to identify proteins involved in the amastigote form apoptosis and to assess the consequences of *Leishmania* apoptosis on the phagocytic host cell.

Proposed approach (experimental / theoretical / computational) and research plan
In this project, we will address the consequences of the inhibition or overexpression of different *L. mexicana* genes on the parasite inside the macrophage and on the macrophage itself. More precisely, we will study genes coding proteins identified as involved in the promastigote form apoptosis, for example the metacaspase, the Endonuclease G or calpains (Basmaciyan and Casanova, 2019). The consequences of gene expression modifications will be assessed: (i) on the **promastigote form** if it is unknown, evaluating cell growth, cell morphology, motility, propidium iodide permeabilization,… ; (ii)

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on the **amastigote form**, evaluating the number of infected host cells and the number of parasites per infected host cell by fluorescence microscopy ; (iii) on the **macrophage**, quantifying gene expression in macrophages, and assessing the macrophage motility following infection. To study host-parasite interactions, the human monocyte THP-1 cell line will be used, after differentiation into macrophage-like cells using PMA. In addition, gene-edited THP-1 will be generated to disrupt pathways involved in parasitophorous vacuole maturation, phagocytosis, or inflammasome signaling (Obeid *et al.*, 2023). To obtain a comprehensive view of both host and pathogen responses, we will conduct **dual transcriptomic profiling**, enabling the simultaneous analysis of global gene expression changes in *Leishmania* and the infected human macrophages. This approach will allow us to characterize the molecular reprogramming events that shape the dynamic interaction between the parasite and its host cell. In the end, this project will bring a better understanding of *Leishmania* cell death and its consequences in the host cells, allowing the development, in the future, of new therapeutic tools targeting the parasite apoptosis, for example by encapsulation of genes/proteins.

Interdisciplinarity and Implication of the two labs

This is a new project that combines complementary expertise of Magali Casanova, a parasitologist, and Sandrine Marquet, a specialist in human genetics and genomics. It also combines biology and physics (fluorescence microscopy and motility assays), as well as experiments and bioinformatic quantitative analysis. In this project, LAI lab will bring MC's expertise on *Leishmania* gene modifications, on the biology of the parasite and on fluorescence microscopy. LAI will also bring the expertise of the other researchers on immune cells and on cell migration. TAGC lab will bring expertise on human gene modifications, conducting experiments and analyzing transcriptomic data. TAGC will also contribute the expertise of two engineers in genomics and bioinformatics. Overall, the LAI/TAGC implication in this project will be 50/50, segmented as follows:

Year 1: 70/30: *Leishmania* gene modifications (episomal overexpression or inhibition by CRISPR/Cas9) (LAI) and consequences on the promastigote forms (LAI) + macrophage gene modifications (TAGC)

Year 2: 50/50: consequences of the inhibition/overexpression of the different *Leishmania* genes on the amastigote form (LAI) and on macrophages (motility)(LAI) + dual transcriptome profiling (TAGC)

Year 3: 70/30: bioinformatic analysis of the transcriptomic results on infected macrophages (TAGC) and *Leishmania* cells (LAI)

Specify with whom the person recruited will collaborate and on what aspects

At LAI, the PhD student will be supervised by M. Casanova. He/she will collaborate for this project with engineers (Thi Thien Nguyen, Martine Pélicot-Biarnes) and will be able to count on the support of permanent researchers in immunology (mainly Philippe Robert) and on immune cell migration (Marie-Pierre Valignat and Olivier Théodoly). At TAGC, the PhD student will be supervised by S. Marquet. He/she will interact with engineers (Magali Torres and Aurélie Bergon). In addition, LAI actively collaborates with other laboratories that could benefit from the project: (i) Yvon Sterkers at the National Reference Center for leishmaniases (UMR MIVEGEC, Montpellier University) for *Leishmania* gene modifications; (ii) Sébastien Pomel (UMR BioCIS, Paris-Saclay University) for macrophage infection by *Leishmania* cells.

PhD student's expected profile

Biologist with an interest in cellular biology, quantitative biology, biophysics, and bioinformatics. Curiosity, independence and tenacity are some of the "must have" for such an interdisciplinary project.

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PhD PROJECT PROPOSAL

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This project is an entirely new one.

Two to five references related to the project

- Obeid S, *et al.* Deciphering the mechanism of action of VP343, an antileishmanial drug candidate, in *Leishmania infantum*. iScience. 2023;26(11):108144. doi: 10.1016/j.isci.2023.108144.
- Basmaciyan L, Casanova M. Cell death in *Leishmania*. Parasite. 2019;26:71. doi: 10.1051/parasite/2019071
- Basmaciyan L, *et al.* Temporal analysis of the autophagic and apoptotic phenotypes in *Leishmania* parasites. Microbial Cell. 2018;5(9):404-17. doi: 10.15698/mic2018.09.646
- Casanova M, *et al.* Implication of different domains of the *Leishmania major* metacaspase in cell death and autophagy. Cell Death Dis. 2015;6:e1933.

Two main publications from each PI over the last 5 years

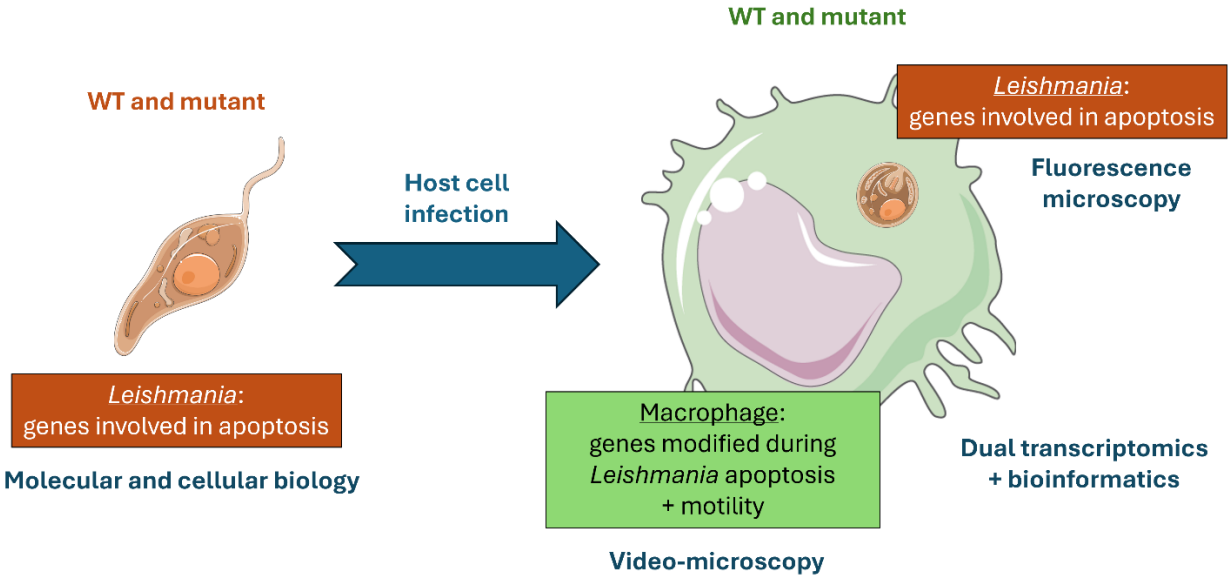
M. CASANOVA

- Klionsky DJ *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy 2021;17(1):1-382. doi: 10.1080/15548627.2020.1797280
- Basmaciyan L, Casanova M. Cell death in *Leishmania*. Parasite. 2019;26:71. doi: 10.1051/parasite/2019071

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- Adjemout M, *et al.* Unraveling an enhancer-silencer regulatory element showing epistatic interaction with a variant that escaped genome-wide association studies. Cell Genom. 2025;5(7):100889. doi: 10.1016/j.xgen.2025.100889.
- Adjemout M, *et al.* Concurrent PIEZO1 activation and ATP2B4 blockade effectively reduce the risk of cerebral malaria and inhibit *in vitro Plasmodium falciparum* multiplication in red blood cells. Genes Dis. 2023 Mar 27;10(6):2210-2214. doi: 10.1016/j.gendis.2023.02.029.

Project's illustrating image



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