2021-2024 – PhD project
"Role of extracellular vesicles in host-pathogen interactions and virulence of *Mycobacterium tuberculosis*"

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Tuberculosis is one of the 10 leading causes of death in the world (WHO, 2018) (1). The etiological agent, *Mycobacterium tuberculosis* (Mtb), infects alveolar macrophages and establishes a chronic infection in humans through its ability to adapt to intracellular conditions and host immune responses (2). A better understanding of the host-pathogen interactions of this infection is necessary for the development of more effective means of control. Every living cell releases extracellular vesicles (EV) that shuttle diverse molecules (lipids, proteins, nucleic acids) and play important roles in intercellular communication, regulation of immune responses or bacterial virulence (3). Similarly, infected cells as well as the bacillus release EV during Mtb infection. If the host-pathogen interaction of this infection is essentially studied at the direct interface between the 2 organisms, the diffusion of bacterial and eukaryotic factors within EV most likely contribute significantly to these interactions at the infection site and beyond (4, 5). However, the characterization of the composition and immunomodulatory properties of these EV remains largely incomplete.

In this context, the aim of this project is to better characterize the interactions of these vesicles with the preferred target of Mtb: the macrophages. Their capacity to regulate the inflammatory and microbicide properties of macrophages. In particular, we will study the capacity of EV to activate and induce cytokine production and autophagy, via their interaction with innate immunity receptors or Pattern Recognition Receptors (PRRs), such as Toll Like Receptors (TLRs) or C-type lectins. Their intracellular trafficking, which conditions their immunomodulatory properties, will be analysed by super-resolution microscopy. The vesicles released in presence of mycobacteria of variable virulence will be studied comparatively in these different assays. In order to decipher the molecular basis of their immunomodulatory properties, we will undertake to characterize their content in PRR ligands using omic-type methods, among others.

Given their relevance for the study of host-pathogen interactions, a better characterization of EV will provide important insights into Mtb pathogenesis.

Methods: cell and mycobacterial culture, vesicle purification (density gradient, exclusion chromatography), TEM and super-resolution microscopy, activation assays of reporter and functional bioassay on primary cells, flow cytometry, Western blot, ELISA, mass spectrometry.

Application: This PhD project, starting Sept-Oct 2021, is funded by an ANR grant. Motivated candidates with a Master's degree should apply on the CNRS website at https://bit.ly/3exTRqY by providing a CV (including mail contact of references) and a cover letter.

(**1**) WHO. Global Tuberculosis Report 2018. https://www.who.int/tb/. (**2**) Ernst J. D. Mechanisms of *M. tuberculosis* Immune Evasion as Challenges to TB Vaccine Design. Cell Host Microbe 2018, 24(1):34-42. (**3**) Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol. 2014, 14(3):195-208. (**4**) Prados-Rosales R, et al. Mycobacteria release active membrane vesicles that modulate immune responses in a TLR2-dependent manner in mice. J Clin Invest. 2011, 121(4):1471-83. (**5**) Layre E. Trafficking of Mycobacterium tuberculosis Envelope Components and Release Within Extracellular Vesicles: Host-Pathogen Interactions Beyond the Wall. Front Immunol. 2020, 11:1230.