2021-2024 – PhD project

"Structural and functional characterization of new immunomodulators of *Mycobacterium tuberculosis*"

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According to the World Health Organization, Tuberculosis remained one of the top 10 causes of death worldwide in 2018 (1.4 million deaths) (1). *M. tuberculosis (Mtb)* infects alveolar macrophages and is extremely successful in establishing a chronic infection in humans. Indeed, it has evolved strategies to evade immune responses and to persist within the hostile intracellular environment (2). The current lack of efficient anti-tuberculosis strategies is largely due to our incomplete understanding of the host-pathogen interactions of Mtb infection. One characteristic of mycobacteria is the biosynthesis of a highly diverse repertoire of lipids and glycolipids capable to regulate host immune responses, acting as pathogen-associated molecular patterns, virulence factors or T cell antigens (3, 4). However, this repertoire of potent immunomodulators has been essentially characterized by studying bacteria broth cultures and using conventional biochemistry methods of moderate resolution and sensitivity. We have set-up a last-generation lipidomic approach, comprising high sensitivity mass spectrometry and bioinformatics tools, which allows studying this repertoire in a global and exhaustive way in diverse conditions (5, 6). Applied to the analysis of *in vitro* cultures but also to infected cells, such approach has already highlighted several new molecules of unknown structure and functions including molecules that are specifically produced by virulent *M. tuberculosis* strains. The characterization of such molecules might reveal new immunomodulatory mechanisms and give rise to new concepts for the development of anti-TB tools. Hence, this project aims at:

- purifying molecules highlighted by lipidomic analyses (cell/mycobacterial cultures, normal and reverse phase chromatography)
- characterising their structure through the use of state-of-the-art biochemistry tools available at the IPBS and at the MetaToul platform (bioinformatics, mass spectrometry, MS/MS, NMR, capillary electrophoresis)
- achieving the functional characterisation of the newly identified bacterial molecules, in particular their capability to interact with innate immune receptors and to regulate macrophages inflammatory and microbicide properties (ELISA, reporter cell lines activation assays and functional bioassays on primary cells)

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(**1**) WHO. Global Tuberculosis Report 2020. 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**) Ishikawa E et al. Recognition of Mycobacterial Lipids by Immune Receptors. Trends Immunol. 2017, 38(1):66-76. (**4**) Neyrolles O, Guilhot C. Recent advances in deciphering the contribution of Mycobacterium tuberculosis lipids to pathogenesis. Tuberculosis. 2011; 91(3):187-95. (**5**) Layre E, et al. A comparative lipidomics platform for chemotaxonomic analysis of Mycobacterium tuberculosis. Chemistry & biology. 2011, 18(12):1537-49. (**6**) Layre E, et al. Molecular profiling of Mycobacterium tuberculosis identifies tuberculosinyl nucleoside products of the virulence-associated enzyme Rv3378c. Proc Natl Acad Sci U S A. 2014, 111(8):2978-83.