M2 project – 2021-2022

Supervisors: Emeline Fabre & Jérôme Nigou
Team "Immunomodulation by Mycobacterial Lipids & Glycoconjugates" (Resp: J. Nigou)
Institut de Pharmacologie et de Biologie Structurale (IPBS), UMR 5089 CNRS/UPS
205 Route de Narbonne, 31077 Toulouse cedex 4
Tel: 0561175507, Emeline.fabre@ipbs.fr ; Tel: 0561175554, jerome.nigou@ipbs.fr;
http://www.ipbs.fr/index.php/immunomodulation-mycobacterial-lipids-and-glycoconjugates

Title: **Identification of *Mycobacterium tuberculosis* binding site on the C-type lectin Dectin-1**

Innate immunity is the first line of host defence against invading microorganisms. It is based on the detection of invariant molecular signatures (PAMPs) that are unique to microorganisms by pattern recognition receptors (PRRs). Dectin-1 is a C-type lectin receptor that recognizes 1,3-β-glucans from yeast and fungi cell walls.

Several reports indicate that Dectin-1 is also involved in the recognition of *Mycobacterium tuberculosis*, the aetiological agent of human tuberculosis, although this major human pathogen does not produce 1,3-β-glucans. Using a recombinant soluble form of murine Dectin-1, composed of the receptor extracellular domain fused to a Fc fragment (mDectin-1-Fc), our team has identified a glycolipid ligand in the cell envelope of *M. tuberculosis*. Moreover, we have found that this ligand is recognized by a binding site that is distinct from that of 1,3-β-glucans.

The objective of the Master internship will be to identify and map this new binding site on Dectin-1 receptor. To achieve this, the three-dimensional structure of mDectin-1, resolved in 2007, will be observed in details, and compared with that of other lectin receptors, capable of binding ligands belonging to the same structural family as the mycobacterial glycolipids. Predictions will be made regarding the specific role of the amino acids composing the putative binding site(s), in the anchoring of the different parts of the ligand molecule. These predictions will then be validated experimentally, by site-directed mutagenesis, production (in CHO cells) and purification of the murine and human m/hDectin-1-Fc mutants, and finally by testing the ability of the latter to bind *M. tuberculosis* glycolipid ligand as well as whole bacteria. In parallel, the recombinant production of mDectin-1 and hDectin-1 in *E. coli* will be developed for the purpose of co-crystallization with the mycobacterial ligand.

(The project will be performed in collaboration with the Toulouse biotech InvivoGen)

Techniques: site-directed mutagenesis, cell cultures of (*E. coli*, CHO), protein purification, western-blot, ELISA, FACS.

Selected publications of the team on this topic:

- Decout A *et al.* (2017) PNAS, 114(10):2675.
- Blanc L *et al.* (2017) PNAS, 114(42):11205.
- Vergne I *et al.* (2015) Front Cell Infect Microbiol, 4:187