Principal Investigator: Cedric Laguri

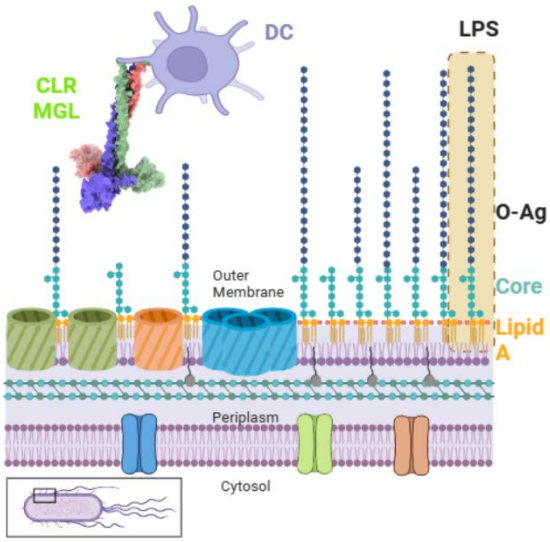
Grant Title: Innovative membrane mimetics systems to study LPS-lectin binding specificity.

Abstract.

The surface of Gram-negative bacteria is covered with LPS glycolipids in which lipid and glycans are detected by the immune system. C-type Lectins Receptors (CLRs), at the surface of dendritic cells, interact with the glycan part of LPS and trigger immune responses. CLRs are multimeric proteins for which multivalence enables high affinity interaction with glycans, especially at the surface of pathogens. The intent of the present project is to use nano-objects resembling the bacterial surface to study CLRs interactions with LPS from different bacterial strains including pathogens in an experimental setup mimicking interaction at the surface of cells. A combination of biophysical methods will investigate the determinants of the interactions of a CLR in term of LPS structure and organization.

Objectives.

Our objectives are mainly to understand and quantify, at the molecular level, the recognition of LipoPolySaccharides at the surface of bacteria by human C-type lectin receptors (CLR) by biophysical methods. In order to obtain information about these interactions we must tackle the complexity of manipulating LPS, glycolipids which, isolated, assemble into diverse membrane structures. Furthermore, LPS structures are heterogeneous depending on the bacteria strain and the presence or absence of O-antigens on LPS alters the recognition of bacteria by immune proteins.



Methods used.

To investigate recognition of the bacterial surface we use fluorescence microscopy and flow cytometry to visualize and quantify CLR binding at the bacterial surface of different bacterial strains. To have access to the molecular determinants, LPS from different bacteria are extracted, purified, and used as ligands in either membrane form, detergent micelles or nanoparticles. Immobilized interaction methods allow the determination of the affinity and kinetic parameters and Nuclear Magnetic Resonance the determinants at the molecular level.

Results[1]

Through integrated structural biology methods, we could determine the 3D structure of Macrophage Galactose Lectin and its interaction surface with LPS. The structure of MGL dictates its recognition of glycans at cell surfaces, and we could also show it is able to differently bind to LPS depending on the presence and absence of O-antigens in laboratory and pathogenic *E. coli* strains.

Abbas, M., Maalej, M., Nieto-Fabregat, F., Thépaut, M., Kleman, J.-P., Ayala, I., Molinaro, A., Simorre, J.-P., Marchetti, R., Fieschi, F., & Laguri, C. (2023). The unique three-dimensional arrangement of macrophage galactose lectin enables *E. coli* LipoPolySaccharides recognition through two distinct interfaces. *PNAS Nexus*. https://doi.org/10.1093/pnasnexus/pgad310