PhD Curriculum: Cellular and Molecular Physiopathology

Title: **Combating inflammation: uncovering the mechanism of secretion of the leaderless pro-inflammatory cytokine IL-1β**

Interleukin-1-beta (IL-1β) is a leaderless cytokine mainly secreted by Toll Like Receptor (TLR)-stimulated monocytes through a non-classical pathway\(^1\). In spite of many efforts, the molecular mechanisms underlying IL-1b processing and secretion are largely unknown: their understanding is the goal of this project. Explaining the non classical mechanism of IL-1β secretion would not only solve a crucial puzzling problem in biology, but also have a great translational relevance. In fact, IL-1β-mediated inflammation underlies many acute and chronic diseases\(^2\).

Data from our labs indicate that in monocytes stimulated by TLR agonists IL-1β is externalized through vesicles belonging to the endo-lysosomal\(^3\) and/or autophagic compartments, expressing the Chaperone-mediated autophagy (CMA) marker Lamp2a\(^4\). These vesicles undergo exocytosis releasing IL-1β outside the cells. Recently, a different mechanism has been proposed, suggesting that following stronger stimuli, higher amounts of IL-1β are externalized through pyroptosis-induced membrane holes\(^5\)\(^,\)\(^6\). These findings suggest the activation of different secretory routes according to the nature of the stimulus. In fact, we have found compounds that specifically affect one mechanism or the other.

Objectives and Experimental plan:
On these bases, we will pursue the following goals:
1. Characterization of IL-1β-containing vesicles in TLR-stimulated human monocytes: a. morphological, by microscopy (confocal, TIRF, super-resolution analyses); b. biochemical, by immunoisolation of vesicles using Ab to lysosomal or autophagic markers, followed by proteomic analyses.
2. Demonstration that LAMP2a mediates translocation of IL-1β across the vesicle membrane. Using CRISPR-Cas9 technology in a monocytic cell line, we will delete: a. the KFERQ like motifs in pro-IL-1β, which mediates cytosolic protein translocation in LAMP2a+ vesicle for CMA; b. The cytosolic tail of the LAMP2a isoform. The ability of WT and mutants to translocate IL-1β into vesicles and secrete it will be evaluated.
3. Definition of the involvement of pyroptosis in the mechanism of IL-1β release: we will mimick the strong stimulus with the simultaneous stimulation of three different TLR and study vesicles dynamics and number, paralleled by the measurement of the extracellular IL1-β and lactate dehydrogenase.
4. Characterization of compounds inhibiting the above mechanisms and their development as anti inflammatory drugs. These studies will be done on monocytes from normal subjects and from patients with diseases characterized strong inflammation (Autoinflammatory syndromes; Chronic Granulomatous Disease; sepsis)\(^7\).

References

of the leaderless protein interleukin 1beta involves exocytosis of endolysosome-related vesicles. Mol Biol Cell. 1999, 5: 1463-75.


