Mitochondrial Ca2+-dependent inlfammasome activation exacerbates the *P. aeruginosa*-driven inflammatory response

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**Background.** The chronic airway infection by *Pseudomonas aeruginosa* (*P. aeruginosa*) in Cystic Fibrosis (CF) is a pathological manifestation associated with an excessive inflammatory response. The NOD-like receptors (NLRs) are intracellular pattern-recognition receptors that recognize pathogen-associated molecular patterns activating pro-inflammatory response through the assembly of large caspase-1-activating complex, called inflammasome. Recent data suggest that the mitochondria integrate distinct pro-inflammatory signals and relay this information to a key molecular complex related to inflammation, the inflammasome. It has been demonstrated that mitochondria are the main source of inflammasome-activating radical species and as such may constitute the signal-integrating organelle for inflammasome recruitment and activation in *P. aeruginosa*-driven inflammation in CF. Moreover, in the previous project we have demonstrated that mislocalized CFTR is associated with an increase in intracellular Ca2+ content and that it favored the mitochondrial Ca2+ uptake, predisposing the organelle to a major stress responsivity.

**Hypothesis and objectives.** The overall goal of this project is to broaden our knowledge on role of mitochondria to decode and integrate the pro-inflammatory signals generated during *P. aeruginosa* infection in CF. We have performed experiments aimed at obtaining a deeper insight into the complex mechanism that controls the mitochondrial homeostasis, in particular the Ca2+ signal, and the inflammasome activation induced by pathogen in CF.

**Methods.** The mitochondrial dysfunction is direct link between *P. aeruginosa* infection and inflammasome recruitment in CF epithelial airways cells. On these topics: Ca2+ signaling and mitochondrial function; the expertise gained and the previous works by the PI and collaborators represent the methodological and cultural background of this grant.

**Results.** Summarizing, in these two years we have demonstrated that:
- *P. aeruginosa* affects mitochondrial Ca2+ signaling and physiology.
- Flagellin is an inducer of mitochondrial dysfunction.
- The degree of *P. aeruginosa*-dependent inflammatory response depends on defective CFTR.
- *P. aeruginosa* promotes NLRP3 activation in CF cells
- Mitochondrial Calcium Uniporter (MCU) links *P. aeruginosa*-dependent mitochondrial dysfunction to NLRP3 activation.

**Spin-off for research and clinical purposes.** The translational aim of this project is identified alternative strategy for treating exacerbated *P. aeruginosa*-triggered inflammation in CF. Our results indicate MCU as target for therapeutic approach focused to rescue the mitochondrial physiology, counteracting tissue degeneration and mitigating the inflammatory response.

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**References**


5. Rimessi, A., et al. (2015), Mitochondrial Ca(2+)-dependent NLRP3 activation exacerbates the Pseudomonas aeruginosa-driven inflammatory response in cystic fibrosis, Nature communications 6, 6201 https://doi.org/10.1038/ncomms7201 PMid:25648527