Mitochondrial Ca2+-dependent inflammasome 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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