ImmunoTools *special* Award 2015

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Possible therapeutic implications of the individual and combined effects of different cytokines on the sterile inflammatory and stress signalling pathways occurring in CFTR-deficient human cell lines.

I recently joined a laboratory committed since 20 years to the study of the cystic fibrosis disease (CF) in models of human cell lines. These cells are employed to study CF-dependent transcripts and inflammatory/oxidative stress signalling. For the same purposes, we started to use transgenic KO mice as a CF model. CF is triggered by genetic defects in the CFTR (cystic fibrosis transmembrane conductance regulator) gene. It encodes a trans-membrane protein whose main function is to be a chloride channel. CF affects many organs, although the main impact is in the airways, pancreas and intestine. Dysfunction of mutant CFTR in the airway epithelial cells results in abnormal chloride transport leading to decreased mucus clearance, to ionic misbalance, and subsequent colonization by different bacteria. This results in chronic pro-inflammatory signalling. CF is a lethal genetic disease that typically results in bronchial inflammation, bronchiectasis, altered innate immunity, progressive loss of lung function and, ultimately, in death. Patients with CF have elevated concentration of cytokines in the sputum and a general inflammatory condition. In addition, CFTR-deficient cells in culture produce diverse cytokines in excess, including IL-1β (see references in 2).

Our laboratory reported in 2012 that the mitochondrial complex I (mCx-I) activity is reduced in cell lines with impaired CFTR function (1, 3). In 2014 we reported that this is mainly due to an increase in the level of IL-1β (2). Results suggested that in CF cells IL-1β, through an autocrine effect, connects the defective CFTR with the reduced mCx-I activity and the increased oxidative stress (reactive oxygen species, ROS). The results also suggest that reagents blocking the IL-1β pathway and inhibitors of NF-kB or p38MAPK, acting downstream of the IL-1 receptor, might have therapeutic significance for CF. The IL-1β autocrine effects over mCx-I and ROS were observed in cells in the absence of bacteria. Thus, it appears to be a primary, default, spontaneous and intrinsic characteristic of CF cells which generate a *sterile inflammation vicious circle*. Therefore, CFTR defects may lead, *per se*, to a basal inflammatory insult which then might be further exacerbated by infection and/or cytokines other than IL-1β. To distinguish precisely between sterile and *infection-dependent* inflammatory pathways, is relevant for the search of CF-specific protein biomarkers and also for diagnostic, prognostic and therapeutic purposes.

CF is a complex disease and it is not clear which event(s) is a cause and which one is(are) a consequence of the chronic inflammation. In humans, the temporal and causal relations between the airway inflammation and its infection have remained uncertain. Besides, each patient´s genetic background may affect the inflammatory outcome. At the cell level, miss-folding of defective CFTR protein leads, in parallel, to activation of many other stress signalling pathways initiated by or converging on the endoplasmic reticulum stress pathway(s). These stress pathways increase and are increased by cytokines (like IL-1β). Therefore, to find drug targets and biomarkers for CF it will be ideal to study the bidirectional and multi-directional cross-talks between the cytokine networks and the other stress pathways altered simultaneously in CFTR-deficient cells. The complexity of CF is further increased by infection which activates many other pathways overlapping with the initiated by pro-inflammatory cytokines. Therefore, precise characterization of the *infection-independent pathways* and the effects on CF cells of individual cytokines, other than IL-1β, will improve our knowledge of the spatio-temporal regulation of the altered cytokine cascades and stress pathways occurring in CF.

Due to the expected therapeutic importance for CF, we will determine, among other tasks, if IL-1β is the only cytokine able to trigger the events reported by us (1, 2, 3) or if other NF-κB- and p38-activating pro-inflammatory cytokines are able to do so (or are even synergic with IL-1β). This will be relevant when the IL-1β pathway will get neutralized in our models. Hopefully, the data collected will be useful to get a deeper understanding of CF and, indirectly, of other chronic inflammatory diseases in which pro-inflammatory cytokines and oxidative/ER stress promote each other generating a vicious circle. Altogether, this will be useful for future studies of the possible influences of the cell-type/tissue affected and of the patient´s genetics.

The cytokines requested will be invaluable tools to materialize our projects concerning inflammation and stress in cell models of CF disease. The ImmunoTools Award will be extremely useful for the development of our research and for the reproducible dissection of the contribution of different cytokines to the inflammatory phenotype of CF cells. It certainly will allow us to quickly move forward with our cytokine-related projects.

References.

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2- Clauzure M, Valdivieso AG, Massip Copiz MM, Schulman G, Teiber ML, Santa-Coloma TA. (2014). **Disruption of Interleukin-1β Autocrine Signaling Rescues Complex I Activity and Improves ROS Levels in Immortalized Epithelial Cells with Impaired Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Function**. PLoS One. 2014 Jun 5;9(6):e99257. doi: 10.1371/journal.pone.0099257.

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