

169* Bactericidal activity of human cystic fibrosis macrophages against *Pseudomonas aeruginosa*

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Chronic inflammation of the lung, as a consequence of persistent bacterial infections by several opportunistic pathogens represents the main cause of mortality and morbidity in cystic fibrosis. Mechanisms leading to increased susceptibility to bacterial infections in CF are not completely known, although the involvement of cystic fibrosis transmembrane conductance regulator (CFTR) in microbicidal functions of macrophages is emerging. Tissue macrophages differentiate *in situ* from infiltrating monocytes, additionally, mature macrophages from different tissues, although having a number of common activities, exhibit variation in some molecular and cellular functions. In order to define possible intrinsic macrophage defects due to CFTR deficiencies we have analyzed the bactericidal activity against *P. aeruginosa* of human monocyte derived macrophages from CF patients. First, by real time PCR, immunofluorescence and patch clamp recordings we demonstrated CFTR expression and functional activity in control macrophages. Subsequently, we evaluated the bactericidal activity of control and CF macrophages infected by *P. aeruginosa*. Our results demonstrate that control and CF macrophages do not differ in the phagocytic activity but, although a reduction of intracellular live bacteria was detected in both groups, the percentage of surviving bacteria was significantly higher in CF macrophages. These findings further support the role of CFTR in the bactericidal activity of innate immune cells.

170 BPI-ANCA correlates better with lung function impairment than bacterial serology

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Background: Autoantibodies against the neutrophil derived bactericidal/permeability increasing protein (BPI-ANCA) have been shown to exhibit a strong correlation with lung function impairment among CF patients chronically colonized with *Pseudomonas aeruginosa* (Pa). However, the test has never been compared with different serological assays for Pa colonization.

Methods: After informed consent all non-transplanted CF patients belonging to the CF centre in Lund, Sweden, who could perform standard spirometry were included in the study. Bacterial colonisation was classified according to the Leeds criteria. IgA and IgG BPI-ANCA was measured with ELISA and results were compared to commercially available assays for Exotoxin A, Alkaline protease and Elastase.

Results: BPI-ANCA was measured in 118 patients, of whom 51 (43%) were classified as chronically colonized with Pa. The median age in the chronically colonized group was 20.2 years and 17 patients (33.3%) were below the age of 18. One patient was too young to have the FEV1% calculated. The median FEV1.0 was 63% of predicted. Overall the serology tests correlated better to each other (r values: 0.39; 0.46 and 0.56) than they did with IgA-BPI-ANCA (r values: 0.02; 0.14 and 0.22). The correlation between serological test results and lung function impairment (100-FEV1.0%) was 0.45 for BPI-ANCA compared with 0.08 (exo), 0.24 (ela) and 0.39 (alk pr) for the three bacteriological tests.

Conclusion: BPI-ANCA measures something different from regular serological tests and seems to correlate better with disease severity.

171* The chitinase-like protein YKL-40 modulates cystic fibrosis lung disease

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Background: YKL-40 – a chitinase-like protein – was increased in patients with severe asthma and chronic obstructive pulmonary disease (COPD). Neutrophils secrete YKL-40. We hypothesized that increased YKL-40 levels in lung diseases reflect neutrophilic inflammation and that YKL-40 accumulates in the airways of cystic fibrosis patients (CF), a prototypic neutrophilic airway disease. We aimed to analyze YKL-40 levels in human and murine CF lung disease compared to COPD, asthma and control subjects.

Methods: YKL-40 protein levels were measured in serum and sputum samples of CF, asthma, COPD and healthy individuals. Protein levels of the murine homologue BRP-39 were quantified in airway fluids from CF-like bENaC-Tg mice.

Results: YKL-40 levels were significantly increased in human and murine CF airway fluids compared to asthma, COPD and healthy individuals. In both CF patients and bENaC-Tg mice, YKL-40/BRP-39 airway levels correlated with severity of obstructive lung disease.

Conclusion: YKL-40 is increased in CF airway fluids and is negatively associated with pulmonary function. These findings suggest YKL-40 as potential biomarker and therapeutic target in CF lung disease.

172 Alpha-lipoic acid as a potential anti-inflammatory treatment in cystic fibrosis

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Increased levels of Reactive Oxygen Species (ROS) and Tissue Transglutaminase (TG2) SUMOylation are present in CF airway, leading to persistence of high TG2 levels. This drives cross-linking and aggresome accumulation of TG2 substrate proteins, as PPAR γ and beclin1, leading to inhibition of autophagy and airway inflammation. ROS scavengers or TG2 inhibitors restore the cellular homeostasis by rescuing autophagy, controlling airway inflammation and favoring surface localization of mutant CFTR [1].

Objectives: Our aim is to demonstrate the efficacy of α -lipoic acid (α LA), already reported as an antioxidant which is also involved in autophagy [2], in controlling airway inflammation *in vitro* and *in vivo* in CF animal models [1].

Methods: IB3-1 and C38 cells were added with 500 μ M α LA. CF mice and control littermates were administered with α LA (100 mg/kg *i.p.* for 7 days). MIP2, adhesion molecules, inflammatory cytokines were assessed by ELISA; TG2, PPAR γ proteins by western blots, TG2 SUMOylation and activity and leukocyte infiltration by confocal microscopy and FRET both *in vitro* and *in vivo*.

Results: α LA decreased TG2 SUMOylation, TG2 protein and activity and increased PPAR γ protein levels both *in vitro* and *in vivo*. Moreover α LA reduced MIP2 and adhesion molecule (p < 0.005) and leukocyte infiltration (p < 0.05) in CF mice.

Conclusion: The treatment with α LA is effective in reducing sustained TG2 activation and their downstream effects in CF epithelia and in ameliorating airway inflammation in CF mice. This could open the door for the implementation of a new class of drugs to control chronic lung inflammation in CF patients.

Reference(s)

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