**Analysis of CFTR expression in immune cell subsets of peripheral blood**


**Background:** Cystic fibrosis lung disease is characterized by recurrent pulmonary infections and a chronic inflammatory response. The underlying mechanisms responsible for the inflammatory phenotype are still debated. CFTR dysfunction in epithelial cells is generally accepted to play a role in disease. Several reports also suggest CFTR mutations to contribute to chronic inflammatory conditions due to an intrinsic defect of the immune system. Limited reports directly showed CFTR expression in human PBMC using RT-PCR, western blot and indirectly by patch clamp analysis. Here, we studied CFTR expression in multiple immune cell subsets isolated from PBMC using a variety of techniques.

**Methods:** To examine CFTR expression in immune cell subsets, we separated PBMC and granulocytes from peripheral blood of healthy donors using ficoll density centrifugation. CD4, CD8, CD19 and CD14 positive immune cells were sorted from PBMC using flow cytometry. CFTR expression was measured by RT-PCR, western blot, immunofluorescence, and invitro phosphorylation by PKA.

**Results:** Preliminary results show no detectable CFTR expression in any immune cell subset.

**Conclusions:** Thus far, we cannot confirm previous reports indicating CFTR expression in PBMC.

**Decreased expression of Nod2-receptors in cystic fibrosis airway epithelial cells**


The innate immunity system has a primary role in the defence of the lung against invasive pathogens. Nod-like receptors, an important family of receptors that forms part of this defence system, have been an area of increasing interest lately. Activated Nod-like receptors give rise to the formation of inflammasomes, protein complexes that eventually lead to the secretion of pro-inflammatory cytokines. Changes in Nod-like receptors have been associated with a number of diseases. In the current study, we investigate the possible expression of Nod1 and Nod2 receptors on cystic fibrosis (CF) and non-CF bronchial epithelial cells.

Cystic fibrosis (CFBE) and normal (16HBE) airway epithelial cells were cultured under standard conditions in M199 medium at 37°C. Reverse transcription of RNA to cDNA was performed with TaqMan Reverse Transcription Reagents (Applied Biosystems). Samples were analyzed by RT-PCR. The expression of Nod1 and Nod2 was analyzed by Western blot (using a goat-anti human antibody and a rabbit-anti human antibody, respectively).

No difference in the expression of Nod1 could be observed with either method between CF and non-CF cells. However, an approximately 30% lower mRNA and protein expression for Nod2 were found in CF cells compared to non-CF airway epithelial cells.

Defective Nod2 signalling may contribute to the pathogenesis of chronic lung disease observed in CF patients.

**Influence of CFTR mutations on bacterial activity of human macrophages**

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It has been recently reported that the absence of CFTR alters the bactericidal activity of murine alveolar macrophages. In order to evaluate the role of CFTR in human macrophages we have first analyzed the CFTR expression in monocytederived macrophages and subsequently we compared the bactericidal activity of macrophages from CF and control subjects. Analysis of CFTR mRNA expression by real time PCR in macrophages and parental monocytes isolated from twelve healthy donors demonstrated an up-regulation of the mRNA levels in macrophages from eight subjects.

Additionally we demonstrate the presence of the CFTR protein in human monocyte-derived macrophages by confocal microscopy.

The bactericidal activity of in vitro differentiated macrophages from nine CF subjects and seven healthy subjects was determined by the antibiotic protection assay and the clinical isolate of Pseudomonas aeruginosa, PA27853. Viable bacteria in cell lysates were determined at the end of gentamicin treatment (60), and after 2 (t2) and 4 (t4) hours of incubation in antibiotic-free medium. Overall we observed that CF and control macrophages did not differ in the ability to internalize and kill P pseudmonas although, when the data were analysed together, the mean value of live bacteria 4 hr post infection, was higher in CF than in HD macrophages (P=0.032). Our results demonstrate the expression of CFTR in human monocyte-derived macrophages and suggest that CFTR might be involved in bactericidal activity.

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