

216* Allergic inflammation produces a hypersecretory airway ion transport phenotype in mice

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Abnormal airway ion transport plays a central role in the pathogenesis of cystic fibrosis (CF) lung disease. Cytokines released in allergic airway inflammation (IL-4 and IL-13) have been reported to be potent mediators of ion transport in airway epithelia in vitro. To determine the impact of allergic airway inflammation on airway ion transport in vivo, we challenged BALB/c mice with a natural aeroallergen, i.e. *Aspergillus fumigatus* extract (Af), by intratracheal instillation, and studied the effects on ion transport in freshly excised airway tissues in Ussing chambers, and on mRNA expression of the epithelial Na⁺ channel (ENaC) by real-time RT-PCR. Af challenge induced a robust allergic airway inflammation with elevated IL-13 and eosinophils in the airways. Amiloride-sensitive ENaC-mediated Na⁺ absorption was significantly reduced ($\Delta I_{sc-allergic} = 22.2 \pm 3.2 \mu A/cm^2$ vs. $\Delta I_{sc-naive} = 32.7 \pm 3.8 \mu A/cm^2$, n = 18–21 per group, P < 0.05) and Ca²⁺-activated (UTP-mediated) Cl⁻ secretion was increased ($\Delta I_{sc-allergic} = 72.8 \pm 11.0 \mu A/cm^2$ vs. $\Delta I_{sc-naive} = 45.8 \pm 7.0 \mu A/cm^2$, n = 18–21 per group, P < 0.05) in lower airway tissues from allergen-challenged compared with naive mice. Moreover, allergic inflammation caused a significant reduction in α , β , and γ ENaC expression. We conclude that allergic inflammation inhibits ENaC-mediated Na⁺ absorption and enhances CaCC-mediated Cl⁻ secretion thereby producing a hypersecretory phenotype in vivo. This could be of relevance for CF patients with confounding allergic airway disease including allergic bronchopulmonary aspergillosis. Supported by: Deutsche Forschungsgemeinschaft (DFG MA 2081/3–2), Ernest-Solvay-Stiftung (T077/17752/08).

218* Azithromycin is not able to decrease inflammatory process in cystic fibrosis human bronchial epithelial cells

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Objective: The aim of this study was to explore the effects of macrolides: azithromycin (AZM), clarithromycin (CAM) and erythromycin (EM) on molecular mechanisms involved during inflammation in bronchial epithelial cystic fibrosis (CF) cells.

Methods: Human CF bronchial epithelial (IB3–1) and CFTR-corrected bronchial epithelial cell lines (S9) were pre-treated 30 minutes with the macrolide (10 μ g/ml) and TNF- α (10 ng/ml) was added for additional 16 h of culture. To confirm results, we have treated with the same protocol human bronchial gland cells (HBG) isolated from non-CF patients with or without the CFTR inhibitor Inh-172 (10 μ M, 72 h). IL-8 concentrations were evaluated in culture supernatants by ELISA and NF- κ B pathway was investigated by a p65-luciferase plasmid at 4 h and 8 h of treatment.

Results: CAM and EM were not able to modulate TNF- α induced IL-8 secretion. Interestingly AZM significantly reduced TNF- α induced IL-8 secretion in non-CF cells (S9 and HBG cells) but not in CF cells (IB3–1 cells and HBG cells treated with the CFTR inhibitor Inh-172). Next, we have investigated molecular mechanisms after 4 h and 8 h of treatment. We have demonstrated that AZM significantly decreased TNF- α induced NF- κ B transcriptional activity in S9 but not in IB3–1 cell line.

Conclusion: Our study demonstrates that azithromycin is not able to decrease inflammation in CF bronchial epithelial cells and suggests that CFTR chloride function may be involved in normal cells response to AZM.

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217* Accumulation of NKT cells in tissues of cystic fibrosis mice

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In lung tissues of patients suffering from cystic fibrosis (CF), a hereditary disease caused by mutations in the membrane bound chloride channel CFTR, and in uninfected CF mice, we have detected an abnormal accumulation of ceramide (Teichgräber et al., Nature Med 2008;14:382). Ceramide accumulation provoked an age-dependent recruitment of macrophages and neutrophils in lungs of uninfected CF mice which was abrogated by administration of amitriptyline, a blocker of acid sphingomyelinase. Since endogenous glycolipids are known to trigger NKT cell maturation and proliferation, we hypothesized that ceramide accumulation also triggers NKT cell accumulation in tissues of CF mice, expressing mutated CFTR such as the intestine and lungs, but not in CFTR-negative tissues such as the liver. We homogenized in-testine, liver and lungs of CF and control mice at various ages (12–91 weeks), iso-lated the T cell fraction and quantitatively determined NKT cells using CD1 d-aGalCer tetramer-staining. We present evidence that the numbers of NKT cells present in the intestine and lungs of CF mice are significantly higher compared to wild type (WT) mice (NKT intestine CF: 5.36×10^4 NKT cells; WT: 3.33×10^3 ; lung CF: 8.14×10^4 NKT cells; WT: 1.92×10^4). No difference was observed in the liver (NKT liver CF: 7.76×10^5 NKT cells; WT: 7.16×10^5). The results suggest that CF mice represent a new animal model for testing NKT cell functions and drugs with anti-NKT cell activity.

219* Weak anti-inflammatory effects of glucocorticoids on CF bronchial epithelial cells

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Background: Lung dysfunction is the main cause of mortality in CF patients. Infection combined with inflammation lead to progressive destruction of respiratory epithelium. Glucocorticoids (GC) are powerful anti-inflammatory molecules commonly used to treat inflammation but with controversial efficiency among studies. Recent clinical trials showed that inhaled and oral corticosteroids have no significant effect on lung function or markers of inflammation. These results were confirmed in in vitro studies using cell lines and primary cell cultures from CF patients.

Our project focuses on the key steps in GC activation pathway in bronchial epithelial cells to characterise the molecular basis of such dysregulation.

Methods: CF and non CF bronchial epithelial cell lines were incubated with IL-1 β or TNF- α (10 ng/ml) at 4 h, 8 h, and 16 h with or without dexamethasone (dex, 1 μ M). Rates of secreted IL-8 were assessed by ELISA 16 h after treatment. To evaluate NF- κ B or AP-1 activation at 4 h and 8 h, we transfected cells using plasmids containing NF- κ B or AP-1 promoter coupled to luciferase.

Results: In presence of IL-1 β , basal secretion of IL-8 was restored by dex at 16 h in non CF cells, whereas CF cells barely respond. Other results showed there is no effect of dex on NF- κ B activation at 8 h in CF cells.

Conclusions: This study shows a lack of GC efficiency to decrease IL-8 secretion via NF- κ B pathway in CF bronchial epithelial cells.

Identify the origin of the GC resistance in CF will allow to adapt the anti-inflammatory treatments of patients.

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