89* Long acting β2-agonist and corticosteroid restore airway glandular cell functionality altered by *Staphylococcus aureus* virulence factors

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Staphylococcus aureus (S. aureus) releases in the airway lumen virulence factors (VF) that may impair the functionality of airway epithelium. The aim of our study was to determine whether a corticosteroid (fluticasone propionate: FP) combined with a long-acting β2 adrenergic receptor agonist (salmeterol: Sal) was able to regulate CFTR localization, ion content and cytokine expression after exposure of airway glandular cells to *S. aureus* VF.

A human airway glandular cell line was exposed to 2% *S. aureus* VF for 1 h and then incubated with Sal/FP for 4 h. The expression of actin and CFTR proteins was analyzed by immunofluorecence. Videomicroscopy was used to evaluate chloride secretion and X-ray microanalysis to measure the intracellular ion and water content. Pro-inflammatory cytokine expression was assessed by RT-PCR and ELISA.

When the cells were incubated with *S. aureus* VF and then with Sal/FP, CFTR recovered an apical localization compared to the cytoplasmic localization in cells incubated with *S. aureus* VF alone. Sal/FP treatment significantly increased the chloride content ($p < 0.05$) and decreased the S and K content ($p < 0.001$ and $p < 0.05$, respectively) in the intra-cytoplasmic secretory granules, compared to the *S. aureus* VF-treated cells. We also observed that Sal/FP treatment downregulated *S. aureus* VF-induced IL-8 and TNFα expression and release.

Our results demonstrate that treatment with the combination of a corticosteroid and a long-acting β2 adrenergic receptor agonist after bacterial infection may restore the airway glandular cell functionality.

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90* Are basal cells progenitors of the human bronchiolar epithelium?

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In numerous airway diseases such as cystic fibrosis, the bronchiolar epithelium is frequently injured and has to regenerate in order to restore its defense functions. In animal models, it has been proposed that Clara cells could be the bronchiolar stem cells. In human, we have previously demonstrated that bronchial epithelial basal cells expressing specifically CD151 and Tissue Factor (TF) were able to reconstitute a mucociliary differentiated and functional epithelium and then, could be considered as, at least progenitor cells of the bronchial epithelium (Hajj et al., 2007). The aim of our study was to determine the stem/progenitor potential of basal cells of human bronchiolar epithelium.

Human bronchioles were microdissected from lung pieces obtained from surgery and processed either for histological examination and immunohistochemical detection of basal cell markers, or for bronchiolar cells dissociation. After enzymatic dissociation, bronchiolar epithelial cells were prepared for flow cytometry analysis of constitutive cell populations. Our results demonstrated that the human bronchiolar epithelium showed a basal cell layer. Bronchiolar basal cells, expressing specifically cytokeratin 13, exhibited a CD151 staining on their plasma membrane. Analysis by flow cytometry confirmed the CD151 expression by bronchiolar epithelial basal cells which represented 20 to 30% of the total bronchiolar cells. Based on their CD151 expression, bronchiolar basal cells will be sorted in order to determine if they act as progenitor cells during the bronchiolar epithelial regeneration both in vitro in air-liquid interface cultures, and in vivo in humanized xenografts in nude mice.

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91* Epithelial factors produced during the remodelling of the human airway epithelium

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In numerous respiratory diseases such as cystic fibrosis, the airway epithelium is frequently injured and remodelled. It must rapidly regenerate its structure. During the regeneration process, various molecules are produced by the epithelial cells. We have previously shown that epithelial IL-8, matrix metalloproteinases (MMP)-7 and -9 and their inhibitor TIMP-1 were modulated during the non-CF regeneration, MMPs playing a crucial role in epithelial mucociliary differentiation. Moreover, CF bronchial epithelial regeneration, in absence of infection, gave rise to a remodelled epithelium and was associated with deregulation of these molecules. The aim of our study was to determine the expression and secretion of epithelial factors during processes of remodelling such as squamous metaplasia or goblet cell hyperplasia. Human airway epithelial cells from nasal polyps were cultured at the air-liquid interface, and culture conditions were modified to induce remodelling. Cultures were collected at different culture steps for immunohistochemical and electrophysiological studies. Total RNAs were extracted for RT-PCR, and secreted liquids collected for bactericidal activity assessment and zymography. Despite remodelled airway epithelia were able to kill *Staphylococcus aureus*, their bioelectric properties were disturbed. We also showed that IL-8, TIMP-1, MMP-7 and -9 mRNA levels, as well as MMPs activities were modulated during the processes of remodelling. Our results demonstrate that airway epithelial remodelling leads to altered epithelial functions and is associated to deregulation of epithelial factors expression. It will be now important to determine the specific involvement of deregulated factors in the genesis of squamous metaplasia and goblet cell hyperplasia.

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92* Mitochondrial glutathione and decreased complex I activity in cystic fibrosis models

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Cystic fibrosis transmembrane regulator protein (CFTR) transports anions across apical membranes of epithelial cells; among these anions is glutathione (GSH) a major antioxidant. Previous studies show a deficit in mitochondrial GSH (mGSH) in cystic fibrosis (CF) cells. In other cellular models a similar decrease has been shown to lead to complex I (CI) inhibition. Our aim was to investigate the possible relationships between mitochondrial GSH and CI activity in CF models. Mitochondria were isolated from murin colonic epithelia from $cftr^{-/-}$ *vs* $cftr^{+/+}$ mice. We also used mitochondria from two epithelial cell lines derived from CF patients (CFBE Par and IB3) *vs* their corrected cell lines (CFBE WT and C38, respectively). GSH was measured by HPLC with electrochemical detection. CI activity was measured by spectrophometry and native PAGE was used to study its expression. Our results show a significant decrease in CI activity in all three CF models. Interestingly, we were able to mimic this decrease in corrected cells by treating them with a CFTR inhibitor (Inh 172). Decrease in CI activity could not be explained by a decrease in CI expression. Furthermore, no difference in expression was observed after Inh 172 treatment. mGSH measurements confirmed a decrease in CF cells *vs* corrected cells. Using a mitochondrial specific GSH mono ethyl ester we were able to restore mGSH levels in CF cells; this allowed partial recovery of CI activity. Similar results were observed when mitochondria were reduced with DTT. These results suggest that oxidative modifications of certain mitochondrial proteins and possibly CI proteins could be involved in CI inhibition in CF models and are currently under investigation.

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