Short Communication

Effect of Th2 type cytokines on hCLCA1 and mucus expression in cystic fibrosis airways

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Abstract

Correlations between expression of interleukin (IL)-9, the calcium-activated chloride channel hCLCA1 and mucus expression in cystic fibrosis (CF) airways have suggested a causal relationship. To verify this hypothesis mucosal tissue from upper airways of CF patients (N=5) was stimulated with the Th2 type cytokines IL-4, IL-9, or IL-13. Expression of hCLCA1 mRNA and protein as well as mucus and mucin (MUC5AC) gene expression was quantified using real time PCR, immunohistochemistry (hCLCA1) and PAS staining (mucus). Th2 type cytokines significantly increased hCLCA1 protein expression (P<0.05) whereas increase in hCLCA1 mRNA expression failed to reach statistical significance (P>0.05). Mucin protein and MUC5AC mRNA expression were not significantly changed (P>0.05). These data suggest that Th2 type cytokines may increase hCLCA1 expression in CF but may not have a significant effect on mucus expression. Therefore the role of hCLCA1 as a mediator of mucus overexpression in CF has to be questioned.

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Increased mucus production (mucus hypersecretion) is a hallmark of cystic fibrosis (CF) lung disease [1]. Excessive amounts of mucus lead to airway obstruction and support bacterial infection and colonization leading to inflammation and loss of lung parenchyma. Previous studies have described several mediators that induce mucin gene expression and mucus protein production. These mediators include neutrophil elastase (NE) and bacteria such as Pseudomonas aeruginosa (PA) as well as bacterial products [2,3].

In addition, Th2 type cytokines have recently been implicated in mucus induction in CF [4]. Previous studies have indicated that a Th2 predominated immune response in CF may favour PA colonization and infection [5,6]. A strong Th2 type immune response is also found in allergic bronchopulmonary aspergillosis (ABPA) that is frequently observed in CF patients. We and others have shown Th2 type cytokine expression in the airways and blood cells of CF patients [4,7,8]. The Th2 type cytokines interleukin (IL)-4, IL-9, and IL-13 important mediators of mucus expression in bronchial asthma [9–11]. Atherton and colleagues demonstrated increased MUC5AC mRNA expression via a direct effect of IL-13 in epithelial cells [12]. Th2-induced mucus expression is at least in part mediated via the so-called calcium-activated chloride channel hCLCA1. In CF airways the IL-9 receptor has been co-localised with mucus expressing cells. Moreover, positive correlations between IL-9, mucus and the calcium activated chloride channel hCLCA1 have been reported [4]. However, no functional studies have been conducted so far to find out whether Th2 type cytokine stimulation can really increase hCLCA1 and mucus expression in CF airways.

Therefore we used explanted mucosal tissue from the upper airways of five CF patients who underwent sinus surgery (3 male, 2 female). As described previously mucin protein...
distribution is similar in the upper and lower airways [13]. Tissue was cultivated as previously described (37 °C, 5% CO₂) [14] for 24 hours without and with stimulation of recombinant human (rh)IL-4, rhIL-9 or rhIL-13 at a concentration of 50 ng/ml. Immunohistochemistry using a standard APAAP technique was applied to differentiate and count inflammatory cells (CD68: monocytes/macrophages; CD: lymphocytes; NE: neutrophils; major basic protein, MBP: eosinophils) and hCLCA1 (anti-hCLCA1 antibody; provided by Genaera Corp.) positive epithelial cells. Periodic acid Schiff (PAS) staining was used to quantify mucus protein expression although this method is not specific for mucin proteins since it stains for glycoproteins. Gene expression of hCLCA1 and MUC5AC (an important secretory mucin in airways) in airway mucosa was measured using real time PCR (aminolaelvulinat synthetase, ALAS-1 served as housekeeping gene). Primer sequences were as follows: ALAS-1 5’-CCC ATG AGT TG GAG CAA TC –3’ and 5’-ATT TTC CAA CAC AAC CAA AG -3’, MUC5AC 5’-TGA CCC ACC AGT GTG AGA AG -3’ and 5’-GGA TGA TCA GCC TCC TAT CG -3’, and hCLCA1 5’-GCA AGG TGG CTT TGT AGT GG -3’ and 5’-GGA ATT GAG CAA TC –3’. Inflammatory cells were counted per high powered field. Mucus and hCLCA1 expressing epithelial cells were quantified as percentage of total epithelial cells. The difference in the number of inflammatory cells and cells expressing hCLCA1 as well as staining for mucus (PAS), were compared with the nonparametric Kruskal-Wallis test. Statistically significant differences between groups were subsequently analyzed with the Wilcoxon matched pair test (Systat version 7.0; SPSS, Chicago, Ill). Results are expressed as means±SEM and are significant at a P value of less than 0.05.

Fig. 1. Change in hCLCA1 and mucin protein expression after stimulation with Th2 type cytokines. Bars represent mean values±SEM. *= P<0.05 vs control.

Stimulation with Th2 type cytokines did not significantly change inflammatory cells counts (Table 1). RhIL-4, rhIL-9, and rhIL-13 decreased mucus expression compared to unstimulated tissue (28.1±9.1% vs 37.5±8.8%, 33.3±10.2%, and 45.8±13.5%, respectively). However, this effect was not statistically significant (P>0.05). In contrast, all Th2 type cytokines significantly increased hCLCA1 protein expression (53.1±12.3% vs 66.7±13.5%, 79.2±18.4%, and 91.7±10.2%, respectively) (P<0.05) (Fig. 1). A small but not significant change in hCLCA1 mRNA expression was observed with Th2 type cytokine stimulation (P>0.05) (Fig. 2). There was also no increase in MUC5AC gene expression following stimulation with Th2 type cytokines (data not shown).

Our experiments show that Th2 type cytokines can induce hCLCA1 mRNA and protein expression in CF airway mucosa whereas mucus expression was not significantly affected. Previous reports have demonstrated a strong correlation between IL-9, its receptor, hCLCA1 and mucus expression in upper and lower airways of CF patients [4]. However, those studies did not provide functional data showing a clear induction of hCLCA1 and mucus after IL-9 stimulation. In the present study we found that IL-4, IL-9, and IL-13 increased hCLCA1 expression in CF mucosa. This finding agrees with data from the literature on non-CF airway mucosa [15].

Interestingly we did not observe any significant changes in MUC5AC gene and mucus expression. It is possible that other mucin genes such as MUC5B or MUC2 may behave differently but this would not explain that mucus protein expression did not significantly change. It may be due to the short time frame of 24 hours. Longer stimulation may lead to increased mucus expression. In fact, Th2 type cytokines can be regarded as milieu modifiers meaning that long-term stimulation eventually leads to goblet cell metaplasia. On the other hand data from the literature have shown increased mucin gene expression in cell culture after 24 hours of stimulation [9,10]. Another possible explanation is that the effect of Th2 type cytokines is attenuated by the plethora of other mucus secretagogues in CF airway mucosa (e.g. neutrophil elastase) [2,3].

Despite being primarily described as a chloride channel it has now become clear that hCLCA1 facilitates chloride currents but it is not an ion channel itself [16]. Moreover, hCLCA1 may play an important role during exocytosis of mucin proteins. Although its exact role remains to be further studied several studies have shown that inhibition of hCLCA1 leads to decreased mucus expression in both allergic and non-allergic disease [17,18]. Based on the findings in the present study the role of hCLCA1 in CF-associated mucus hypersecretion has to be questioned since we did not observe a significant change in mucus expression after stimulation with IL-4, IL-9 or IL-13 but a significant increase in hCLCA1 expression. It is possible that Th2 type cytokines and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Inflammatory cell counts.</th>
<th>Control</th>
<th>+rhIL-4</th>
<th>+rhIL-9</th>
<th>+rhIL-13</th>
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<tbody>
<tr>
<td>CD68+ve cells</td>
<td>20.1±2.1</td>
<td>24.3±6.4</td>
<td>19.0±1.3</td>
<td>13.2±4.2</td>
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<tr>
<td>CD3+ve cells</td>
<td>25.5±9.9</td>
<td>38.0±14.6</td>
<td>44.5±18.9</td>
<td>40.2±16.5</td>
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<tr>
<td>NE+ve cells</td>
<td>23.4±8.2</td>
<td>26.8±15.2</td>
<td>34.0±18.0</td>
<td>18.7±4.9</td>
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</tr>
<tr>
<td>MBP+ve cells</td>
<td>2.7±2.8</td>
<td>1.2±1.2</td>
<td>1.1±0.1</td>
<td>3.7±1.3</td>
<td></td>
</tr>
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CD68: monocytes/macrophages. CD3: lymphocytes. NE: neutrophils. MBP: eosinophils. Mean values±SEM.
hCLCA1 become more important in special conditions such as ABPA. This has to be further evaluated.

Although mucus hypersecretion in CF has to be considered to be mainly due to neutrophil inflammation and release of NE Th2 type cytokines may also play an important role in certain conditions (e.g. ABPA). Thus, blocking Th2 type cytokines or more specific hCLCA1 (e.g. with niflumic acid) may be beneficial in selected patients.

References