221* Elevated IL-17 and IL-23 mRNA levels in sputum of stable CF patients

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The role of the innate immunity in lung inflammation of cystic fibrosis (CF) has been well described but T lymphocytes might also be important. In particular, T helper 17 (Th17) cells can recruit neutrophils to an inflammatory site through production of IL-17, which induces chemokine release. IL-23 is an important inducer of IL-17 production. Elevated protein levels of IL-17 and IL-23 were found in bronchoalveolar lavage (BAL) fluid and sputum of CF patients during exacerbation (McAllister et al., J Immunol 2005;175:404). Our aim was to study the role of Th17 cells in CF lung disease by measuring IL-17 and IL-23 mRNA in sputum of stable CF patients and by comparing these levels with healthy controls. Sputum induction was performed in 39 stable adult CF patients (age: 25.6±6.2 years; FEV_1: 65.8±18.6% predicted) outside of exacerbation and 11 controls (age: 27.5±10.9 years). Neutrophil counts ranged from 91.6±8.3% in the CF group and 42±23.7% in the controls. RNA was isolated and quantitative real-time RT-PCR was performed. mRNA levels were normalised to the house keeping gene 18s rRNA. We found significantly higher levels of IL-17 ($p < 0.0001$) and IL-23 ($p < 0.0001$) in the CF group compared to controls. There was a correlation between IL-17 and IL-23 mRNA expression in the CF group ($p < 0.0001$) and to a lesser extent in the controls ($p = 0.0426$). The elevated levels of IL-17 and IL-23 mRNA could explain the persistent neutrophil infiltration present in sputum of stable CF patients. The correlation between IL-17 and IL-23 mRNA supports the potential role of the IL-23-IL-17 axis in the pathophysiology of CF lung disease.

222 *Aspergillus fumigatus*-induced IL-8 synthesis by bronchial epithelial cells: lack of involvement of the TLR−MyD88 pathway

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Aspergillus fumigatus (Af) pulmonary infections are common in cystic fibrosis. A crucial feature of the host defense is to recruit neutrophils into the airways to eradicate the invader. It is established that the Toll-like receptors (TLR) 2 and 4 play a role in the recognition of Af. In the present study, we show that bronchial epithelial cells (BEC) synthesize the neutrophil chemoattractant interleukin (IL)-8 in response to Af. Upon Af stimulation, NF-κB activates a short IL-8 promoter (−133) containing a binding site for NF-κB, and fused to the luciferase (Luc) reporter gene. We looked for the involvement of MyD88, a common adaptor for TLR2 and 4. BEC expressing a dominant negative form of MyD88 did not activate NF-κB upon Af challenge but paradoxically still synthesized IL-8. PI3 kinase, p38 MAPK, and ERK1/2 are also activated upon Af challenge, and the use of specific inhibitors showed that they are involved in IL-8 synthesis. As PI3 kinase and p38 MAPK inhibitors did not prevent activation of the IL-8-luc reporter gene, we suggest that these two kinases act downstream of NF-κB, e.g. histone modifications or mRNA stabilization. By contrast, the ERK1/2 inhibitor suppressed the IL-8 reporter activation but did not alter the NF-κB activity, suggesting that the ERK1/2 pathway activates a nuclear factor other than NF-κB, and essential for IL-8 synthesis. It is concluded that Af-induced IL-8 production by BEC is not triggered through the classical TLR-MyD88 signaling pathway, but is dependent on other pathways involving kinases such as PI3 kinase, p38 MAPK and ERK1/2.

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223* A new candidate biomarker of inflammation in CF airway: Amphiregulin

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Background: Identifying markers of inflammation relevant to CF airway disease are necessary to monitor disease progression and could help determine novel drug therapies.
Objective: To define biomarkers of the activation state of neutrophils (PMNs) collected from sputa and blood in the same CF patients, devoid of bacterial contamination.
Methods: We compared PMNs collected from airway and blood of CF children to blood PMNs from healthy subjects. We performed a macroarray analysis of 1050 genes coding for cytokines and their receptors, apoptosis-related and signaling molecules.
Results: We demonstrate an upregulation of 62 genes and downregulated expression of 27 genes in CF blood PMNs compared to healthy blood PMNs. Among upregulated genes were vitronectin, chemokines, particularly CCL17 and CCL18, interleukin (IL) receptors (IL-3, IL-8, IL-10, IL-12), all three colony stimulating factors (G-, M-, GM-CSF) and genes involved in NF-κB/IκB-α signaling. The upregulation of 6 genes in CF blood PMNs (thrombospondin-1, CXCL10, CCL17, IKKε, IL-10Ra and G-CSF) was confirmed by qPCR. High levels of G-GSF in CF blood PMNs supernatants were observed. When comparison was performed between blood and airway PMNs in CF child, there was a limited difference in terms of gene expression. Only the mRNA expression of amphiregulin and tumor necrosis factor (TNF) alpha receptor p55 were higher in airway PMNs. Significant amounts of amphiregulin were detected in CF sputa. Amphiregulin is an EGF receptor ligand that mediates TNF-α induced IL-8 secretion in airway epithelial cells.
Conclusion: Our findings make amphiregulin a new biomarker of CF-associated airway inflammation.

224 Serum surfactant protein D: a possible marker for lung disease in cystic fibrosis

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Aims: Surfactant protein D (SP-D) is a defence lectin of the innate immune system with implications for pulmonary inflammation and clearance of microbes. SP-D interacts with *Pseudomonas* and promotes phagocytosis and killing of spores of *Aspergillus fumigatus* in vitro. SP-D levels are decreased in bronchoalveolar lavage from patients with cystic fibrosis, and there is increased proteolytic degradation of SP-D. It is not known if serum SP-D likewise might reflect disease activity and we examined the serum SP-D as a marker for CF lung disease.
Methods: We measured serum SP-D at a routine visit on 111 CF patients (children and adults). We measured the forced expiratory volume in 1 second (FEV-1) at sampling date (2002–2004) and again in 2005.
Results: In adults (⩾19 y-old), the ln(se SP-D) level and the FEV-1 were linearly correlated (−0.64, $p < 0.001$). The correlation remained significant in adults two to three years after the serum sampling (−0.72, $p < 0.001$). The corresponding correlations were weaker in children, (Pearson = −0.29, $p = 0.057$) and (−0.26, $p = 0.045$) for the initial and the follow up measurement, respectively.
Conclusion: Our results suggest that serum SP-D may be a valuable marker of lung disease in CF patients. The correlation to FEV-1 is clear in adults. In children it is acknowledged that FEV-1 is a relatively poor marker of lung disease compared to CT scans. Further studies are warranted to investigate if SP-D improves evaluation of disease activity in children, when compared to FEV-1.