

204 Is 3-nitrotyrosine a marker of inflammation in airways of patients with cystic fibrosis?

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Nitrated tyrosine (3NT) has been detected in inflammatory diseases including cystic fibrosis (CF). Tyrosine nitration is thought to be mediated by the interaction of the activated oxygen species, superoxide anion radical, with nitric oxide leading to the generation of the nitrating species peroxynitrite. We analysed BAL fluids from patients with asthma and CF for 3NT-positive proteins. Concentrated BAL fluids were incubated with magnetic beads, coated with antibodies against 3NT, and antibody-bound proteins identified after desorption by Western blotting and MALDI-TOF. Human eosinophil peroxidase (EPO) was the only protein identified. 3NT staining of various immune cells in lung transplant tissues from CF patients revealed that only eosinophils were positive for 3NT. Four eosinophil granule toxins, including EPO were identified as 3NT-positive and high resolution MS demonstrated the presence of a single surface-exposed nitrosylated tyrosine residues in three of these toxins. However, 3NT-positive eosinophil granule toxins were also present in blood eosinophils from healthy individuals. Further experiments using EPO^{-/-} mice, mice with a defect in NADPH oxidase, nitric oxide synthase KO mice and patients with CGD demonstrated that the mechanism of tyrosine nitration is mediated by EPO in the presence of hydrogen peroxide and a minute source of NO or nitrite. These data suggest most if not all of the post-translationally modified 3NT-positive proteins are derived from eosinophils, suggesting that 3NT is not a marker of inflammation but rather a marker of eosinophils in CF airways.

205 8-isoprostaglandinF-2-alfa in urine as a marker of oxidative stress in cystic fibrosis patients. A longitudinal study

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Several publications have shown that a state of oxidative stress exists in the lungs of cystic fibrosis(CF) patients. As the clinical effect on lung destruction is a long term process we found it of interest to follow a marker of oxidative stress (8-isoprostaglandinF-2-alfa) in the urine at monthly controls over a 6 months period, to see if it might help to:

1. Evaluate which patients have a high degree of oxidative stress.
2. Evaluate urine samples as a practical paediatric tool to monitor effect and optimal dosage of a future glutathion inhalation study.

As reactive oxygen species can be formed for example by the activation of neutrophil granulocytes during bacterial infection, we found it important to have as precise a description as possible of the inflammatory state of the patients. Therefore the following parameters were followed monthly: CRP, Leucocyte count, Neutrophil count, NO in expiration, Laryngeal suction or sputum culture, Antibiotics administered, FEV1, FVC, FEF 75/25, Urine 8-isoprostaglandinF-2-alfa.

With the knowledge that 8-isoprostaglandinF-2-alfa is a bronchoconstrictor we have also made reversibility tests on all patients. Results will be presented.

206 Sputum eosinophils are elevated in CF patients with asthma

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Asthma complicating cystic fibrosis (CF) is difficult to diagnose due to similar features of both lung diseases. CF lung inflammation is characterised by neutrophilic inflammation, while asthma is regarded as an eosinophilic disease. Whether measures of inflammation differ and can be used for diagnosing CF-asthma remains to be determined.

The aim of this study was to evaluate inflammatory markers in sputum in CF patients with vs. without CF-asthma (CF-A).

CF-A was diagnosed by the presence of significant reversibility of FEV1 with β_2 -agonists and symptoms of bronchial hyperreactivity or frequent episodes of wheezing. CF patients with allergic bronchopulmonary aspergillosis (ABPA) were excluded. Induced sputum samples were obtained. Sputum eosinophil percentages were calculated on total leukocyte count. IL-5, IL-17A and IFN- γ mRNA levels were determined with quantitative real-time RT-PCR.

39 adult patients were recruited: 13 (33.3%) had CF-A and 21 did not have CF-A, 5 patients were excluded for ABPA. FEV1 was 70.4 \pm 17.1% in CF-A patients versus 66.7 \pm 18.8% in the non CF-A group (p=0.48). We found significantly higher eosinophil percentages in the CF patients with CF-A compared to those without (0.37 \pm 0.5% and 1.5 \pm 2.7%, respectively, p=0.047). CF patients with asthma also had a trend towards higher IL-5 and IL-17A mRNA levels (p=0.16 and p=0.12 respectively). IFN- γ mRNA levels were comparable between the two groups (p=0.35).

We found elevated sputum eosinophil percentages and a Th2 and Th17 phenotype in CF patients with CF-A. Measuring eosinophils in sputum could be a useful tool to help diagnosing asthma in CF patients.

207 Neutrophil Extracellular Traps impair the inhibition of elastase, protease 3 and cathepsin G in neutrophil suspensions from CF patients

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Objectives: The proteolytic degradation of CF lung is mainly due to the serine proteases secreted by activated neutrophils at inflammatory sites. But anti-inflammatory therapies using antiproteases have not led to clinical application. Improvement of the targeting of deleterious proteases requires a better understanding of how they are regulated in the extracellular environment. We analyzed how the secretion of Neutrophil Extracellular Traps (NETs) may influence activity of neutrophil serine proteases and their regulation by protease inhibitors.

Methods: Neutrophils purified from control blood and CF sputum were challenged with phorbol myristate acetate, A23187 or IL-8. Peptidase activities were measured using specific FRET substrates. Extracellular DNA was quantified using a non-permeant cell fluorophore. DNA and proteases of NETs were analyzed by confocal microscopy. The ATP dependence of NETs secretion was assayed using myxothiazol and oxamate. Inhibition of NETs-bound proteases was measured using natural inhibitors, α 1-PI and antichymotrypsin.

Results and Conclusions: The three serine proteases (elastase, protease 3 and cathepsin G) are bound to NETs secreted by metabolically active neutrophils. NETs impair the inhibition of elastase and cathepsin G by natural inhibitors. Inhibition is significantly improved by DNase treatment in spite of the overall increase of proteolytic activity. These results suggest that a combination of DNase and antiproteases that target elastase and cathepsin G would improve the anti-inflammatory therapies of lung inflammation.

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