E3 ubiquitin ligases: A potential role in regulating the inflammatory phenotype of cystic fibrosis?

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Cystic Fibrosis (CF) is characterized by an aggressive inflammatory response. One important marker of CF lung disease, the pleiotropic cytokine TGF-beta, is negatively regulated by E3-ubiquitin ligases, which have been found to be dysregulated in previous studies of F508del-CFTR-related gene expression. To understand the role of E3-ubiquitin ligases in CF, we studied the effects of:
1. F508del mutation and
2. exposure to TGF-beta and TNF-alpha cytokines on mRNA and protein expression of the E3-ubiquitin ligases SMURF1, SMURF2 and NEDD4L4 in polarized CF bronchial epithelial cell models.

Using real-time quantitative PCR, we demonstrated that the F508del mutation is partly responsible for the increased pro-inflammatory mediators that characterize C. Baseline transcriptome levels in BALF from CF patients were examined for the presence of endogenous WAP2 via Western blot.

Conclusion: Recombinant WAP2 inhibited cathespin G but not elastase activity. Monocytic cells pre-treated with rWAP2 before LPS stimulation showed significantly lower levels of IL-8 and MCP-1 production compared to cells given LPS alone. Recombinant WAP2 was conjugated to fibronectin with a transglutaminase and retained anti-protease activity. WAP2 was detected at variable levels in BALF from CF patients. Together these results suggest a role for this lesser known WFCDF protein in the regulation of inflammation; therefore further investigation is warranted to determine its merit as a possible therapeutic agent.

Neutrophil elastase-mediated increase in airway temperature during inflammation

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Objectives: SLPI and elafin are members of the WAP Four-Disulphide-Core (WFDC) family of proteins and have multiple contributions to innate immunity including inhibition of neutrophil serine proteases and inhibition of the inflammatory response to LPS. The aims of this research were to explore potential activities of WAP2, a previously uncharacterised WFDC protein expressed in the lung.

Methods: Recombinant expression and purification of WAP2 were optimised in E. coli. Cathespin G and elastase activity assays were used to test anti-protease activity of rWAP2. To investigate anti-inflammatory activity, THP-1 monocytes were given LPS alone or rWAP2 in combination with LPS. Cytokine levels in cell-free supernatants were subsequently analysed by ELISA. To test if WAP2 could become cross-linked to extracellular matrix proteins, rWAP2 was incubated with fibronectin in a transglutaminase-mediated reaction and retained anti-protease activity. WAP2 was detected at variable levels in BALF from CF patients. Together these results suggest a role for this lesser known WFCDF protein in the regulation of inflammation; therefore further investigation is warranted to determine its merit as a possible therapeutic agent.

Table 1: Samples Mean Mean %CRP Age %FEV1 %SHP (n) SAA CRP negative (mg/dl) positive (mg/dl) (ys) (Annual positive average)
Group 1
(SAA <64 mg/l) 26 2.8 0.17 100.0% 20.29 88.0% 25.0%
Group 2
(SAA 64–128 mg/l) 72 27.5 0.56 64.3% 23.68 64.3% 73.2%
Group 3
(SAA 128–256 mg/l) 21 86.2 0.99 47.6% 28.50 47.5% 100.0%
Group 4
(SAA >256 mg/l) 13 433.0 4.13 0.0% 27.00 36.6% 100.0%

8. Immunology/Inflammation

8. Immunology/Inflammation Posters

Serum amyloid A as a useful serum marker of lung inflammation in cystic fibrosis

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Anti-inflammatories are an attractive therapeutic target in CF with a proven ability to slow down lung disease. Up to now, a serum biomarker able to define inflammation baseline state and to determine therapy efficacy, is not available. Serum amyloid A (SAA) is an acute-phase protein, whose serum concentration represents a useful inflammatory marker, particularly in rheumatic diseases. We decided to compare SAA values with CRP values, that are strongly related with acute infective exacerbations, and clinical parameters, useful to define baseline lung condition. We tested SAA (normal value: <64 mg/l) in 147 serum samples from 107 CF patients, followed by Genova CF Centre in 2012. We divided SAA results in 5 groups and we collected for each patient age, average annual FEV1%, P. aeruginosa colonization and CRP value at the time of every SAA collection (Table 1). Pa. positivity is highly related with SAA values (median value in Pa. positive 50.1 mg/l, median value in Pa. negative 5.5 mg/l; p<0.0001). No correlation was observed between SAA values and age (r=0.29). A strong correlation was observed between SAA and CRP values (n=147; r=0.77) and a moderate inverse correlation emerged between SAA and FEV1% (n=133; r=−0.58).

In conclusion, SAA, can be useful to define, as CRP, acute inflammatory events, but also to characterize lung inflammatory baseline condition.

Neutrophil elastase-mediated increase in airway temperature during inflammation

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Objectives: P. aeruginosa is the dominant pathogen in chronic lung infections in CF. The reason(s) for this selection is unclear.

Methods: We determined airway and sputum temperatures in CF patients and healthy controls. Additionally, we measured the enthalpy of the binding reaction between purified human neutrophil elastase (NE) and its endogenous inhibitor α1-PI and pI in temperatures in wild type and isogenic NE−/− mice. We cultured bacterial pathogens at 30°C, 38°C and 39°C for 96 h aerobically, and measured their density. We compared the transcriptome of P. aeruginosa after anaerobic growth at 30°C and 38°C and sequenced the microbiota in 8 paired early and late CF sputum specimens.

Results: PLET temperatures in 56 CF patients were inversely correlated to lung function. Within mucus plugs 37.9±0.8°C was measured while airway luminal temperatures were 36.62±0.91°C. NE bound to α1-PI in an exothermic reaction with a binding enthalpy of ~18 ± 1.3 kcal/mol. Temperatures in pouch airspaces of infected WT mice were significantly higher compared to controls. After 96h at 38°C, the densities of many bacterial species were significantly lower (or remained constant) vs. 30°C while only P. aeruginosa grew at 38°C or higher. We identified 858 differentially expressed genes at 38°C vs 30°C including virulence genes, quorum sensing system genes and thermotolerance genes. The mean P. aeruginosa relative abundance was 0.19± in early sputum samples and 0.59± in late samples.

Conclusion: NE mediates a temperature increase in mucus plugs of CF patients which favours the selection of P. aeruginosa.