161 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 NEDD4L in polarized CF bronchial epithelial cell models.

Using real-time quantitative PCR, we demonstrated that the F508del mutation is not sufficient to induce significant differential mRNA expression of E3-ubiquitin ligases. However, the F508del-CFTR genotype altered the responsiveness of E3-ubiquitin ligases to both inflammatory cytokines. Our results showed that both TGF-beta and TNF-alpha increased the expression of *SMURF2* mRNA in F508del-CFTR CFBE cells, suggesting an up-regulation of this E3-ubiquitin ligase under inflammatory status. In addition, this increased expression was consistent with an observed decrease in *SMAD2* and *SMAD3* mRNA expression. These results suggest that increased expression of E3-ubiquitin ligases in CF under inflammation could be partly responsible for the increased pro-inflammatory mediators that characterize CF disease, via an inhibition of the Smad-dependent anti-inflammatory effects of TGF-beta. Our preliminary data enlighten the potential implications of differential expression of enzymes involved in ubiquitination on modulation of CF inflammatory responses.

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162 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: <6.4 mg/l) in 147 serum samples from 107 CF patients, followed by Genoa 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). P.a. positivity is highly related with SAA values (median value in P.a. positive 50.1 mg/l, median value in P.a. negative 5.5 mg/l: $p < 0.0001$). No correlation was observed between SAA values and age ($r_s = 0.29$). A strong correlation was observed between SAA and CRP values ($n = 147$; $r_s = 0.77$) and a moderate inverse correlation emerged between SAA and FEV_1% ($n = 133$; $r_s = -0.58$). In conclusion SAA, in CF, can be useful to define, as CRP, acute inflammatory events, but also to characterize lung inflammatory baseline condition.

Table 1.

	Samples (n)	Mean SAA (mg/l)	Mean CRP (mg/dl)	%CRP negative (<0.46 mg/dl)	Age (yrs)	%FEV1 (Annual average)	%*P. aeruginosa* positive
Group 1 (SAA <6.4 mg/l)	26	2.0	0.17	100.0%	20.29	88.0%	25.0%
Group 2 (SAA 6.4–64 mg/l)	72	27.5	0.56	64.3%	23.68	64.3%	73.2%
Group 3 (SAA 64.1–128 mg/l)	21	86.2	0.99	47.6%	28.50	47.5%	100.0%
Group 4 (SAA 128.1–256 mg/l)	15	176.4	1.95	6.6%	27.21	50.5%	92.9%
Group 5 (SAA >256 mg/l)	13	433.0	4.13	0.0%	27.00	36.6%	100.0%

163 A role for the WFDC protein, WAP2, in the regulation of inflammatory response in the lung

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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*. Cathepsin G and elastase activity assays were used to test anti-protease activity of rWAP2. To investigate anti-inflammatory activity, THP-1 monocytic cells 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 +/– transglutaminase, and then assessed by SDS-PAGE and Western blotting. BALF samples from CF patients were examined for the presence of endogenous WAP2 via Western blot.

Conclusion: Recombinant WAP2 inhibited cathepsin 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 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 WFDC protein in the regulation of inflammation; therefore further investigation is warranted to determine its merit as a possible therapeutic agent.

164 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 α_1-PI and pouch temperatures in wild type and isogenic $NE^{-/-}$ mice. We cultured bacterial pathogens at 30ºC, 38ºC and 39ºC for 96 h anaerobically, and measured their density. We compared the transcriptome of *P. aeruginosa* after anaerobic growth at 30ºC and 38ºC 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 37.98±0.80ºC was measured while airway lumenal temperatures were 36.62±0.91ºC. NE bound to α1-PI in an exothermic reaction with a binding enthalpy of –18.5±1.3 kcal/mol. Temperatures in pouch airspaces of infected WT mice were significantly higher compared to controls. After 96 h at 38ºC, the densities of many bacterial species were significantly lower (or remained constant) vs. 30ºC while only *P. aeruginosa* grew at 38ºC or higher. We identified 858 differentially expressed genes at 38ºC vs 30ºC including virulence genes, quorum sensing system genes and thermotolerance genes. The mean *P. aeruginosa* relative abundance was 0.191 in early sputum samples and 0.592 in late samples.

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