

108 Reduced effect of intravenous antibiotic treatment on sinonasal compared to pulmonary inflammatory markers

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Objectives: Chronic rhinosinusitis promoted by upper airway (UAW) colonization with pathogens like *P. aeruginosa* and *S. aureus* is a hallmark of CF. While pulmonary therapy with antibiotics has been intensively studied, UAW effects received little attention. Aims of the present trial are to compare early dynamics of inflammatory markers in the UAW and lower airways (LAW) during IV-antibiotic therapy.

Methods: Changes of inflammatory markers in both airway segments were assessed in 16 CF patients receiving IV-antibiotic treatment against *P. aeruginosa* and/or *S. aureus* over ≥ 14 days (median after 7.5 days). Non invasive sampling methods were applied (nasal lavage with 10 mL of isotonic saline/nosril, respectively induced sputum). Cytology and interleukins (IL)-4, IL-8, IL-13, MPO, MMP-9, TIMP-1, CTSS, NE were analyzed.

Results: Antibiotic therapy for 7.5 days significantly reduced total cell count in LAW but not in UAW. MPO and IL-8 decreased significantly in both, the UAW and LAW. Interestingly, this was much more pronounced for LAW (median decrease of MPO: LAW 9.8fold resp. UAW 1.75fold; IL-8: LAW 3fold resp. UAW 1.9fold).

Conclusion: Although some inflammatory markers decreased in both airway levels early during IV-antibiotic treatment responses in the UAW were relevantly reduced, compared to pulmonary effects. Our findings highlight limitations of systemic antibiotic treatment in the sinonasal compartment. Obviously, paranasal sinuses, which are hollow organs often filled with pus, mucocoeles, and polyps in CF are not reached effectively by systemic antibiotic treatment. This finding is important for treatment of bacterial rhinosinusitis in CF and other diseases.

109 Increased IL-8 production in human CFTR-deficient macrophages

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Exaggerated inflammatory responses to bacterial infections contribute to tissue damage and pulmonary disease in cystic fibrosis. Recently a growing body of evidence suggests that dysregulated production of pro-inflammatory mediators by macrophages might contribute to cystic fibrosis lung pathology. While excessive production of IL-6, GM-CSF, MCP-1 by human CF macrophages has been ascribed to abnormal signalling and trafficking of TLR4 following stimulation with LPS, dysregulation in the constitutive secretion of other pro-inflammatory mediators, such as IL-8, has been also reported. These data suggest that, dysfunctional CFTR may affect multiple molecular pathways involved in cytokine expression. Thus the aim of this study was to assess the impact of CFTR dysfunction in the production of IL-8 by human macrophages. To this aim we quantified constitutive or LPS-induced IL-8 secretion by human CF macrophages. The resulting data demonstrated a significant increase in the constitutive production of IL-8 by CF macrophages with respect to control cells while no differences were observed following LPS stimulation between the two groups (CF versus non-CF). Quantitative analysis of IL-8 mRNA by real time PCR confirmed an increase in the chemokine transcript in CF macrophages. Similarly, treatment of non-CF macrophages with the CFTR inhibitor CFTR_{inh-172} led to a significant increase in IL-8 transcripts indicating that the absence of CFTR function is sufficient to alter IL-8 transcription. Current studies focus on the identification of transcription factors responsible for the augmented IL-8 expression in human macrophages carrying dysfunctional CFTR.

110 Connectivity mapping: an advanced bioinformatics approach to predict A20-inducing small molecules to reduce inflammation

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Objectives: The zinc finger protein A20 negatively regulates inflammatory NF- κ B signalling, but in CF A20 expression/function is significantly reduced (Kelly *et al.* 2012, 2013) and preliminary data suggest that A20 up-regulation can normalise inflammation.

Methods: To predict further small molecules that would induce A20, we employed an advanced bioinformatics technique (Connectivity Mapping, CM). CM can establish the connection between different biological states based on their gene expression profiles. Using six independent public genome expression data sets A20 was used as the seed gene to create a gene expression profile. CM analysis then identified its closest associates (genes behaving similar to A20 in CF).

Results: The 'A20 associated' genes are activating transcription factor 3 (ATF3), intercellular adhesion molecule-1 (ICAM1), a member of the RAS oncogene family (RAB5C), nuclear envelope pore membrane protein (POM121), presenilin 1 (PSEN1) and domain containing protein 4A (DENND4A). Quantitative RT-PCR analyses in 16HBE14o- and CFBE41o- confirmed reduced basal expression and a delayed and lower LPS-induced expression of these genes, similarly to A20 (Kelly *et al.* 2012), given further confidence in the approach.

Conclusion: Together with A20, these 'A20 associates' then served as an input to the CM process (www.broadinstitute.org) to predict small molecule compounds already licensed for the use in humans that enhance A20 expression. Future work will determine A20-inducing and anti-inflammatory effects of these drugs, which could then be used as alternative anti-inflammatory therapies (drug repositioning).

111 Defective A20 signalling in cystic fibrosis: anti-inflammatory action of giberrellin

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Pathogen-induced acute and chronic airway inflammation is driven by activation of the transcription factor NF- κ B. To ensure termination of the inflammatory response NF- κ B signalling is tightly regulated. The zinc finger protein A20 negatively regulates NF- κ B signalling through interaction with various signalling partners. In Cystic Fibrosis chronic airway inflammation, A20 expression and function are significantly reduced (Kelly *et al.* 2012) and this lack is associated with reduced lung function (Kelly *et al.* 2013). The plant diterpene giberrellin (GA₃) has been described to induce Zn-Finger like proteins in plants and have anti-inflammatory properties in mice.

We hypothesized that GA₃ works via an A20-dependent mechanism and examined the effects on airways inflammation in CF in-vitro.

14HBE16o- and CFBE41o cells were stimulated with LPS in the presence or absence of GA₃. IL-8 release and NF- κ B activation were determined. Further studies employed siRNA.

Our results show that in CF and non-CF cell lines GA₃ (30 μ M) protects cells against LPS-stimulated IL-8 release by induction of A20. In 14HBE16o- this action was accompanied by reduced p65 expression and increased I κ B α abundance. Silencing of A20 using siRNA showed that this effect was A20-dependent. The effect and mechanism of GA₃ was confirmed in non-CF primary nasal epithelial cells.

Future work will determine the efficacy of GA₃ to overcome defective A20 signalling in CF chronic airways disease.