165 An immunomodulatory role for the serine protease inhibitor, eppin, in the lung

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Objectives: Small molecular weight proteins involved in innate host defence, for example SLPI, have demonstrated some efficacy in clinical CF trials as a way of controlling inflammation. We investigated the immunomodulatory and anti-bacterial activity of a related protein, eppin, *in vitro*.
Methods: THP-1 monocytes and monocyte-derived macrophages were given LPS alone or pre-incubated with recombinant eppin prior to LPS activation. Expression and secretion of cytokines were measured by RT-PCR and ELISA respectively. Nuclear extracts were prepared and p65 activity compared using a TransAm activity ELISA. Binding of eppin to the consensus NF-κB binding site was evaluated by EMSA. IκBα, phosphorylated IκBα and polyubiquitinated protein levels in cytoplasmic lysates were assessed by Western blot. Membrane permeabilisation assays were used to test the bactericidal effects of eppin. CF sputum samples were screened for endogenous eppin via Western blotting.
Conclusion: THP-1 monocytes and macrophages showed impaired cytokine expression and secretion if given recombinant eppin before LPS stimulation. Further investigation showed that NF-κB activity was significantly reduced in cells pre-treated with eppin, but eppin did not appear to bind the NF-κB consensus site. Levels of phosphorylated IκBα and total ubiquitinated protein were increased in cells pre-incubated with eppin prior to LPS. Recombinant eppin permeabilised the membranes of lung-relevant bacteria. Endogenous eppin was detected in CF sputa. The results from this study point towards a role for eppin in the innate immune response in the lung and it could therefore prove valuable as a therapeutic option in CF.

166 Beneficial immunomodulatory effect of vardenafil in mouse CF macrophages

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Objectives: We previously showed that F508del-CFTR homozygous mice display increased numbers of alveolar and peritoneal macrophages. We also demonstrated that Vardenafil, a phosphodiesterase type 5 inhibitor, reduces LPS-induced inflammatory responses in CF mice. We hypothesized that the activity of macrophages is altered in CF and that macrophages represent target effectors of the anti-inflammatory effect of vardenafil.
Methods: Macrophages from lung homogenates and peritoneal lavages were isolated from CF and WT mice. To determine if the activity of macrophages is altered in CF, LPS-induced inflammatory responses were first evaluated. Then, macrophage differentiation in pro/anti-inflammatory effectors was studied using a M1 (LPS and IFN-γ) or M2 (IL-4 and IL-13) polarization protocol. Pro- (TNF-α, IL-1β and CCL-2) and anti-inflammatory (FIZZ-1 and mYm1) mediators were quantified by ELISA (supernatants) or qRT-PCR (macrophages). In each condition, the effect of vardenafil (50 μM) was tested.
Conclusion: CF macrophages displayed an exaggerated pro-inflammatory response to LPS. M1/M2 polarization was altered in CF macrophages. M1 responses were at least 4-fold larger and M2 responses were reduced in CF compared to WT cells, which confirms the pro/anti-inflammatory unbalance in CF macrophages. Vardenafil reduces the expression of TNF-α and IL-1β and corrects FIZZ-1 overproduction in CF macrophages. These results suggest that the differentiation of macrophages from different body compartments is oriented towards both an increased pro-inflammatory and a decreased anti-inflammatory status. The immunomodulatory effect of vardenafil could be beneficial in CF pharmacotherapy.

167 Molecular mechanisms of enhanced expression of interleukin 8 (CXCL8) in cystic fibrosis (CF) airway epithelial (AE) cells

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Objectives: The Aim of this study was to investigate the transcriptional mechanisms of CXCL8 production basally and following induction by IL-1β in immortalized CFTR-deficient AE (CFTR-AE) cells and their corrected counterparts.
Methods: CXCL8 secretion/expression was measured by ELISA/RT-PCR respectively. Transcriptional regulation was assessed by transient transfection of wild type (WT) CXCL8 promoter constructs. The involvement of individual transcription factors was assessed using CXCL8 promoter luciferase constructs with mutated binding sites for NF-κB, AP-1, and C/EBPβ. Chromatin immunoprecipitation (ChIP) measured binding of transcription factors to the CXCL8 promoter.
Conclusion: CFTR-AE cells secreted higher levels of CXCL8 compared to their corrected counterparts both basally and after stimulation with IL-1β. CFTR-AE cells expressed increased levels of CXCL8 transcript and exhibited increased WT CXCL8 promoter luciferase activity. Mutation of NF-κB, AP-1 and C/EBPβ binding sites abolished CXCL8 promoter luciferase activity suggesting that all 3 factors are required for CXCL8 expression. ChIP assay showed increased C/EBPβ binding to the CXCL8 promoter in CFTR-AE cells. These studies suggest that CFTR-AE cells produce increased levels of CXCL8 due to higher binding of C/EBPβ to the CXCL8 promoter. Current studies focus on determining the underlying epigenetic mechanisms responsible for increased transcription factors binding.

169 Comparison of inflammatory response from CF bronchial epithelial cells following stimulation with aerobic and anaerobic *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is one of the most common bacteria found in sputum from people with Cystic Fibrosis (CF). The bacteria can grow in aerobic and anaerobic environments resulting in an immune response in the host involving the release of inflammatory cytokines and proteolytic enzymes. Cycles of persistent infection and inflammatory response leads to lung injury and decreased lung function. This study investigates whether *P. aeruginosa* cultured under aerobic and anaerobic conditions induce a similar inflammatory response in CF airway epithelial cells.
Methods: Aerobic and anaerobic lysates or heat inactivated *P. aeruginosa* type strains (PA01 & PA14) and clinical isolates (B003, B008, B021) were co-incubated with CFBE cells for 2 hours, 6 hours and 24 hours. The pro-inflammatory signalling molecules IL-6, IL-8 and G-CSF were measured in the cell-conditioned media by ELISA, whilst the activity from the remodelling lung degrading proteases, Cathepsin B and Cathepsin S were measured by kinetic fluorogenic assay.
Results: When compared to unstimulated control cells, the heat inactivated bacteria induced the greatest secretion of IL-8 (435%) and IL-6 (922%). However, G-CSF levels (1306%) and Cathepsin B (19889%) and S (390%) activity were highest following stimulation by bacterial lysates. In 3 of the biomarkers (Cat B, IL-8 and IL-6) the anaerobic bacteria induced a greater response in comparison to the aerobic bacteria.
Conclusion: The data demonstrates variability in inflammatory response from CFBE cells. However in the majority of biomarkers the anaerobic bacteria invoke a greater inflammatory response.