The unfolded protein response orchestrates low-grade chronic inflammation, by IL-6 production, in patients with cystic fibrosis arthropathy.

Samuel Lara Reyna[1, 2], Thomas Scambler[1], Jonathan Holbrook[1 ,2], Chi Wong[1], Camilla West[1], Sinisa Savic[1, 3], Daniel Peckham[2, 4] and Michael F. McDermott[1].

[1]Leeds Institute of Rheumatic and Musculoskeletal Medicine, [2]Leeds Institute of Biomedical and Clinical Sciences, [3]Department of Clinical Immunology and Allergy, St James's University Hospital, [4]Adult Cystic Fibrosis Unit St James' University Hospital.

Introduction: The cystic fibrosis transmembrane regulator (CFTR) is a transmembrane protein, involved in transport of chloride ions and bicarbonate into the cell. When mutated, the reduced function and expression of the CFTR protein on the cell surface is responsible for the pathophysiology of cystic fibrosis (CF). As patients with CF are living longer, complications such as CF arthropathy (CFA) and hypertrophic pulmonary osteoarthropathy (HPOA) are becoming more prevalent, with a negative impact on morbidity and quality of life. Episodes of joint pain are well-recognised in CF, usually starting after age 10, with a prevalence of 5-10%. The CFTR is assembled and modified in the endoplasmic reticulum (ER); accumulation of misfolded CFTR protein leads to activation of the unfolded protein response (UPR), in order to resolve ER stress. The UPR comprises three ER transmembrane resident proteins, known respectively as PERK, IRE1, and ATF6. It has been reported that UPR activation is linked to the production of proinflammatory cytokines, including IL-6 and TNF. We hypothesised that CFA is associated with low-grade, chronic inflammation perpetuated by accumulation of misfolded CFTR protein within the ER, which activates IRE1α, causing XBP1 splicing (XBP1s) and increased production of IL-6 and TNF. The main objective of this project was to investigate the presence of increased UPR activation in human bronchial epithelial cell (HBEC) lines harbouring different CFTR mutations, and whether the proinflammatory consequences of UPR activation can be reduced to restore normal cellular function and reduce systemic inflammation in CFA.

Methods: HBEC lines harbouring different CFTR mutations (Beas-2b (wild-type; WT), IB3-1 (ΔF508/W1282X), CUFI-1(ΔF508/ΔF508), CUFI-4 (ΔF508/G551D)), were used to evaluate the presence of UPR activation, by real time qPCR and flow-cytometry. IL-6 production was measured by real time qPCR and ELISA assays. Tunicamycin (Tn), and thapsigargin (Tg) were used as cellular UPR stimulants to assess the extent of the UPR activation.

Results: Gene and protein expression revealed a significant increase (P = <0.05) in *IRE1α* expression in the CuFi-1 and -4 HBEC lines, both at basal conditions and after Tn and Tg stimulation. Furthermore, *XBP1s* expression was also significantly higher under basal conditions, with 19-fold and 12-fold increases, respectively. *XBP1s* was associated with increased levels of *IL-6* and a progressive decrease of *XBP1-unspliced (XBP1u)* production in the CuFi-1 and -4 HBEC lines. Interestingly, the heterozygous HBEC cell line, IB3-1, with one CFTR missense mutation and hence reduced amounts of misfolded CFTR protein, did not show any increase in *IRE1α, XBP1s,* or *IL-6*, but had significantly increased levels of *XBP1u* after Tg stimulation (P = <0.05). Finally, we observed a significant increase in IL-6 secretion in CuFi-1 and -4 HBECs compared to WT HBECs after Tg stimulation. These data suggest that misfolded CFTR protein induces ER stress and UPR-dependent inflammation that may be CFTR genotype specific