WS9.5 CF epithelial cells are a source of pulmonary cathepsin S via increased IRF-1

S. Weldon[1], P. McNally[2], D.F. McAuley[1], C.J. Scott[3], M.A. Mall[4], S.H. Randell[5], C. Wohlford-Lenane[6], J.A. Bartlett[6], P.B. McCray Jr.[6], C.C. Taggart[1]. *[1]Queen's University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom; [2]Our Lady's Children's Hospital Crumlin, National Children's Research Center, Dublin, Ireland; [3]Queen's University Belfast, School of Pharmacy, Belfast, United Kingdom; [4]University of Heidelberg, Department of Pediatrics, Heidelberg, Germany; [5]University of North Carolina, Department of Cell and Molecular Physiology, Chapel Hill, United States; [6]University of Iowa, Department of Paediatrics, Iowa, United States*

Cysteine cathepsins are a family of proteases relatively unexplored in the area of CF lung disease. In agreement with previous work, cathepsin S (CTSS) activity is increased in CF bronchoalveolar lavage (BAL) fluid, even in the absence of *Pseudomonas aeruginosa* infection, compared to healthy control BAL fluid. In this study, we investigated the hypothesis that epithelial cells contribute to the protease burden of the CF lung by secreting CTSS. Basal expression and secretion of CTSS were significantly increased in CF bronchial and tracheal epithelial cell lines as well as primary bronchial epithelial cells compared to non-CF controls in the absence of stimulation or infection. In addition, levels of the transcription factor, IRF-1, correlated with increased levels of its target gene, CTSS, in CF epithelial cells. Knockdown of IRF-1 using siRNA gene silencing significantly decreased expression and secretion of CTSS. This novel data not only identifies airway epithelial cells as a source of CTSS in the CF lung, but also highlights a mechanism for the increased CTSS via IRF-1. The role of CTSS in CF lung disease has long been overshadowed; however, we believe that the IRF-1/CTSS pathway may play a functional role in the pathogenesis of CF lung disease.

WS9.6 A role for TLR9 in *Pseudomonas aeruginosa*-induced lung inflammation

F. BenMohamed[1], M. Medina[1], Y. Wu[1], S. Mashalidi[2], M. Chignard[1], M. Bénédicte[2], T. Lhousseine[1]. *[1]Unité de Défense innée et Inflammation INSERM U874, Institut Pasteur, Paris, France; [2]Inserm U. 1013, Hôpital Necker, Paris, France*

Background: *Pseudomonas aeruginosa* (*PA*) is present in lung of cystic fibrosis (CF) patients. TLR9 is functional upon cleavage by asparagine endopeptidase (AEP) but its role in host response to *PA* is unknown.
Aims: Determine the roles of TLR9 in host defense during acute lung infection by *PA*.
Methods: Wild type (WT) and TLR9$^{-/-}$ mice were infected i.n. with 10^7 CFU of a laboratory strain of *PA* (PAK). 24 h post-infection, we compared: mouse survival, pro-inflammatory cytokine levels in broncho-alveolar lavages (BALs), alveolar macrophages (AMs) and PMN recruitment and *PA* loads in lungs, cytokine secretion by a macrophage cell line (MHS) and primary AMs.
Results: (*i*) TLR9$^{-/-}$ showed less mortality than WT mice, (*ii*) significantly lower number of AMs, PMN and bacteria were detected in BALs of TLR9$^{-/-}$ compared to WT mice; (*iii*) decreased levels of IL-6, KC and TNFα were detected in BALs of TLR9$^{-/-}$ mice; (*iv*) stimulation of MHS by either PAK DNA or CpG triggered both TLR9 cleavage and cytokine secretion; (*v*) both an AEP inhibitor and concanamycin B, a vacuolar H(+)-ATPase inhibitor that increases endosomal pH, reduced CpG- and PAK DNA-induced IL-6 and TNFα secretion by MHS.
Conclusions: Signalling through TLR9 plays a role in inflammation induced by *PA* acute lung infection. This may help to better understand the mechanisms involved in *PA*-induced inflammation and to development of potential drugs for the treatment of CF.
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WS9.7 Modulatory effects of *Aspergillus* colonization and ABPA on blood and sputum eosinophils and neutrophils in CF

Y. Gernez[1], C. Everson[2], C.E. Dunn[2], N. Fernandez-Becker[3], L. Gudiputi[1], Z.A. Davies[2], L.A. Herzenberg[1], R. Tirouvanziam[4], R.B. Moss[2]. *[1]Stanford University, School of Medecine, Genetics, Stanford, United States; [2]Stanford University, School of Medecine, Pediatrics, Pulmonary, Palo Alto, United States; [3]Stanford University, School of Medecine, Gastroenterology and Hepatology, Stanford, United States; [4]Emory University, School of Medicine, Pediatrics, Atlanta, United States*

Introduction: Fifteen to sixty percent of CF patients are colonized with *Aspergillus fumigatus* (*Af*) [CF-AC] and are at risk for *Af* infection or allergic bronchopulmonary aspergillosis [CF-ABPA]. Although airways inflammation in CF is typically characterized by neutrophilia, ABPA-associated inflammation is defined by eosinophilia. We hypothesized that blood and sputum eosinophils and/or neutrophils may be primed or activated in CF-ABPA or CF-AC patients when compared to CF patients without *Af* colonization or ABPA [CF].
Methods: Using flow cytometry, we measured surface CD16, CD63 and CD66b on blood and induced sputum neutrophils as well as surface CCR3 (eotaxin receptor) on eosinophils from CF-ABPA (N = 11), CF-AC (N = 9), and CF (N = 10) patients. We also studied the blood granulocytes from patients with celiac disease as controls.
Results: Whereas no differences were observed within the three groups of CF patients in any activation surface markers on blood neutrophils, the levels of surface CCR3 on blood eosinophils were increased in CF-ABPA patients compared to CF-AC ($P = 0.046$). In the sputum, the levels of surface CD66b were higher on the neutrophils from patients with CF compared to CF-AC ($P = 0.03$).
Conclusions: Blood neutrophil activation profiles are similar in CF, CF-AC and CF-ABPA patients, while the observed increased expression of CCR3 on blood eosinophils from patients with CF-ABPA indicates the systemic immunopathology of this complication, complements our previously described activation of blood basophils in CF-ABPA (*JCF* 2011; Suppl 1: S46). CCR3 may represent a novel ABPA therapeutic target.

WS9.8 Toll like receptor 4 is not targeted to the lysosome in cystic fibrosis airway epithelial cells

C. Kelly[1,2], P. Canning[1], P.J. Buchanan[1], M.T. Williams[1], J.S. Elborn[1], M. Ennis[1], B.C. Schock[1]. *[1]Queen's University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom; [2]Keele University, Institute for Science and Technology in Medicine, Keele, United Kingdom*

Objectives: LPS activation of Toll like receptor 4 (TLR4) triggers internalisation of the receptor-ligand complex which is directed towards lysosomal degradation or endocytic recycling. The robust and uncontrolled inflammatory response to bacterial infection in CF suggests a defect in this regulation. This study examined the intracellular trafficking of TLR4 in CF and non-CF airway epithelial cells following stimulation with LPS.
Methods: We employed cell lines (16HBE14o– and CFBE41o–) and confirmed selected experiments in primary nasal epithelial cells from non-CF controls and CF patients (F508del homozygous). Markers of the endosome, lysosome and Golgi were assessed by Western Blot, while co-localisation of TLR4 with the lysosomal marker LAMP1 was investigated by immunocytochemistry. Finally, TLR4 expression was determined by flow cytometry.
Conclusions: All cells expressed markers of the early (EEA1) and late (Rab7b) endosomes at basal levels. However, only CF cells displayed persistent expression of Rab7b and the 58K Golgi protein following LPS stimulation. TLR4 co-localised with the lysosomal marker LAMP1 in 16HBE14o– cells, suggesting that TLR4 is targeted for lysosomal degradation in these cells. However, this co-localisation was not observed in CFBE41o– cells, where we also found persistent expression of p65 and release of pro-inflammatory cytokines. Consistent with the apparent inability of CF cells to target TLR4 towards the lysosome for degradation, we observed persistent surface and cytoplasmic expression of this pathogen recognition receptor. This defect may account for the prolonged cycle of chronic inflammation associated with CF.
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