**Variability of toll like receptor mediated innate immune response in patients with cystic fibrosis and their relationship with clinical phenotype**

O.J. O’Connell1, C. Goss2, F. Radella3, F. Shanahan1, M.T. Henry1, M.M. Wurfel2, B.J. Plant1, 1Cork Adult Cystic Fibrosis Centre, University College Cork, Cork, Ireland; 2Division of Pulmonary and Critical Care, University of Washington, Seattle, United States; 3Department of Internal Medicine, University College Cork, Cork, Ireland

**Introduction:** Toll like receptor (TLR) response has been suggested as a factor in the inter-individual variability in clinical phenotype for patients with cystic fibrosis (PWCF). This study aims to compare the relationship between whole blood TLR responses in PWCF and healthy controls, and their association with clinical phenotype.

**Methods:** Using an ex-vivo whole blood stimulation model, blood from stable PWCF and healthy controls was incubated with different purified pathogen associated molecular patterns (PAMP’s) including *Pseudomonas* hexa and penta-acyl LPS (TLR4), *Burkholderia cenocepacia* (TLR4), Flagellin (TLR5), Pam3CYS (TLR1/TLR2) and *Zymosan* (TLR2/6/Dectin) over 6 hrs, and pro & anti-inflammatory cytokines measured in the supernatant (IL-6, IL-8, TNF-α, IL-1β and IL-10). Associations between log transformed, monocye-normalized TLR agonist-induced cytokine production and FEV1% predicted, gender, serum [1,25 OH D3] and azithromycin use at enrolment were calculated.

**Results:** 68 PWCF and 57 controls were recruited. All basal circulating cytokines were non-significantly higher in PWCF. All stimulated pro-inflammatory cytokine responses were significantly attenuated in PWCF versus controls for TLR4 and TLR5 PAMP’s (p < 0.001). There was a direct relationship between cytokine response and FEV1% predicted (p < 0.001). There was no significant difference in stimulated cytokine responses based on gender, serum [1,25 OH D3] or azithromycin use.

**Conclusion:** PWCF have an attenuated ex-vivo whole blood cytokine response to TLR stimulation suggestive of a chronic endotoxin tolerance state. This reduced TLR response is directly associated with worse lung function.

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**Nitrous oxide production in sputum from cystic fibrosis patients with chronic *P. aeruginosa* lung infection**

M. Kolpen1, M. Kühl2, T. Bjarnsholt1, C.R. Hansen3, T. Pressler3, L.L.H. Møller2, N. Husby1, P.O. Jensen1, 1Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark; 2University of Copenhagen, Marine Biological Section, Department of Biology, Copenhagen, Denmark; 3Copenhagen CF Center, Rigshospitalet, Copenhagen, Denmark

**Objective:** Chronic lung infection with *Pseudomonas aeruginosa* is the major severe complication in cystic fibrosis (CF) patients, where *P. aeruginosa* proliferates in biofilms on bronchial epithelial mucus under hypoxic conditions. Numerous polymorphonuclear leukocytes (PMNs) surround the biofilm and are major consumers of O2. We hypothesized that *P. aeruginosa* can acquire energy for growth in anaerobic endobronchial mucus by denitrification, which can e.g. be demonstrated by production or degradation of nitrous oxide (N2O), an intermediate in the denitrification pathway.

**Methods:** We measured concentration profiles of O2 and N2O with microsensors in fresh expectorated sputum from CF patients with chronic *P. aeruginosa* infection. The concentration of PMNs was estimated by flow cytometry. The Griess reagent assay was used to measure the concentration of NO2 and NOx in sputum samples.

**Results:** In purulent sputum from 9 CF patients, N2O production occurred only in layers where O2 was absent or at low concentrations (median 30.8 μM N2O, range 1.4–157.9 μM N2O). During the initial period of measurements the concentration of N2O increased followed by a period of decreasing N2O concentration. In addition, the concentration of PMNs correlated to the concentration of NO2 (P < 0.04, r2: 0.66, n = 10) and NO2 (P < 0.006, r2: 0.78, n = 11).

**Conclusion:** The present study demonstrates for the first time production of N2O in human sputum. Our results thus show that *P. aeruginosa* can acquire energy for growth via denitrification in hypoxic endobronchial mucus of CF patients. As a source of endobronchial NO2 and N2O we suggest the summoned PMNs.