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

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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 (PAMPs) including Pseudomonas hexa and pentacyl-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-10) and IL-10. Associations between log transformed, monocyte-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.

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

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Objective: Chronic lung infection with Pseudomonas aeruginosa is the major severe complication in cystic fibrosis (CF) patients, where P. aeruginosa proliferates in biofilms on the bronchial 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 Giess reagent assay was used to measure the concentration of NO₃ and NO₂ 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±1μM 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 NO₃ (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 NO₃ and NO₂ we suggest the summoned PMNs.

Immunoglobulin levels in cystic fibrosis patients: influence of age and disease severity

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Introduction: Abnormal immunoglobulin (Ig) levels, particularly elevated IgG levels, have been described in cystic fibrosis (CF) patients.

The aim of the study was to assess Ig levels among CF patients who regularly attend the CF center at the University Pediatric Clinic in Skopje, Macedonia and relate them to parameters of disease severity.

Methods: IgG, IgA and IgM levels were measured in 100 patients. Hyper- and hypo-IgG were compared with age and sex-matched control group with normal IgG levels.

Results: Median age of patients was 9.6 years (0.1−28), 61 males. Chronic Pseudomonas aeruginosa (PA) status was 20% with mean FEV1 61.7%. Six patients (6%) had hypo-IgG, all infants. Twenty patients had hyper-IgG (20%), mainly males and more than 18 years old (70%). No difference was found in any parameters of severity between hypo-IgG and controls. Hyper-IgG patients had lower FEV1 and worse Shwachman scores than the control group (55.9% versus 87.8%, p < 0.05).

Conclusions: Hyper-IgG as a marker of worse clinical status among CF patients is a useful tool for monitoring the severity of the disease and the presence of chronic PA infection. Hypo-IgG is present in some young CF patients and does not seem to have consequences.

The inflammatory response of in vitro co-culture models of normal and cystic fibrosis (CF) airways to CF-relevant pathogens

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Objectives: To complement research using CF animal models, we have developed and characterized a multi-cellular model of human airways, using co-cultures of fibroblasts with bronchial epithelial cells at air-liquid interface (ALI). The aim of this work was to determine if our model of CF airways displays the hyper-inflammatory responses characteristic of the disease.

Methods: Cells were grown at ALI for at >14 days to allow differentiation, as measured by transepithelial electrical resistance. Mono- and co-cultures were challenged with LPS (extracted from P. aeruginosa and B. cenocepacia), heat inactivated bacteria or live bacteria. Challenges were applied either to the apical side of polarised cultures, into the basal compartment or to both sides at once. Supernatants were harvested after 24 hours and ELISA analysis performed for IL-8.

Conclusion: Epithelial monocytes did not respond to challenge with LPS or heat inactivated bacteria; in fact IL-8 secretion was only increased following application of live bacteria to the apical compartment. Under these conditions, for non-CF epithelial cultures, apical release of IL-8 was ~400pg/ml, approximately double basolateral levels, suggesting directed release of IL-8. At baseline, CF epithelial cell monocytes secreted equivalent levels of IL-8 to non-CF, but importantly, the response to live bacteria was exaggerated in CF cultures (~900 pg/ml IL-8). The non-CF co-culture model also only responds to live bacteria applied apically. We are now investigating the response of CF co-culture models to confirm the presence of the hyper-inflammatory phenotype. We would like to thank the Humane Research Trust for funding.