

WS5.1 A high degree of variation in virulence of clonal *Burkholderia cenocepacia* ST32 isolates from cystic fibrosis patients in zebra fish embryos

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Strain *Burkholderia cenocepacia* sequence type (ST) 32 caused infections in ca. 30% of the Czech cystic fibrosis (CF) patients and in some cases ended up with a fatal cepacia syndrome. To evaluate ST32 virulence and differences in virulence among individual isolates, we tested 31 *B. cenocepacia* ST32 isolates obtained at different phases of chronic infection from 14 patients (28 isolates from sputum, 3 from blood cultures) in a zebra fish embryo infection model.

Results from survival assay in zebra fish embryos split the isolates into following virulence groups: group A contained 14 isolates, which were able to kill 100% of infected embryos within 64–96 hours post infection (hpi). Group B was composed of 13 isolates with intermediate virulence that killed 40–75% of embryos by 120 hpi. Four isolates (group C) were able to kill 0–20% of all embryos by 120 hpi. Blood isolate 1 (group B) and 2 (group C) were analyzed in more detailed. Real time analysis revealed that isolate 1 was able to cause systemic infection and only 30% of embryos were alive at 120 hpi. Isolate 2 did not cause systemic infection, embryos could control infection and they were alive at 120 hpi. To perform kinetics assay, five embryos were collected at 4, 24 and 48 hpi and treated for bacterial enumeration. Isolates differed each other in growth kinetics at 24 and 48 hpi as follows: for isolate 1, 3.70 ± 0.24 and 4.17 ± 0.13 log CFU and for isolate 2, 0.25 ± 0.16 and 0.83 ± 0.13 log CFU, respectively.

Our results indicate variation in virulence potential between *B. cenocepacia* ST32 isolates originating not only from different patients, but also from one patient. Supported by GAUK307311; IGA MZ NT12405–5; MS MT LD11029.

WS5.3 GSNOR inhibitors as potential, novel anti-inflammatory therapy in cystic fibrosis

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Small molecule inhibitors of the enzyme, S-nitrosoglutathione reductase (GSNOR) discovered and developed by N30 Pharmaceuticals, Inc., (N30 Pharma) may provide a novel therapeutic strategy in cystic fibrosis (CF). These inhibitors preserve endogenous levels of S-nitrosoglutathione (GSNO), an important signaling molecule that exerts its effects through modulation of protein function. GSNO levels have been found to be lower than normal in the CF lung, possibly through increased GSNOR activity. N30 Pharma's GSNOR inhibitors have demonstrated improved chloride channel function in *in vitro* and *in vivo* models of CF as well as potent anti-inflammatory effects in experimental models of chronic obstructive pulmonary disease (COPD). The inflammatory pathways through which GSNOR inhibitors exert their effects have relevance to the pathogenesis of CF. In an elastase-induced model of emphysema, GSNOR inhibition resulted in significant effects on preserving lung architecture and decreasing airway hyper-responsiveness compared to vehicle controls. In an 11-day tobacco smoke-induced model of COPD, GSNOR inhibition significantly reduced bronchoalveolar lavage neutrophils and decreased airway hyper-responsiveness. The anti-inflammatory effects of GSNOR inhibitors have been shown to be mediated in part via down regulation of NFκB through S-nitrosation of the p65 subunit. Other effects include direct antioxidant effects of GSNO and inhibition of a variety of chemokines and cytokines. The first of N30 Pharma's novel inhibitors is currently being studied in patients homozygous for *F508del-CFTR*. This study includes a comprehensive evaluation of biomarkers of inflammation and CFTR function.

WS5.2 Toll-like receptor 9 deficiency protects mice against *Pseudomonas aeruginosa* pulmonary infection

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Pseudomonas aeruginosa is an opportunistic pathogen involved in nosocomial infections. While a number of studies have demonstrated the roles of TLR2, TLR4 and TLR5 in host defense to *P. aeruginosa* infection, the implication of TLR9 in this process has been overlooked. Here, we showed that *P. aeruginosa* DNA stimulated TLR9 in both alveolar macrophages (AMs) and airway epithelial cells (AECs). This activation required asparagine endopeptidase- and endosomal acidification. Interestingly, TLR9^{-/-} mice resisted to lung infection by *P. aeruginosa* as compared to WT mice. The resistance of TLR9^{-/-} mice to *P. aeruginosa* infection was associated with:

- i. a higher ability of TLR9^{-/-} AMs to kill *P. aeruginosa*
- ii. a rapid increase in the production of pro-inflammatory cytokines TNFα, IL-1β and IL-6 and
- iii. an increase in nitric oxide (NO) production and inducible NO synthase (iNOS) expression in both lungs and AMs.

Interestingly, inhibition of both IL-1β and NO production resulted in a significant decrease of *P. aeruginosa* clearance by AMs. Altogether these results indicate that both IL-1β and NO play a key role in AM-mediated clearance of *P. aeruginosa*. TLR9 plays a detrimental role in pulmonary host defense toward this pathogen by downregulation IL-1β and NO production by AMs.

WS5.4 Increased production of IL-17A in circulating Th17 and dysfunctional Tregs in adults with cystic fibrosis-related diabetes

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Cystic fibrosis-related diabetes (CFRD), a frequent complication of CF, is associated with decreased lung function and increased mortality. Our preliminary data showed that CF patients, regardless of their glucose tolerance, have increased glucose fluctuations (GF) and hyperglycemia (HG) when compared to healthy controls (HC) that could be associated with altered immune reactivity. IL-17A, produced by Th17 and dysfunctional regulatory T-cells (Treg), is a pro-inflammatory cytokine associated with diabetes and CF lung disease. Its role in CFRD is unknown.

Objectives: Determine if Th17 and Treg cells of CF patients, particularly CFRD patients, produce increased levels of IL-17A.

Methods: Peripheral blood was drawn from HC (n = 7), non-diabetic (n = 6) and pre-diabetic (n = 2) CF and CFRD patients (n = 7). Lymphocytes were isolated, incubated (24 h, 5 mM glucose), stimulated or not with phorbol myristate acetate (PMA) and phytohemagglutinin (PHA) and then stained for cell surface markers (CD3, CD4, CD25), FoxP3 and IL-17A. The proportion of Th17 (CD3⁺CD4⁺CD25^{low}FoxP3⁻) and Treg (CD3⁺CD4⁺CD25^{high}FoxP3⁺) cells producing IL-17A and their relative production were determined by flow cytometry.

Conclusions: Stimulation with PMA and PHA increased the proportion of Th17 and Treg cells producing IL-17A and the quantity they produce (p < 0.02). The proportion of Th17 and Treg cells producing IL-17A of CF patients and HC were similar (p > 0.05). However, Th17 cells of CFRD patients (p = 0.01) and Treg cells of all CF patients (p = 0.004) produced increased levels of IL-17A as compared to those of HC. Thus, increased production of IL-17A by circulating Th17 and Treg cells is associated with CFRD.