

WS17.1 Modulation of inflammatory mediators associated with cystic fibrosis by alpha-1 antitrypsin

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Cystic fibrosis (CF) lung disease is defined by a continuous cycle of infection and inflammation which has a deleterious effect on the quality of life and patient survival. Neutrophils and their derived products perpetuate the inflammation associated with CF lung disease due to excessive inflammatory cytokines including TNF- α . A potential therapeutic option for CF is the use of protease inhibitors such as alpha-1 antitrypsin (AAT), which is known to possess novel anti-inflammatory properties. The aim of this study was to evaluate AAT potential to modulate neutrophil function in response to the key inflammatory cytokine TNF- α .

An evaluation of AAT impact on the production of superoxide by TNF- α stimulated neutrophils was carried out using a cytochrome C assay. The HL-60 cells were used to determine AAT impact on TNF- α autocrine signaling through NF- κ B activation, assessed by western blot analysis of I κ B α degradation and gene expression of TNF- α . ELISA was employed to investigate the ability of AAT to inhibit TNF- α binding to the TNF receptors TNF-R1 and TNF-R2.

Our results demonstrate that AAT reduced the production of superoxide in a dose dependant manner in cells stimulated with TNF- α . AAT regulated TNF- α autocrine signaling resulting in reduced I κ B α degradation and subsequent TNF- α gene expression in HL-60 cells. Protein binding studies confirmed that AAT inhibition of TNF- α signalling is in part due to AAT binding thereby preventing TNF- α interaction with TNF Receptor 1 and Receptor 2 ($p=0.02$).

This study has uncovered AAT as novel modulator of the TNF- α inflammatory signaling pathway and highlights the potential for AAT as being a treatment option for CF.

WS17.2 Anti-inflammatory therapy in a mouse model of *Pseudomonas aeruginosa* lung infection leads to bacteraemia

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In cystic fibrosis (CF), neutrophils restrict bacterial infections to the airways, however contribute to excessive airway tissue remodeling/destruction. Therefore, reduction of neutrophils by anti-inflammatory drugs has been proposed. Whether this negatively impacts innate immunity is unknown. We assessed the effects of the orally administered leukotriene B4 (LTB4)-receptor antagonist BIIL 284 on neutrophils, *P. aeruginosa* numbers and cytokines in C57Bl/6 mice. Treatment of *P. aeruginosa*-infected mice with 0.3, 50 or 100 mg/kg body weight of BIIL 284 decreased pulmonary neutrophils significantly after 24 h. While bacterial numbers remained stable in total lung homogenates of animals treated with 0.3 mg/kg body weight of BIIL 284 after 24 h, *P. aeruginosa* cfus increased significantly ($p<0.01$) when 50 or 100 mg/kg body weight of BIIL 284 was administered compared to untreated control animals. Significantly higher bacteraemia rates and lung inflammation were observed in BIIL 284 treated animals compared to placebo treated animals. Because reduction of pulmonary neutrophils by BIIL 284 below a critical level allows enhanced proliferation and transmission of pathogens to the bloodstream in infected animals, care should be taken when administering potent anti-inflammatory compounds in CF patients who already may suffer from bacteraemia during acute exacerbation.

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WS17.3 Alteration of human macrophage functions in cystic fibrosis

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Recently, several reports suggest that CFTR should contribute to altered inflammatory response in cystic fibrosis (CF) by modification of normal macrophage functions. In order to highlight possible intrinsic macrophage defects due to CFTR dysfunction, we have studied macrophages from human peripheral blood monocytes of healthy subjects (non CF) and CF patients.

Ficoll isolated adherent monocytes were differentiated in macrophages with GM-CSF (400 U/mL) during 6 days. Non CF macrophages were treated during 72h with CFTR^{inh-172} (10, 20 μ M). We have focused to study some membrane markers of CD71⁺ macrophages (mCD14, CD16, CD64, CD11b) by flow cytometry. Soluble CD14, IL-1b and IL-8 supernatant levels were quantified by ELISA. Phagocytosis was measured by fluorescein-labeled *E. coli* Bioparticles.

In CF macrophages, we have observed strong inhibition of phagocytosis (70%) as well as 1.3-, 1.8- and 2.0-fold significant decrease of CD16, CD64 and CD11b respectively. However membrane CD14 (mCD14) was slightly increased but mCD14/sCD14 ratio is in favor of sCD14 (1/6 vs 1/270 in CF cells). Elsewhere, concentrations of IL-1b and IL-8 were strongly raised in CF cell supernatant. CFTR inhibition in non CF macrophages also showed alterations in surface expression of CD11b, CD16, CD64, IL-8 level and phagocytosis.

Pathogens recognition and phagocytosis seems to be impaired in CF macrophages, and some alterations could be associated with CFTR defect. Thus, perturbations of CD11b and sCD14, involved in innate immune response, may play a potential important role in CF pathogenesis.

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WS17.4 Anti-pseudomonas IgY antibodies opsonize *Pseudomonas aeruginosa*, augmenting the phagocytosis activity of polymorph nuclear neutrophils

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Background: Oral treatment with egg yolk antibodies (IgY) against *Pseudomonas aeruginosa* (PA) in cystic fibrosis (CF) patients prevents PA infections in a clinical study. IgY seems to acts as anti-adherence factor and bacterial neutralizer. The present study evaluated the opsonizing capacity of IgY and polymorph neutrophils (PMNs) burst and PMN mediated PA killing in vitro.

Methods: Anti-*Pseudomonas* specific (S-IgY) or non-specific (C-IgY) IgY antibodies were obtained from ImmunSystem I.M.S. (Uppsala, Sweden). PA vaccine-strains (PAO1, PAO3, PAO5, PAO6, PAO9, PAO11) and a flagel mutant strain were grown in LB broth.

PMNs were isolated from whole blood. The purified PMNs were resuspended in Krebs-Ringer buffer with glucose and human sera at a density of 10⁷ PMNs/ml. Phagocytosis was commenced by adding 1 \times 10⁸ PA to PMNs with either C-IgY or S-IgY. The phagocyte-derived reactive oxygen species (ROS) was measured by adding luminol and using a luminometer. Mixing (non)opsonized bacteria and PMNs and plating diluted samples overnight followed by colony counting evaluated bacterial killing.

Results: S-IgY and C-IgY increased the chemiluminescence signal in all PA vaccine-strains compared to non-IgY ($p<0.05$). The S-IgY signal was higher than C-IgY ($p<0.05$) except in low concentration (0.005%). No difference between S-IgY or C-IgY was observed in the flagel mutant strain. Bacterial killing in + S-IgY groups ranged from 43%-97%, 21%-86% in + C-IgY groups and 7%-72% in non-IgY groups. S-IgY increased the bacterial killing in all the vaccine-strains compared to non-IgY controls ($p<0.05$).

Conclusions: Specific IgY opsonizes PA resulting in increased clearance by PMNs, thus may explain the observed protective activity.