165 Mannan-binding-lectin-associated serine protease 2 (MASP-2) – a major modifier of CF lung disease?

H.V. Olesen[1], J.C. Jensenius[2], R. Steffensen[3], S. Thiel[2], P.O. Schiøtz[1]. [1]*Dept Pediatrics, Aarhus University Hospital,* [2]*Dept Medical Microbiology and Immunology, University of Aarhus;* [3]*Dept Clinical Immunology, Aalborg Hospital, Denmark*

Aims: Activation of complement through the lectin pathway, part of the innate immune-defence against bacteria, is initiated by either mannan-binding-lectin (MBL) or the ficolins (H, M or L-ficolin) which are all in complex with a common protease, MBL-associated serine protease-2 (MASP-2). Some studies have associated deficiency of MBL with poorer outcome in CF lung disease. We explored the modifying effects of dysfunction of the lectin pathway by analysis of the MASP-2 dysfunction mutation (D105G) as well as MBL-2 genotypes.
Methods: We analysed concentrations and genotypes of MASP-2 and MBL in 109 CF patients (76 children and 33 adults), and correlated the results to lung function (FEV-1, FVC and FEF25–75) and chronic bacterial infections.
Results: Patients heterozygous for the D105G mutation of MASP-2 had low concentrations of MASP-2, but normal MBL pathway function. We found no correlation to poor lung function. We found, however, one child homozygous for the mutation and with no pathway function. She had extremely severe lung disease with no other obvious precipitating factors. We found no correlation between MBL deficiency and poor lung function. Actually lung function was higher in patients with the genotypes resulting in deficiency/dysfunction of MBL (XA/YO+YO/YO) than with the other genotypes ($p < 0.04$).
Conclusion: We describe the first CF patient homozygous for the D105G mutation of MASP-2, with otherwise unexplained severe lung disease. We suspect MASP-2 dysfunction to be a major modifier of CF lung disease. Earlier reports of poorer outcome for MBL deficient patients were not supported by our findings.

166* Mast cell activation and oxidative stress may be key factors in pulmonary destruction in Cystic Fibrosis

P.O. Schiøtz[1], M. Kruhøffer[2], P. Ebbesen[3], V. Zachar[3], H.B. Andersen[1], M. Holm[1], H.V. Olesen[1]. [1]*Ped. Dep. A, Aarhus University Hosp.,* [2]*Dep. of Clin. Chem., Aarhus University Hosp.;* [3]*Lab. of Stem Cell Research, Aalborg University, Denmark*

Aims: To investigate the prostaglandin secretion from mast cells and its dependence on gluthtation concentration and oxygen tension.
Methods: Mast cells from controls and CF patients were cultured from peripheral blood CD133+ stem cells. Mast cells were incubated with IgE for 24 hours and then stimulated with anti-IgE. Before stimulation increasing concentrations of reduced glutathion were added to the cultures. After 30 min. of stimulation prostaglandin was measured in the supernatant. Furthermore mastcells were cultured at 2%, 5% and 21% oxygen tension.
Results: Increasing concentration of reduced glutathion was able to reduce prostaglandin production with up to 50% in mast cells from CF patients as well as normal controls.
Furthermore mast cell culture at 2% and 5% also inhibited prostaglandin synthesis with more than 50% compared to culture at 21% oxygen. This was prostaglandin specific since histamin release and receptor expression was unchanged in cultures at 5 and 21% oxygen.
Conclusions: We hypothesize that prostaglandin synthesis depends on the amount of free oxygen radicals. This could explain why glutathion (a free radical scavenger) and reduced oxygen tension both reduce prostaglandin synthesis. Since concentration of gluthation is reduced in epithelial lining fluid, this is in accordance with the clinical findings of increased mucus production and increased frequency of astmatic symptoms found in CF, both known prostaglandin effects. It further fits the effect of COX inhibitors (Ibuprofen), and the findings of abnormal prostaglandin metabolism previously published in CF.

167 Surfactant protein D in BAL of children with CF

J. Brazova[1], A.P. Pohunek[2], A. Sediva[1]. [1]*Institute of Immunology,* [2]*Pediatric Clinic, University Hospital Motol, Prague, Czech Republic*

Introduction: Surfactant protein D (SP-D) is a member of pulmonary collectins that plays an important role in the first line lung defense mechanisms, enhances opsonization of microbes and limits lung inflammatory responses. Our hypothesis was that insufficient production of SP-D in lungs of CF patients measured in bronchoalveolar lavage fluids (BAL) plays role in defect defense respiratory mechanisms in these patients.
Patients and Methods: BAL from 15 CF children (mean 8.5 years, S.D. 3.9, 10 female, 5 male) and 42 control subjects (mean 9.5 years, S.D. 6.3, 26 female, 16 male) without CF (with respiratory diseases like bronchial asthma, bronchopneumonia and non respiratory diseases especially leukemias) undergoing clinically indicated bronchoscopy were investigated. Levels of SP-D (pg/ml) were measured in BAL supernatants using commercial available ELISA kit. Results were further interpreted in the context of acute state blood investigation.
Results: In our pilot study with 15 CF children and 42 non CF controls production of SP-D was lower in CF (mean 335, S.D. 317) than in controls (mean 750, S.D. 374) being statistically significant ($p < 0.0015$). In CF patients lung inflammation regarded to neutrophil counts was inversely correlated with SP-D levels. Neither age nor gender did affect SP-D levels.
Conclusion: Insufficient production of SP-D in lungs in CF patients resolved in defect defense mechanisms. Therefore supplementation of SP-D might be a useful therapeutical target in CF patients to prevent infectious and inflammatory lung damage in these patients.
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168 *Pseudomonas aeruginosa* flagellin is the major antigen for anti-pseudomonas IgY

E. Nilsson[1], H. Kollberg[2], M. Johannesson[2], B. Wretlind[3], A. Larsson[1]. [1]*Dep. of Medical Sciences, University Hospital,* [2]*CF Center, University Childrens Hospital, Uppsala;* [3]*Dep. of Clinical Bacteriology, Div. of Laboratory Medicine, Karolinska Hospital Huddinge, Sweden*

Aims: *Pseudomonas aeruginosa* (PA) account for high morbidity and mortality in cystic fibrosis (CF) patients. CF patients with a chronic PA infection have more rapid deterioration of lung functions and the bacteria are impossible to eradicate from the lungs. Antibiotic resistant PA strains are steadily increasing. We have previously shown that specific chicken IgY antibodies against PA, anti-pseudomonas IgY (anti-PA IgY), purified from the egg yolk of hens immunized with PA may be an alternative to prevent PA infections in CF (Pediatr Pulmonol 2003; 35: 433). The number of positive PA cultures and the need of antibiotics have decreased significantly while lung function is maintained. Anti-PA IgY reduces PA adhesion to epithelia, but the mechanism has not been fully elucidated.
Methods and Results: The immunoreactivity of anti-PA IgY was investigated to get further insight in the prophylactic effect of these antibodies. Flagellin was identified as the major antigen by 2D electrophoresis of PA strains, western blot and MALDI-TOF mass spectrometry.
Discussion: Flagellin is the main protein of the flagella, involved in chemotaxis, motility, adhesion and inflammation and crucial for establishing infections in hosts. Furthermore, flagellin is secreted by PA, which elicits an inflammatory response. Earlier studies have both shown that flagellin mutants are much less infectious than flagellated strains and that antibodies against flagellin reduce PA infection.
Conclusion: Anti-PA IgY binds flagellin, which may prevent PA infections in CF patients by reduced inflammation and less ability of host invasion.