

European CF Young Investigator Meeting 2008

Tuesday, August 26: Arrival + check-in (16:00 – 20:00)

Wednesday, August 27:

08:30 – 9:00 Welcome

09:00 – 10:30 Session 1: Clinical issues in CF

Introductory lecture

Progression of abnormal Carbohydrate Metabolism to Diabetes Mellitus in Patients with CF
Christine KAMPFERT

Is presence of cystic fibrosis-related diabetes really associated with a faster decline in pulmonary functions?
Annemarie VAN DEN BERG

Recurrent pancreatitis as the first manifestation of cystic fibrosis: a single centre experience
Federico ALGHISI

The use of non-invasive liver elastography (fibroscan) for early detection of cystic-fibrosis-associated liver disease: a cross-sectional study
Peter WITTERS

10:30 – 11:00 Coffee break

11:00 – 13:00 Session 2: The respiratory epithelium

Introductory lecture

Modulation of the pro-inflammatory phenotype of CFTR mutated epithelial cells
Delphyne DESCAMPS

Secretion of neutrophil extracellular traps (nets) by neutrophils isolated from the sputum of cf patients and interaction with neutrophil serine proteases
Alexandre GAUTHIER

IL-6 and IL-8 increase the expression of glycosyltransferases and sulfotransferases involved in the biosynthesis of sialylated and/or sulfated Lewisx epitopes in the human bronchial mucosa
Sophie GROUX-DEGROOTE

Epithelial factors involved in the regeneration and remodelling of the human airway epithelium
Jacqueline ROUX

Regulation of Muc5ac expression by arachidonic acid metabolites
Ignacio GARCIA-VERDUGO

The C-terminus of the transmembrane mucin MUC17 binds to the scaffold protein PDZK1 that stably localizes it to the enterocyte apical membrane in the small intestine
Thafer PELASEYED

13:00 – 14:30 Lunch

14:30 – 16:30 Session 3: Microbiology in CF

Introductory lecture

Molecular epidemiology of the longitudinal course of the *Pseudomonas aeruginosa* infection in cystic fibrosis

Nina CRAMER

Hypermutable expression in *P.aeruginosa* from Cystic Fibrosis patients in the early colonization and in the long-term infection stage

Patrizia MORELLI

Lung pathogenesis of *Pseudomonas aeruginosa* hypermutable strains from patients with cystic fibrosis

Sara MONTANARI

Molecular behaviour of *Pseudomonas aeruginosa* peptidoglycan at different stages to the airways of cystic fibrosis patients

Maria Rosaria LEONE

Development of genetic tools for *Mycobacterium abscessus*, an emerging pathogen, and construction of a strain devoid of glycopeptidolipid

Halima MEDJAHED

Analysis of endotoxins from *Burkholderia*

Teresa IERANO

16:30 – 17:30 Poster session

17:30 – 19:30 Session 4: Immunology in CF

Introductory lecture

Increased susceptibility to allergic airway inflammation in a murine model of cystic fibrosis lung disease

Lu DAI

Antioxidant imbalance in the lung from CFTR-KO mice: abnormal Peroxiredoxin 6 expression levels and phospholipid peroxidation

Stéphanie TRUDEL

Transcription factor "decoy" strategy to silence expression of pro-inflammatory genes in CF bronchial epithelial cell lines

Valentino BEZZERRI

Azithromycin is not able to decrease inflammatory process in cystic fibrosis cells

Vinciane SAINT-CRIQ

Glucocorticoids resistance in Cystic Fibrosis

Carine REBEYROL

CTLA-4-mediated regulatory phenotype of T cells in tolerant lung recipients

Karine BOTTURI-CAVAILLES

19:45 – 22:00 Dinner

Thursday, August 28:

08:45 – 10:30

Session 5: Microbiology in CF

The "P-usher", a novel protein transporter involved in fimbrial assembly and TpsA secretion
Ségolène RUER

The CupE system in *Pseudomonas aeruginosa* is a new gene cluster involved in *P. aeruginosa* community lifestyle
Caroline GIRAUD

Pseudomonas aeruginosa Tad-dependent Flp pilus assembly is transcriptionally controlled by the PprAB two-component system and post-translationally modified by the RcpC protein
Christophe Sébastien BERNARD

Development of a low dose nasal infection model with *pseudomonas aeruginosa* in mice
Florian WOLBELING

Novel virulent genes of *Pseudomonas aeruginosa* by high-throughput screening in a mouse model of chronic infection
Irène BIANCONI

The effects of lectins carbohydrates inhibitors on *Pseudomonas aeruginosa* pathogenesis
Chanez CHEMANI

n-3 long chain polyunsaturated diet improves the resistance to *Pseudomonas aeruginosa* lung infection in CF mice
Hélène TIESSET

10:30 – 11:00

Coffee break

11:00 – 12:30

Session 6: Biology of CFTR

Introductory lecture

Molecular, cellular and functional study of seven rare mutations of CFTR
Fleur FRESQUET

COMMD1 promotes CFTR trafficking through ubiquitination
Loïc DREVILLON

GPact-11a: new CFTR activator
Johanna BERTRAND

Endoplasmic reticulum stress : implications for cystic fibrosis
Mathieu KERBIRIOU

12:30 – 14:00

Lunch

14:00 – 16:00

Session 7: Genetic and gene therapy

Introductory lecture

Mechanisms of cftr pre-mRNA splicing
Gwendal DUJARDIN

Functional interplay between *c/ebpb*, *yy1* and *usf* involved in transcriptional cftr regulation
Estelle LOPEZ

Generation of CFTR mutant pig models for cystic fibrosis

Katrin WALLNER

Imaging tools for the study of gene transfer using synthetic vectors

Olivier LE BIHAN

In vitro screening model for viral vectors for optimal pulmonary transduction

Marianne CARLON

Long term pulmonary gene transfer with a lentiviral vector in a fetal mouse model

Jaan TOELEN

16:00 – 17:00 Posters session

18:30 – 19:30 City tour

19:30 Networking

Friday, August 29:

09:30 – 10h45 Session 8: Ion channels in CF

Differential expression of murine CLCA-Gene family members in cystic fibrosis mouse models

Josephine BRAUN

Normal Ca²⁺-activated Cl⁻ channel (CaCC) activity in native airway tissues of mCLCA3 deficient mice

Bjarki JÓHANNESSON

Towards a rational therapy for Cystic Fibrosis Liver Disease

Mark K DOEVEN

Upregulated expression of ENaC subunits in human CF nasal epithelium

Nadine BANGEL

Enduring inhibition of Na⁺-hyperabsorption in human CF nasal epithelia cells

Katja SOBCZAK

10h45 – 11h15 Coffee break

11h15 – 12h15 Session 9: Clinical issues in CF

Recurrence of PSA after early treatment- a long-term follow-up study

Katharina REBMANN

Own clinical observations with long-term tobramycin inhaled therapy in children with cystic fibrosis

Malgorzata OLSZOWIEC-CHLEBNA

Exhaled leukotriene B₄ and nitric oxide in cystic fibrosis

Karolien BLOEMEN

The effect of vitamin K supplementation on bone formation in children with Cystic Fibrosis

Jeroen VAN HOORN

12h30 – 13h00 Award and Conclusion

13h00 Fly-away lunch

Abstracts

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SESSION 1:
CLÍNICAL ISSUES IN CF

Poster # 1

Progression of abnormal Carbohydrate Metabolism to Diabetes Mellitus in Patients with CF

Kämpfert C¹, Holl R W², Marquart A² and Ballmann M¹ for the CFRD Study Group

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There is an ongoing discussion when to start intervention for abnormal glucose metabolism in CF patients. Nevertheless there are only few data describing the time course from first abnormal OGTT to diabetic OGTT. We prospectively investigated the time course from first abnormal OGTT to diabetic OGTT in a large group of patients. In this study all CF patients were investigated by annual OGTT from the age of 10 years on. We used the data set of the screening phase of a prospective intervention study which compared oral agents (repaglinide) to insulin in the therapy of Cystic Fibrosis-related Diabetes mellitus (CFRD). Between 2002 and 2008, 4514 OGT tests from 1579 CF-patients were available for statistical analysis (Kaplan-Meier-curves, Wilcoxon-test).

The 1st quartile for a first pathological OGTT result was 16.4 years. The 1st quartile for a first diabetic test result was 21.5 years. The 1st quartile for a confirmed CFRD (second diabetic OGTT) averages in male patients 27.6 years, in female patients 24.5 years. In an age of 18 years 32 % of CF patients show a first pathological OGTT. 16.5 % have a first diabetic test result and 11.5 % has confirmed Diabetes. 6 % of male patients in the age of 18 have Diabetes and 16 % of the female patients.

The relatively long interval between abnormal OGTT and diabetic OGTT might be a window in which intervention should take place. Up to now there are no prospective randomized controlled studies related to this issue. The interval from abnormal OGTT to diabetic OGTT is shorter in females. This might be part of the discussion why females are more severe ill with CFRD than males in cross-sectional studies.

Supported by Mukoviszidose e. V., Bonn and NovoNordisk Pharma GmbH, Mainz, Germany.

Poster # 2

Is presence of cystic fibrosis-related diabetes really associated with a faster decline in pulmonary functions?

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Background:

Cystic fibrosis-related diabetes (CFRD) is believed to be associated with faster decline in pulmonary functions and higher morbidity and mortality in CF patients. However, accurate matched patient control studies are missing.

Aims:

We investigated retrospectively the decline in pulmonary functions and BMI in patients with CFRD in comparison with properly matched CF patients without diabetes.

Methods:

27 patients with insulin dependent CFRD and good diabetic management (median HbA1c = 7,0) were matched with 27 CF patients without diabetes according to sex, age, BMI, class of mutation, FEV1% predicted and infection status. Over a period of 5 years the following parameters were studied: FEV1% predicted, FVC% predicted, BMI and in-hospital days.

Results:

At the start of this study, no differences between CFRD patients and CF patients without diabetes were seen. Over a period of 5 years, both patients groups showed a remarkable decline in pulmonary functions. The rate of decline for FEV1% predicted was significantly higher in CFRD patients (11,8% decline in 5 year vs. 8,1% decline in 5 years, p=0,007), though this difference was not significant for FVC% predicted. CFRD patients also spent more days in hospital (36 vs. 17 in-hospital days, p=0,050). BMI remained unchanged in both groups.

Conclusion:

After 5 years, differences were found between CFRD patients and properly matched CF patients without diabetes for FEV1 and in-hospital days, however not for FVC and BMI. This study shows that the clinical impact of CFRD is certainly present, but probably smaller than originally presumed.

Poster # 3

Recurrent pancreatitis as the first manifestation of cystic fibrosis: a single centre experience

Alghisi F¹, Angioni A², Tomaiuolo A C², D'Apice M R³, Gambardella S³, Russo B¹, Bella S¹, De Angelis P⁴, Dall'Oglio L⁴, Novelli G³ and Lucidi V¹

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Background:

Epidemiology of Recurrent Idiopathic Pancreatitis (RIP) is not well understood in pediatry. RIP can also be a manifestation of cystic fibrosis (CF). Several genetic studies have recently demonstrated a role of CFTR, SPINK1 and PRSS1 genes in the pathogenesis of RIP.

Aim:

to report the experience of a single centre in the evaluation of RIP occurrence as the first manifestation of CF.

Patients and Methods:

We studied, retrospectively, 51 young patients (27 F, mean age at diagnosis: 11.1±6.7 yrs) affected by recurrent acute pancreatitis and showing signs of chronic pancreatitis. All patients were submitted to ERCP to exclude bilio-pancreatic malformations. Patients without malformations underwent sweat test and genetic analysis: CFTR gene was evaluated by DHPLC method and the most common mutations of PRSS1 and SPINK1 genes were also studied.

Results:

A bilio-pancreatic malformation was diagnosed in 12 patients (23.5%: 5 choledochus cyst, 2 pancreas divisum, 2 duodenal duplication, 2 choledochus stenosis, 1 duodenal stenosis). Biliary lithiasis was observed in 5 patients (9.8%), whereas 1 developed a post-transplantation, drug-induced pancreatitis. No bilio-pancreatic malformations were detected in 33 patients (64.6%); a family history of chronic pancreatitis was observed in 43.3% of them. Furthermore, genetic analysis showed mutations in CFTR, SPINK1 and PRSS1 genes in 31%, 7.7% and 3.6% of patients, respectively. The sweat test identified border-line values in 23.3% of patients, allowing a diagnosis of atypical CF.

Conclusions:

In 33 pediatric patients affected by RIP we observed high percentages of familial/hereditary pancreatitis (43.3%) and atypical CF (23.3%). Genetic testing confirmed the high frequency of CFTR mutations and has been reported to be useful in the detection of atypical forms of CF. An early diagnosis of atypical CF allows a better quality of life and prognosis.

Poster # 4

The use of non-invasive liver elastography (fibrosan) for early detection of cystic-fibrosis-associated liver disease: a cross-sectional study

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Background:

CF is a multi-organ disease. CF liver disease is the second cause of mortality and morbidity and its prevalence is estimated to be 34%, but sensitive tools for early detection are lacking, as biochemical markers and ultrasound(US) screening are neither sensitive nor specific.

Aim:

We investigated if the Fibrosan could serve as a screening tool.

Methods:

Fibrosan (10 measurements, of which $\geq 60\%$ successful, cut-off for abnormal was $>7.2\text{kPa}$) was performed on 46 patients attending the CF clinic for routine check-up (age range: 1.4y-42.7y). These results were compared to halfyearly biochemistry (liver function: AST, ALT, Alkaline Phosphatase, γGT , bilirubin) and to US (presence of hepato- or splenomegaly and Westaby score), currently considered the goldstandard in this population.

Results:

Fibrosan results were above the cut-off in 15% (7/46) of the patients (median value and range of the whole group 4.65, 2.8-29.10kPa).

Of these, 7 patients (4male, 3 female) with abnormal Fibrosan value (7.5, 7.9, 8.1, 10.8, 11.6, 20.5 and 29.1kPa), two had a history of meconium ileus and all were pancreas insufficient. Four had biochemically demonstrated CF liver disease. On US, 4 had hepatomegaly, 5 had splenomegaly and Westaby scores were 4,?, 5,5,8,8 and 9 respectively.

There was no correlation between liver elasticity and liver biochemistry. The liver stiffness was however significantly increased in the US splenomegaly group (n=12/36) (6.55, 3.7-29.1kPa versus 4.7, 2.8-11.6kPa, $p=0.01$) but not in the hepatomegaly group (n=11/40, 4.8, 3.8-29.1kPa versus 4.8, 2.8-11.6kPa, $p=\text{N.S.}$). There was a positive correlation with the Westaby score ($R=0.55$, $p<0.001$).

Conclusions:

Reliable non-invasive outcome measures of CF liver disease are crucial for use in therapy trials and to evaluate preventive strategies. Fibrosan could provide a valuable tool to detect and quantify CF liver disease. Fibrosan is an objective measure and is easy to perform in CF patients, even in small children.

Session 2:

The respiratory epithelium

Poster # 5

Modulation of the pro-inflammatory phenotype of CFTR mutated epithelial cells

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). In addition to the ionic imbalance conferred by the $\Delta F508$ CFTR mutation, evidence also exists that, even in the absence of infection, $\Delta F508$ CFTR deficient targeting to the cell membrane may activate the cellular stress response and induce a pro-inflammatory phenotype by activating potent transcription factors, including NF- κ B. In the lung, elafin and SLPI (secretory leukocyte protease inhibitor), endogenous mucosal anti-proteases produced by epithelial cells or macrophages, are important anti-proteases known for their anti-microbial and anti-inflammatory (anti-NF κ B) properties.

Aim:

To assess whether correction of $\Delta F508$ -CFTR pro-inflammatory phenotype is possible using endogenous mucosal antiproteases (elafin or SLPI).

Methods:

Human epithelial cells and CFTR over-expression:

a: IB3 ($\Delta F508$ -CFTR over-expressers) and S9 control bronchial epithelial cells ; b: over-expression of WT-CFTR and $\Delta F508$ -CFTR in A549 alveolar cells and BEAS-2B bronchial cells, using recombinant adenovirus (Ad) vectors.

Measurement of 'CFTR cells' pro-inflammatory phenotype:

Sub-confluent epithelial cells (described above) were either mock-infected (with an 'empty' Ad vector) or infected with Ad-NF- κ B-luciferase (multiplicity of infection/MOI 25). 24 hrs later, cells were either mock-stimulated or stimulated with increased concentrations of TNF- α . Secretion of IL-8 and/or luciferase activity were used as pro-inflammatory read outs of the stimulation.

Modulation of CFTR phenotype with antiproteases:

Whenever possible, the modulation of the pro-inflammatory phenotype of 'CFTR cells' (see above) was attempted by using Ad derived elafin or SLPI, two mucosal protease inhibitors. As above, cells were infected at subconfluence with 50 MOI of Ad. After 24 hrs, cells were either further mock-infected or infected with Ad-NF- κ B-luciferase and/or stimulated with TNF- α . As above, secretion of IL-8 and/or luciferase activity were measured.

Results:

We show and confirm that IB3 $\Delta F508$ -CFTR cells secreted 4- to 6-fold higher levels of IL-8 than S9 corrected cells when stimulated with 1, 10 or 50 ng/ml of TNF- α . BEAS-2B- or A549-Ad- $\Delta F508$ -CFTR cells also secreted high levels of IL-8, when stimulated with low levels of TNF- α , when compared to BEAS-2B- or A549-Ad-WT-CFTR controls, respectively. The IL-8 differential output in 'CF cells' seems mediated by the NF- κ B pathway in mutated IB3 cells (2- to 4-fold increase in luciferase activity, when compared to S9-corrected cells).

Conclusion:

We confirm here, using 2 independent methods of over-expressing $\Delta F508$ -CFTR, that the $\Delta F508$ -CFTR mutation confers a pro-inflammatory phenotype (IL-8 secretion) to bronchial and alveolar epithelial cells. The modulation of the described pro-inflammatory phenotype by anti-proteases is currently under study in our laboratory.

This work is supported by the Association Vaincre la Mucoviscidose.

Poster # 6

Secretion of neutrophil extracellular traps (nets) by neutrophils isolated from the sputum of cf patients and interaction with neutrophil serine proteases

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Background :

The secretion of mucus and the release of DNA by lytic/necrotic neutrophils contribute to the high viscosity of bronchial secretions in CF patients. This environment favors bacterial infection that, in turn, promotes massive influx of neutrophils. Activated neutrophils release cationic serine proteases in the airways that interact with negatively charged DNA and may partly escape from control by protease inhibitors.

In addition to this release of DNA by lytic/necrotic neutrophils, an active secretion of chromatin fibers covered by granular proteins, the Neutrophil Extracellular Traps (NETs), has been recently described. The aim of this study is to measure the activity of elastase, protease 3 and cathepsin G in response to the secretion of NETs by neutrophils isolated from the sputum of CF patients.

Materials and methods:

Neutrophils were purified from blood of healthy volunteers and CF patients and from sputum of CF patients. The activity of proteases was measured using specific FRET peptide substrates (Fluorescence Resonance Energy Transfer) and the extracellular DNA was quantified by fluorimetry. NETs were induced by phorbol-myristate acetate (PMA) and interleukin-8 (IL-8) and characterized by immunocytochemistry and scanning electron microscopy.

Results :

Neutrophils purified from blood and sputum of patients produce extracellular DNA filaments bearing similar concentrations of active elastase, protease 3 and cathepsin G. Neutrophils purified from sputum produce 10 times more NETs (DNA and proteases) than neutrophils from blood patients and controls. A significant part of NETs-associated proteolytic activity remained resistant to inhibition. After DNase treatment, we observed an increase of neutrophil elastase, protease 3 and cathepsin G peptidasic activity, but these activities were completely and stoichiometrically inhibited by serpin inhibitors.

Conclusions:

The production of NETs by activated neutrophil contributes to the regulation of proteolytic activities released from sputum neutrophils. Would this phenomenon be of biological relevance, the question will remain to identify the factors in the lung environment that activate NET production and to understand the effect of endogenous bacterial DNases and exogenous rhDNase on the NETs-associated proteolytic activities.

This work is supported by the Association "Vaincre la mucoviscidose".

Poster # 7

IL-6 and IL-8 increase the expression of glycosyltransferases and sulfotransferases involved in the biosynthesis of sialylated and/or sulfated Lewis^x epitopes in the human bronchial mucosa

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Bronchial mucins from patients suffering from CF (cystic fibrosis) exhibit glycosylation alterations, especially increased amounts of the sialyl-Lewis^x (NeuAc α 2-3Gal β 1-4[Fuc α 1-3]GlcNAc-R) and 6-sulfo-sialyl-Lewis^x terminal structures. These epitopes are preferential receptors for *Pseudomonas aeruginosa*, the bacteria responsible for the chronicity of airway infection and involved in the morbidity and early death of CF patients. However, these glycosylation changes cannot be directly linked to defects in CFTR (CF transmembrane conductance regulator) gene expression since cells that secrete airway mucins express no or very low amounts of the protein. Several studies have shown that inflammation may affect glycosylation and sulfation of various glycoproteins, including mucins. In the present study, we show that incubation of macroscopically healthy fragments of human bronchial mucosa with IL-6 or IL-8, two pro-inflammatory interleukins present in the airway of CF patients, results in a significant increase in the expression of several α 1,3/4-fucosyltransferases (FucT-XI and FucT-III), sialyltransferases (ST6Gal II and ST3Gal VI), and GlcNAc-6-O-sulfotransferases (GlcNAc6ST-2 and -5) mRNA. In parallel, the amounts of sialyl-Lewis^x and 6-sulfo-sialyl-Lewis^x epitopes at the periphery of high-molecular-mass proteins, including MUC4, were also increased. These results allow us to propose a pathway for the biosynthesis of sialyl-Lewis^x and 6-sulfo-sialyl-Lewis^x epitopes and indicate that IL-6 and IL-8 may contribute to the increased levels of these epitopes on human airway mucins from patients with CF.

This work was supported by “Vaincre la mucoviscidose”.

Poster # 8

Epithelial factors involved in the regeneration and remodelling of the human airway epithelium

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In cystic fibrosis (CF) the airway epithelium is frequently injured and remodelled, and has to regenerate its structure. We have previously showed that IL-8, matrix metalloproteinases (MMP)-7 and -9 and their inhibitor TIMP-1 were modulated during the non-CF regeneration, playing a crucial role in epithelial differentiation, and that CF bronchial epithelial regeneration, in absence of infection, gave rise to a remodelled epithelium and was associated with deregulation of these factors.

As the CF airway epithelium exhibits remodelling (squamous metaplasia or goblet cell hyperplasia), we aimed to determine the influence of epithelial factors during the process of epithelial remodelling.

The human airway epithelial regeneration and remodelling was studied in the air-liquid interface (ALI) culture model. The cultures were processed at day (D)1 and D5 of ALI culture, when the first ciliated cells appeared (alternatively at D15) and after complete differentiation (alternatively at D30) for immunohistochemistry, RT-PCR and zymography. Analysis of specific epithelial marker expression (cytokeratins, MUC5AC, β -tubulin) allowed to characterize the models of normal and deregulated regeneration. We showed that IL-8 and TIMP-1 mRNA levels, MMP-7 and -9 activities were modulated during the deregulated regeneration, in comparison to results obtained during normal regeneration. The differences of results obtained during the normal or deregulated human airway epithelial regeneration suggest the potential involvement of the studied factors in the epithelial remodelling.

It will now be important to determine their specific roles and their mutual regulation during the non-CF and CF normal and deregulated airway epithelial regeneration.

Supported by Vaincre la Mucoviscidose.

Poster # 9

Regulation of Muc5ac expression by arachidonic acid metabolites

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Background :

Cytosolic phospholipase A2 (cPLA2) is a rate-limiting key enzyme controlling the release of arachidonic acid (AA) from membrane phospholipids. Conversion of AA by cyclooxygenases (COX) and lipoxygenases (LOX) generates prostaglandins (PG) and leukotrienes (LT), respectively. These AA metabolites play an important role in the control of inflammation. We have previously observed that cPLA2 is involved in mucus hyper-secretion and expression of muc5ac mucin in CFTR-deficient mice (Dif et al. Submitted)

Aim:

To identify the signalling pathways involved in PMA-induced Muc5ac expression mediated by cPLA2 in the bronchial epithelial cell line NCI-H292

Methods :

Production of muc5ac was measured in cell lysates and supernatants by specific ELISA, after stimulation with PMA. cPLA2 activity was analyzed in cell lysates, after hydrolysis of phosphatidyl choline labeled with radioactive AA. The role of cPLA2, COX and LOX in muc5ac production was examined after treatment with specific inhibitors. Activation of transcription factors was followed using EMSA.

Results :

PMA-induced muc5ac expression was mimicked by AA and inhibited after pre-treatment of the cells with the cPLA2 inhibitor MAFP. Further, muc5ac expression was inhibited by a LOX inhibitor (NDGA) but not by COX inhibitors (aspirin, NS398). Inhibition of cPLA2 activity by MAFP was related to PPAR activation but not to NF-κB activation. Indeed, AA activated PPARs and PPAR agonist induced muc5ac expression.

Conclusion :

cPLA2 activity is important for the production of muc5ac mucin. This involves LOX metabolites and a signalling pathway resulting in the activation of the transcription factors PPARs.

Acknowledgments:

Work supported by “Vaincre la Mucoviscidose”.

Poster # 10

The C-terminus of the transmembrane mucin MUC17 binds to the scaffold protein PDZK1 that stably localizes it to the enterocyte apical membrane in the small intestine

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Department of Medical Biochemistry and Cell Biology, Göteborg University, Sweden

Background:

Mucins line and cover epithelial surfaces of luminal organs. Deregulation of mucin synthesis and secretion is often coupled to disease, e.g. Cystic fibrosis. We previously showed increased levels of the mouse Muc3(17), the orthologue of human MUC17, in small intestine of Cfr^{-/-} mice.

Aims:

The membrane mucins, MUC3, MUC12 and MUC17, clustered on chromosome 7, are found in the gastrointestinal tract. A common feature is typical C-terminal PDZ-domain-binding motifs. Therefore we sought to identify novel interactions involving mucins and PDZ proteins.

Methods:

Immobilized PDZ domains were screened for binding to mucin C-terminal peptides and GST pull-down assays verified interactions as true PDZ mediated. Immunostaining was used to assess mucin expression and localization in Pdzk1^{-/-} mice.

Results:

MUC17 exhibited a strong binding to PDZK1, whereas MUC3 and MUC12 did not. The PDZ motif of MUC17 was responsible for the interaction, acting as a ligand for three out of four PDZ domains of PDZK1. Immunostaining of Muc3(17) in jejunum revealed strong brush-border membrane staining in the wild type while Muc3(17) was profoundly relocalized to intracellular, vesicular compartments of enterocytes in Pdzk1^{-/-} mice.

Conclusions:

This study suggests a specific role for Pdzk1 in regulation of mucin expression. PDZK1, a well-known PDZ binding partner of CFTR, binds Muc3(17) and stabilizes it at apical membranes of enterocytes. This may suggest a direct connection between CFTR and mucins.

References:

Malmberg, E. K., Pelaseyed, T., Petersson, A. C., Seidler, U. E., De Jonge, H., Riordan, J. R., and Hansson, G. C. (2008) *The Biochemical journal* **410**(2), 283-289.

Session 3:
Microbiology in CF

Poster # 11

Molecular epidemiology of the longitudinal course of the *Pseudomonas aeruginosa* infection in cystic fibrosis

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Sequential *Pseudomonas aeruginosa* isolates from cystic fibrosis (CF) patients treated at two large CF clinics were collected since the onset of colonisation over a period of 20 years and then genotyped by a high-throughput DNA chip that allows the identification of the *P. aeruginosa* genotype by SNP-typing and the repertoire of the accessory genome.

The distribution of the clones from CF lungs was compared with the global population structure of *P.aeruginosa*.

1. Whereas in one CF clinic a convergence of strains towards two uncommon clones was observed, the distribution of clones in the other CF clinic matched that of the global population. In other words dominant clones in the global population were also frequently isolated from CF lungs.
2. The occurrence of microevolution in the gene island pattern, called adaptive radiation, positively correlates with a milder clinical course.
3. Severity of the chronic *P.aeruginosa* infections in CF lungs could be differentiated by clade, i.e. the clinical virulence differed by clade.

These findings suggest that the stratification of *P.aeruginosa* strains by clade and clone is a useful adjunct for bacteriological diagnostics of CF specimens.

Furthermore, we propose that bacterial genome remodelling leads to a better adaptation between host and opportunistic pathogen as well as that radiation decreases the host immune response and leads to a milder course of lung disease in CF.

Poster # 12

Hypermutable (HMP) expression in *P.aeruginosa* (PA) from Cystic Fibrosis (CF) patients in the early colonization and in the long-term infection stage

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Several studies demonstrated the importance of mutations in PA adaptation to the CF lung and the role that the HMP phenotype might play in bacterial pathogenesis. HMP are strains with an increased mutation frequency due prevalently to mutations in mismatch repair and error prevention genes. The prevalence (37%) of HMP in chronic CF infection has been established, but few studies investigated the mutator phenotype in other PA populations such as early clinical isolated from CF patients (pts). The aim of this study is to characterize and compare the HMP strains in early infections and in long-term colonizations. We have preliminary studied the genotypes and phenotypes of 57 PA serial strains collected from 1991/92 to 2007 in 3 pts chronically infected and 8 PA isolates from 2004 to 2008 in 3 pts early colonization. **RESULTS:** Our preliminary results show that pts with long-term colonization harbour strains with different phenotype, but same genotype; for hypermutability the strains examined are greatly weak mutators (mutation frequency of $2 \times 10^{-7} > f < 1 \times 10^{-6}$) but intermittently show strong ($f \geq 1 \times 10^{-6}$) mutation frequency rate, characterized by high antimicrobial resistance (in particular tobramycin and colistin) and associated with SCV morphotype. Concerning the pts with early infection the examined strains show only non-mucoid phenotype with low resistance for antimicrobial agents; about the mutation frequency rate in early PA, all strains studied are strong mutators ($f \geq 1 \times 10^{-6}$). These results confirm that the CF lung is not only the promoting factor in the occurrence of mutators, moreover the presence of HMP in early PA infection in CF strengthen the importance of timely and aggressive approach to antimicrobial therapy. Our purpose is to study the hypermutability in a large collection of PA, serial recovered at different stage of CF and non CF infection, investigating the possible correlations with the other virulence factors and the antimicrobial therapy selective pressure.

Poster # 13

Lung pathogenesis of *Pseudomonas aeruginosa* hypermutable strains from patients with cystic fibrosis

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The respiratory tract of CF patients provides a selective environment favoring development and persistence of multiple *P. aeruginosa* pathogenic variants not eradicable by any known therapy. Whether *P. aeruginosa* clonal variants, including hypermutable strains, differ in their pathogenic potential is not known.

Multiple genotypic analysis were performed in strains isolated from CF patients carrying the same clonal lineage from the onset of colonization over many years. PFGE, ATchip and multilocus SNPs showed intraclonal diversity with genome rearrangements, variations in pathogenic islands and acquisition of mutations in the *muc* genes and *mutS*, *muL* and *uvrD* genes of the mismatch repair system (MMR).

To understand the role of hypermutation in the pathogenesis of chronic lung infection and antibiotic resistance, couples of clinical wild type/hypermutable clonally related *P. aeruginosa* strains and the isogenic laboratory strains PAO1/PAO1 Δ mutS were subjected to competition in murine model. After 14 days, *P. aeruginosa* hypermutable strains were less efficient than wild type in establishing chronic lung infection in C57Bl/6 mice. Under antibiotic treatment the results were opposed. In vitro, the hypermutable strains showed a higher level of resistance to 10 antibiotics and the MIC returned back to the level of the wild type strains when complemented with MMR genes.

Our finding suggests that hypermutation is a key factor in development of multiple-antimicrobial resistance and may favor chronic colonization in CF patients under antibiotics treatment.

Supported by the Italian CF Research Foundation.

Poster # 14

Molecular behaviour of *Pseudomonas aeruginosa* peptidoglycan at different stages to the airways of cystic fibrosis patients

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The establishment of a chronic infection by *P. aeruginosa* (PA) in the airways depends on the efficient and simultaneous suppression of the pathways leading to the activation of the innate immune response, thus, among others, of the Peptidoglycan (PGN) recognition.

PGN is a unique and essential structural component of the bacterial cell wall. Fragments deriving by PGN recycling during cell duplication are well recognised virulence factors and involved in pathways controlling inflammation and host defences. Changing of these bacterial structures influence host reactions and improves bacterial survival in infected tissues as those of patients with Cystic Fibrosis (CF).

In this work we elucidated the structure of PGN fragments, muramyl peptides, released by PA clonal strains isolated at the onset of colonization and after years of chronic infection. The first non-mucoid strain was isolated after 6 month of colonization, the last two (mucoid and non-mucoid) were isolated after 7 years and thus after the persistence of the infection.

The structural elucidation of PGN has been carried out by a combination of biochemical analyses, NMR spectroscopy and MS spectrometry. The presence of muramyl peptides carrying unusual amino acid will be also discussed.

This work was supported by the Italian Cystic Fibrosis Research Foundation (grant FFC# 8/2007) with the contribution of “Furla S.p.A.”, “GVS S.p.A.” and “Delegazione FFC di Bologna”.

Poster # 15

Development of genetic tools for *Mycobacterium abscessus*, an emerging pathogen, and construction of a strain devoid of glycopeptidolipid

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Mycobacterium abscessus is an emerging and rapidly growing mycobacterium that causes chronic lung and disseminated cutaneous diseases in young patients with cystic fibrosis or suppressed immune system. *M. abscessus*, which is characterized by its low susceptibility to antibiotics is equipped, as other mycobacteria, with a complex waxy cell wall. The genome of this species has been sequenced (CNS, Evry) and DNA microarrays have been produced. The main drawback in deciphering *M. abscessus* virulence is the lack of a suitable genetic system, which explains that studies have been restricted so far to the use of natural spontaneous variants.

The aim of our project is to develop a suitable mutagenesis system for *M. abscessus* by taking advantage of counter selectable or phage base systems that have proved their efficacy in other mycobacterial species. The most convenient system will be used to construct mutants in target genes that are believed to play an active role in the virulence of this organism, the glycopeptidolipid biosynthesis pathway. The final goal is to test the virulence of the mutant and complemented strains using various *in vitro* or *in vivo* models. The most recent data will be discussed.

The development of an efficient genetic system will enable the characterization of the virulence of *M. abscessus*, by taking full-advantage of the genomic tools currently available (genome sequence and DNA arrays). This constitutes a pre-requisite for developing new drugs or vaccine candidates against this emerging pathogen

Acknowledgments. We thank “Vaincre La Mucoviscidose” for continuous support (Project N° 110 637) and Jean-Louis Gaillard for research materials and stimulating discussions.

Poster # 16

Analysis of endotoxins from *Burkholderia*

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The *Burkholderia cepacia* complex is a group of Gram negative bacteria that are opportunistic pathogens for humans especially in CF patients. LPS molecules are potent virulence factors of Gram negative bacteria essential for their survival. To understand the chemical basis of the inflammatory process, a complete analysis of the bacterial endotoxins' structure to function relationship is required. In this work we define the structure of the O-antigen and of the lipid A extracted from the LPS produced by *B. multivorans* strain C1576 that is one of the most common bacteria isolated in CF centers and also one of the most virulent species among *Bcc* CF pathogens. The elucidation of the primary structure of the O-chain and of the lipid A has been performed through chemical analysis, mass spectrometry and 2D NMR spectroscopy. The lipid A was constituted by a mixture of species with a different acylation and phosphorylation pattern that can potentially modulate its inflammatory activity. The LPS from this clinical isolate was constituted by two different O-side chain, constituted by different repeating units: [$\rightarrow 2\text{-}\alpha\text{-D-Man-(1}\rightarrow 2)\text{-}\alpha\text{-D-Rha-(1}\rightarrow 3)\text{-}\alpha\text{-D-Man-1}\rightarrow$] and [$\rightarrow 2\text{-}\alpha\text{-D-Man-(1}\rightarrow 2)\text{-}\alpha\text{-D-Aco-(1}\rightarrow 3)\text{-}\alpha\text{-D-Rha1}\rightarrow$]. Further, conformational studies has been performed in order to establish and compare the spatial arrangements of the two polysaccharides. Structural and conformational analysis are essential basis in the discovery of new therapies targeted towards bacterial endotoxins that play a key role in the inflammatory process. This work was supported by the Italian Cystic Fibrosis Research Foundation (grant FFC# 11/2006).

Session 4:
Immunology in CF

Poster # 17

Increased susceptibility to allergic airway inflammation in a murine model of cystic fibrosis lung disease

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Background:

Increased airway Na⁺ absorption is a characteristic abnormality of cystic fibrosis (CF) airways and causes CF-like lung disease in β ENaC-transgenic (Tg) mice. Recent studies demonstrated that juvenile β ENaC-Tg mice also exhibit spontaneous allergic airway inflammation.

Aims:

We hypothesized that airway surface dehydration in β ENaC-Tg mice causes increased susceptibility to allergic airway inflammation due to reduced pulmonary clearance of inhaled allergens.

Methods:

To test this hypothesis, we induced allergic airway inflammation by repeated intratracheal instillations of *Aspergillus fumigatus* extract (Af), and compared total and differential cell counts in bronchoalveolar lavage, pulmonary IL-13 expression, and airway morphology in β ENaC-Tg mice with wild-type littermates.

Results:

We demonstrate that elevation of airway eosinophils and pulmonary IL-13 caused by intrapulmonary exposure to Af was significantly increased in β ENaC-Tg mice compared to wild-type controls. Further, we show that genetic deletion of Stat6 critical for Th-2 signaling protects β ENaC-Tg mice from airway eosinophilia, elevated IL-13, goblet cell metaplasia and airway mucus obstruction.

Conclusions:

Our studies demonstrate that airway surface dehydration characteristic of CF airways plays a critical role in the pathogenesis of Stat6-dependent allergic airway inflammation. These findings are consistent with a high incidence of allergic bronchopulmonary aspergillosis (ABPA) and confounding allergic airway inflammation in CF patients.

Acknowledgements:

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Poster # 18

Antioxidant imbalance in the lung from CFTR-KO mice: abnormal Peroxiredoxin 6 expression levels and phospholipid peroxidation

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Although many reports have showed a decreased antioxidant capacity and the presence of oxidative stress markers in CF airway epithelial lining fluid, few studies have focused on the oxidant/antioxidant balance within the CF cell.

The aim of the current study was to examine the cellular balance between antioxidant defenses and oxidative damages in the lung from just weaned CFTR-knockout mice (CF) by measuring GSH-peroxidase activity and TBARS, respectively.

GSH-peroxidase activity and lipid oxidation index were found significantly increased in lung extracts from CF mice as compared to wild-type (WT). In addition, proteomic analysis revealed that a major intracellular pulmonary GSH-peroxidase, Peroxiredoxine 6 (PRDX6), is differentially expressed in CF lung. As PRDX6 plays a role in the repair of damaged cell membranes by reducing phospholipid hydroperoxides, we examined its expression level and specific peroxidase activity in the lung. Immunoblot and immunohistochemistry analyses revealed that PRDX6 expression is increased in CF as compared to WT lungs. Consistent with this result, a specific PRDX6 assay, using phosphatidylcholine hydroperoxide (PC-OOH) as substrate, showed that PRDX6 activity is similarly increased in CF lung.

The increased lipid oxidation index and PRDX6 antioxidant activity suggest the presence of a constitutive redox imbalance in CF pulmonary cells and raise the possibility that the intracellular antioxidant defense mechanism could be overwhelmed when exposed to a pro-oxidative environment.

To assess this hypothesis we evaluated the level of PC-OOH together with PRDX6 expression in the lung from mice exposed to an oxidative challenge induced by paraquat (PQ). After PQ treatment, CF lungs exhibited a dramatic PC-OOH increase and a marked decrease in PRDX6 protein expression.

This impaired PL-OOH detoxification mechanism may enhance oxidative stress-related signalling and damage, contributing to an exaggerated inflammatory response in CF lung.

This work was supported by Vaincre la Mucoviscidose.

Poster # 19

Transcription factor “decoy” strategy to silence expression of pro-inflammatory genes in CF bronchial epithelial cell lines

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Background:

Chronic inflammation in the lungs of patients affected by CF is characteristically dominated by a huge intra-bronchial infiltrate of neutrophils (PMNs), which are responsible for tissue damage, self-inactivation of their killing capacity and release of DNA, which facilitates *P.aeruginosa* infection. *P.aeruginosa* binds to respiratory epithelial cells leading to activation of pro-inflammatory genes, mainly IL-8, GRO α/γ , ICAM-1, IL-1 β and IL-6.

Aims:

Regulating PMN chemotaxis to levels that are beneficial to lung defences with reduced adverse effects, e.g. by controlling IL-8 expression, is presently considered a therapeutic target, complementary to the correction of the basic CFTR defect.

Methods:

To obtain controlled silencing of genes encoding PMN chemokines, we explored the Transcription Factor (TF) “decoy” strategy. This is based on the intracellular delivering of double stranded oligodeoxynucleotides (ODNs) causing inhibition of the binding of TFs to the different consensus sequences localized in the promoter regions of these genes. We studied the transcription of IL-8, IL-1 β , IL-6, GRO, ICAM-1 in bronchial epithelial cells (IB3-1, CuFi-1, Calu-3, BEAS-2B) challenged with *P.aeruginosa*, IL-1 β and TNF- α .

Results:

In silico studies of the promoter region of these genes brought us to identify different consensus sequences for TFs, e.g. NF- κ B, AP-1, NF-AT, CREB, NF-IL6 and Sp1. Therefore we designed TF “decoy” ODNs to investigate the role and hierarchy of potency of the single TFs in respect to the transcription regulatory machinery induced by *P.aeruginosa*. We found that IL-8 is regulated by a complex machinery involving the TFs NF- κ B, NF-IL6, AP-1 and CREB, but not Sp1, whereas IL-6 is mainly regulated by NF- κ B and Sp-1.

Conclusions:

TF decoy strategy is a promising tool with mechanistic and applicative implications for CF lung disease.

Acknowledgments:

Supported by Italian CF Research Foundation.

Poster # 20

Azithromycin is not able to decrease inflammatory process in cystic fibrosis cells

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Objective:

The aim of this study was to explore the effect of macrolides: azithromycin (AZM), clarithromycin (CAM), erythromycin (EM) and josamycin (JOSA) on molecular mechanisms of inflammation in CF.

Methods:

Two CF bronchial epithelial cell lines (IB3-1 and CFBE41o-) and two non-CF bronchial epithelial cell lines (S9 and 16HBE14o-) were pre-treated 30 minutes with the macrolide (10µg/ml) and the inducer (IL-1β or TNFα – 10ng/ml) was added for additional 16h. IL-8 concentrations were evaluated in culture supernatants by ELISA and multiplex assays directed against phosphoproteins were performed on total protein extracts. NF-κB pathway was also investigated by a p65-luciferase plasmid at 2 and 4h of treatment.

Results:

AZM was only able to significantly reduce TNFα-induced IL-8 secretion in S9 cells. CAM decreased IL-1β-induced IL-8 secretion in 16HBE14o- but increased TNFα-induced IL-8 secretion in CFBE41o-. Moreover, JOSA decreased TNFα-induced IL-8 secretion in both IB3-1 and S9 cells.

Multiplex assays showed us that AZM and TNF-α treatment increase phospho(p)-ERK, p-STAT6, p-CREB and p-p65, compared to TNFα alone. After 2h and 4h treatment, AZM decreased NF-κB dependent-transcriptional activity induced by TNFα in S9 but not in IB3-1 cells.

Conclusion:

Our study demonstrates that azithromycin is not able to decrease inflammation in CF bronchial epithelial cells.

We thank Pfizer for their generous gift of azithromycin.

Poster # 21

Glucocorticoids resistance in Cystic Fibrosis

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Background:

Lung dysfunction is the main cause of mortality in CF patients. Infection combined with inflammation lead to progressive destruction of lung respiratory epithelium. Glucocorticoids (GC) are anti-inflammatory molecules commonly used to treat inflammation but with controversial efficiency among patients. This could be due to resistance to GC already observed in others respiratory diseases. To test this hypothesis, we study the key steps in GC activation pathway in the lung to identify the molecular basis of such dysregulation. Our present studies focus on the regulation of the expression of the GR isoforms.

Methods:

CF bronchial epithelial cell lines (IB3-1) and CF corrected (S9) were incubated with IL-1 β (10 ng/ml) at 30min, 1h, 2h, 4h, 8h, 16h, with or without dexamethasone (dex, 1 μ M). Rates of secreted IL-8 were assessed by ELISA 16h after treatment. NF- κ B activation was measured using NF- κ B promoter coupled to luciferase. Expression of GR isoforms was measured by quantitative RT-PCR.

Results:

In presence of IL-1 β , dex can restore basal secretion of IL-8 at 16h in S9, whereas IB3-1 barely respond. Moreover, no effect of dex on NF- κ B activation is observed in CF cells. There is a different regulation of the GR isoforms expression at 16h in both cell lines. The study of the GR activation window of activation in inflammatory conditions is currently in progress.

Conclusions:

These preliminary results show a resistance to GC treatment in CF cell line and suggest a dysregulation of GR isoforms expression. The present results require to be assessed in other cellular models and from CF lung biopsies. We will subsequently investigate the possible dysregulation of other key steps in GC activation pathway such as GR phosphorylation and nuclear translocation.

Supported by Inserm and the french Cystic Fibrosis association Vaincre La Mucoviscidose.

Poster # 22

CTLA-4-mediated regulatory phenotype of T cells in tolerant lung recipients

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Background:

Lung transplantation remains the last therapeutic option for selected patients with end stage pulmonary disorders like cystic fibrosis. Obliterative Bronchiolitis (OB), considered as a chronic graft dysfunction, still occurs in 40 % of lung transplant recipients (LTR). Immune characteristics distinguishing tolerant LTR from OB patients are largely unknown. We compared the effect of dendritic cells (DC) on T-cell activation in healthy LTR (non OB) and in OB patients.

Methods:

Monocyte-derived DC from 35 non OB and 14 OB were cultured with autologous T cells. T regulatory (Treg) cell (CD4⁺CD25^{high}Foxp3⁺), co-receptors, cytokine production, DC phenotype and indoleamine 2,3 dioxygenase (IDO) expression were assessed by flow cytometry. Experiments were repeated in the presence of *P. aeruginosa* or anti-CTLA-4 antibodies.

Results:

DC from non OB up-regulated Treg cells, CTLA-4 and IL-10 (p<0.05). By contrast CD28 and ICOS decreased concomitantly to IL-13 and IL-4 (p<0.03). Compared to OB, DC from non OB displayed an immature phenotype, with lower CD83, CD80 and higher IDO levels (p<0.05). Stimulation by *P. aeruginosa* did not abolish the tolerogenic effect of DC on non OB T-cells. Finally, decreased Treg cells and IL-10 were detected when adding anti-CTLA-4 in non OB recipients (p<0.05).

Conclusion:

In contrast with OB recipients, DC from non OB induce a tolerant T cell phenotype, which is dependant on CTLA4 engagement.

This work was supported by Vaincre la Mucoviscidose.

Session 5:
Microbiology in CF

Poster # 23

The “P-usher”, a novel protein transporter involved in fimbrial assembly and TpsA secretion

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The opportunistic pathogen *Pseudomonas aeruginosa* responsible for severe lung function deterioration in CF, is equipped with at least five genes encoding TpsA-like proteins and five loci encoding a full set of components belonging to the chaperone-usher pathway (Cup), the both secretion and fimbrial assembly dependent-processes contributing to bacterial pathogenesis. We previously described the functionality of two of the Cup systems from *P. aeruginosa*, CupB and CupC (Ruer,2007) which expression is under the control of the RocS1-RocR-RocA1 two-component regulatory system. In addition to genes encoding components of the Cup, the *cupB* gene cluster contains the *cupB5* gene, which encodes a protein with similarities to TpsA proteins such as the filamentous haemagglutinin FHA from *B. pertussis*.

We identified in *P. aeruginosa* a new type of protein transporter, CupB3, which is an outer membrane usher protein involved in pili assembly. In CupB3, the usher domain has fused during evolution with a POTRA-like domain found in two-partner systems transporters, TpsBs. In TpsBs, the POTRA captures TpsA passenger protein, which is then transported across the outer membrane through the β -barrel domain of TpsB. We called CupB3 a “P-Usher” for POTRA-like domain-containing usher. We showed that CupB3 assembles CupB1 fimbrial subunits into pili but also secretes CupB5, a TpsA-like protein encoded within the *cupB* cluster. The CupB3 usher domain plays the role of the TpsB β -barrel for CupB5. We revealed that the POTRA-like domain is neither essential for CupB1 fimbriae assembly nor for CupB5 secretion, but is crucial to coordinate bona fide transport of CupB1 and CupB5 through the usher domain. This reveals a bacterial strategy to build optimized transport pathways by making new molecular machines with old spare parts.

This work was supported by the French Cystic Fibrosis Foundation VLM.

Poster # 24

The CupE system in *Pseudomonas aeruginosa* is a new gene cluster involved in *P. aeruginosa* community lifestyle

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The opportunistic pathogen *Pseudomonas aeruginosa* responsible for severe lung function deterioration in CF, is equipped with redundant molecular systems that contribute to bacterial pathogenesis. Those assembling fimbrial structures that promote complex organized community lifestyle are important elements to study since this bacterial strategy renders them resistant to antibiotic treatment and host defences within CF airways conducting to subsequent antimicrobial therapeutic failure. We recently identified in *P. aeruginosa* genome a novel *cup* gene cluster which exhibits some similarities with the well-conserved chaperone-usher pathway. Moreover, it encodes atypic pilins and until now was not characterized

We engineered a deletion mutant of the *cupE5* gene, encoding OM usher protein through which the fimbriae are assembled. The macroscopic and microscopic observations of the biofilm formed show that *cupE5* mutation clearly attenuates the bacterial ability to structure early step development of the bacterial community with respect to the parental wild-type (WT) strain. An antibody raised against the putative major pilin CupE1 allowed us to visualize the assembly of the CupE pili at the *P. aeruginosa* surface. In classical laboratory culture conditions, this cluster is poorly expressed as evaluated with a *cupE-lacZ* transcriptional fusion. To identify the regulatory processes that control the expression of this *cupE* gene cluster, we are currently developing a transposition mutagenesis strategy in the *P. aeruginosa* strain containing the *cupE-lacZ* fusion searching for mutants that clearly show a *cupE-lacZ* expression monitored by X-gal β -Gal-dependent degradation. Prospects will be dedicated to involvement of other CupE pilins in CupE-dependent *P. aeruginosa* community lifestyle as well as of the role of the CupE pili on the interaction with the host cells.

This work was supported by the French Cystic Fibrosis Foundation VLM.

Poster # 25

Pseudomonas aeruginosa Tad-dependent Flp pilus assembly is transcriptionally controlled by the PprAB two-component system and post-translationally modified by the RcpC protein

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We recently identified a novel Type IVb pilus (de Bentzmann, 2006) in *Pseudomonas aeruginosa* which contributes for the complex organized community lifestyle that the bacteria adopt within CF airways. This biofilm renders bacteria resistant to antibiotic treatment and host defences and therefore conducts to subsequent antimicrobial therapeutic failure. *P. aeruginosa* Tad machinery, which assembles Flp type IVb pili at the surface of the bacteria exhibits several unique features that we further characterized.

Chromosomal flp gene expression occurs late in the growth phase under aerobic conditions and leads to single Flp pilus assembly, even in the absence of the flp2, rcpB and tadE pseudopilin genes in the *P. aeruginosa* tad locus. Five independent transcriptional units were identified in the *P. aeruginosa* tad locus and are positively controlled by the response regulator PprB of the PprA-PprB two-component system, close to the tad locus, thus acting as a main regulator of the identified transcriptional units (TU). Using gel shift assays, we clearly showed that PprB his-tagged version binds specifically to the DNA sequences corresponding to putative promoter regions of the tad locus in a dose-dependent way. Furthermore, the regions between rcpC and flp genes on one hand and between the fppA and tadF genes on the other hand clearly showed two distinct binding sites for PprB. Thus, PprAB TCS controls the activation of the different TU of the *P. aeruginosa* tad locus through a direct way. The unique pseudopilin, TadF, does not affect Flp production, pilus assembly or Flp pilus-dependent adhesion phenotypes. On the other hand, the RcpC protein that belongs to a bacterial proteins family containing two α -clip domains, controls a Flp pilin post-translational modification but not its assembly into pilus. The RcpC-dependent Flp pilin modification which is under characterization, affects the efficiency of the Flp-host receptor interaction.

This work is supported by the French Cystic Fibrosis Foundation VLM.

Poster # 26

Development of a low dose nasal infection model with *Pseudomonas aeruginosa* in mice

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Strategies that prevent chronic *Pseudomonas aeruginosa* infection in Cystic Fibrosis patients are desirable. Vaccination would be a sensible approach. A meaningful animal infection model that represents protection at the airway mucosa surface is still lacking, since present models tend to mimic chronic or lethal airway infection with high doses of *P. aeruginosa*. Consequently, we pursue a model of low dose nasal inoculation and longitudinal investigation of physiologic parameters to monitor the onset of infection. Using head-out spirometry we are able to monitor *P. aeruginosa* infections as low as $1,6 \cdot 10^4$ CFU PA14 in C57BL/6 mice. 18 relevant respiratory parameters including respiration rate, tidal volume and midtidal expiratory flow at 50% are analyzed over a course of 14 days post infection. One of the most sensitive parameters is tidal volume which dropped by 62% within 4 hours post infection with recovery after 10 days. Additional parameters like body temperature, weight, as well as a behavior score and throat swabs are applied to monitor the state of infection. In the next weeks the protectivity of a vaccine against *Pseudomonas* will be investigated in C57BL/6 mice, also CFTR-deficient mice currently bred will be used in the future. Additionally, to monitor the infection *in vivo*, bioluminescent pseudomonads, including the clinical important strains PAO1 and PA14 are being developed using transposon mutagenesis (mini-ctx-lux). PAO1 has successfully been transformed and exhibits high luminescence of about $5 \cdot 10^8$ photons/sec/sr/cm². We conclude that this infection model holds promise for assessment of airway pathology more close to the clinical situation.

This work is performed with the participation of Imke Glass and Britta Gewecke under the supervision of Ulrich Baumann.

Poster # 27

Novel virulent genes of *Pseudomonas aeruginosa* by high-throughput screening in a mouse model of chronic infection

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Most patients with CF suffer from transient airway infections with *P. aeruginosa* followed by permanent chronic infection with persistent respiratory symptoms and decline in lung function. Chronic infection is maintained by *P. aeruginosa* microevolution of the bacterial genome within the CF lung. Here, we searched for *P. aeruginosa* pathogenicity-adaptive mutation by exploiting a new positive selection Signature Tagged Mutagenesis approach (STM). A collection of 6912 *P. aeruginosa* mutants was screened in the agar beads mouse model which mimics the chronic infection as in patients with CF. We have previously showed that the micro-anaerobic conditions provided by the agar beads resemble those in the mucus of CF patients, providing an invaluable tool to test pathogenic potential of *P. aeruginosa* mutants. Three consecutive screenings were carried out which reduced to 17 the *P. aeruginosa* mutants with a selective advantage. Surviving *P. aeruginosa* mutants were recovered and processed for gene identification by sequence analysis. We identified insertions in virulence genes implicated in motility and attachment, heat shock proteins, amino acid biosynthesis and metabolism, secreted factors and transport system. Seven of the 17 STM mutants identified had insertions in hypothetical proteins or proteins of unknown function. Validation of our panel of genes in terms of genetic diversity in *P. aeruginosa* clinical strains of different origins is under investigation. These results should help direct the development of new models and identification of targets required for *P. aeruginosa* persistence.

Supported by the Italian CF Research Foundation.

Poster # 28

The effects of lectins carbohydrates inhibitors on *Pseudomonas aeruginosa* pathogenesis

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Background: Specific oligosaccharide-mediated adhesion, including the two lectins LecA and LecB, are major determinants in *P. aeruginosa* (PA) pathogenesis. Inhibition of these lectins by specific carbohydrates could therefore be beneficial in PA treatment.

Aims: To evaluate *in vitro* and *in vivo* the specific effect of PA lectins carbohydrates inhibitors.

Methods: We examined the effects of 3 carbohydrates: N-acetyl-D-galactosamine (Gal-NAc), α -methyl-D-galactoside (Me-Gal), specific binders of LecA, and α -methyl-L-fucoside (Me-Fuc), a specific binder of LecB. *In vitro* cytotoxicity was performed on A549 cells at 4h and 6h after exposition to PAO1 or its purified lectins with addition of specific carbohydrates at 15mM. For the *in vivo* study, the mice received 15 and 50mM of carbohydrates intratracheally co-instilled with 10^8 or 10^9 CFU of PA (PAO1 strain) or its purified lectins. Mortality, lung injury (LI), lung bacterial clearance (LBC) and bacterial translocation (BT) were then studied at 6 and 16h post-infection (p.i).

Results: *In vitro*, cell cytotoxicity obtained with PA or the purified lectins was significantly decreased when specific carbohydrates were added. *In vivo*, co-instilled carbohydrates, Gal-NAc and Me-Gal improved mouse survival after PA challenge. At 6h p.i, carbohydrates co-instillation significantly reduced LI. The improvement in LI was observed with Gal-NAc and Me-Fuc at a concentration of 50mM but not 15mM. At 16h p.i, LI was significantly lower with Gal-NAc and Me-Gal. The combination of the purified lectins with the specific carbohydrates was associated with a significant reduction of LI at 6h. Me-Gal at the concentration of 15mM was associated with the higher improvement. Gal-NAc and Me-Fuc at the concentration of 50mM were also associated with a significant increased of LBC. The BT from the lung was significantly reduced with Me-Gal at 6h. No translocation was observed with Me-Fuc.

Conclusion: The competitive inhibition of PA lectins by specific carbohydrates is effective and may offer new therapeutic approach for CF patients suffering from infection with PA.

Acknowledgments: We are grateful to the French Association Vaincre la Mucoviscidose for financial support.

Poster # 29

n-3 long chain polyunsaturated diet improves the resistance to *Pseudomonas aeruginosa* lung infection in CF mice

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In two previous works, we demonstrated that a diet enriched with *n*-3 long chain polyunsaturated fatty acids (LC-PUFA) to wild-type (Wt) mice for 5 weeks prior to acute or chronic lung infection with a *Pseudomonas aeruginosa* decreased the local inflammation, improved the alveolar response and reduced the mortality.

The aim of this study was to analyse the role of *n*-3 LC-PUFA diet in a model of acute *P. aeruginosa* infection in *Cftr*^{-/-} mice (CF mice). The resistance against infection was evaluated by the bacterial load in lungs, the local neutrophil cell recruitment, the pulmonary injury measured by the alveolar-capillary barrier permeability and the pulmonary oedema, and the rate of survival mice for the first 24 hours. This study was performed with male and female mice (Wt and CF furnished from CDTA, Orléans, FRANCE). They were randomized to be fed with either a control diet or a diet supplemented with *n*-3 LC-PUFA for 6 weeks. Afterwards, they were infected intratracheally by 5×10^7 *P. aeruginosa*, and sacrificed at 24h for analysis.

We show that the response to infection was different between Wt and CF mice. Local inflammation, pulmonary injury, and death rate were higher in CF mice than in Wt mice. Besides the alveolar-capillary barrier permeability and the death rate were significantly higher in CF female than in CF male mice. By focusing our results on the effect of the diet, we demonstrate that *n*-3 LC PUFA increased the neutrophil recruitment and improved mainly the survival rate of CF male and CF female mice for the first 24 hours. The bacterial load did not decrease but the pulmonary injury was reduced even if this decrease was principally significant for CF female mice.

In conclusion, CF mice especially female mice are more susceptible to *P. aeruginosa* infection than Wt mice. *n*-3 LC-PUFA diet has beneficial effect on *P. aeruginosa* infection in CF mice and represents an interesting tool in diseases where this bacterial specie is frequently observed such as in cystic fibrosis.

Acknowledgments: We are grateful to the french association Vaincre la Mucoviscidose and CDTA.

Session 6:
Biology of CFTR

Poster # 30

Molecular, cellular and functional study of seven rare mutations of CFTR

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Background:

To date, about 1500 mutations have been found within human CFTR sequence. These mutations could be classified according to their degree of severity in CF disease (severe, mild or polymorphic).

Aims:

While the most common mutations (F508del and the 30 mutations routinely screened in CF patients), are well characterized, few data are available for rarer mutations. So genetic counseling is particularly difficult when fetuses or CF patients present these orphan variations.

We have developed *in vitro* biology assays to characterize mutation impact upon CFTR maturation process, trafficking and activity in order to establish a genotype/phenotype correlation. We present here our results obtained with seven rare mutations which have been isolated in our lab.

Methods:

We have used a GFP-tagged CFTR construct to generate these mutations by site-directed mutagenesis. Each plasmid was transfected in COS-7 cells to express the mutated proteins. We visualized CFTR trafficking by confocal microscopy and its cellular localization was determined using several markers. By western blot, we studied CFTR maturation process by quantifying the relative amount of mature and non mature CFTR (C and B bands). We evaluated CFTR channel activity by efflux assays, using notably pharmacoperones (MPBs or Miglustat).

Results:

Our results indicate a clear physiopathological effect of the studied CFTR mutations (retainment in ER, abnormal maturation, null CFTR activity). These data, in relation with clinical survey of the patients should be useful for genetic counseling. Moreover, this work could contribute to the improvement of pharmacological studies of CFTR mutations.

Conclusions:

In conclusion, we recommend that each new or orphan CFTR mutation is subjected to this type of study.

Acknowledgments:

This work was supported by Vaincre La Mucoviscidose.

Poster # 31

COMMD1 promotes CFTR trafficking through ubiquitination

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Cystic fibrosis is mainly caused by mutations that interfere with the biosynthetic folding of the CFTR protein. The aim of this study was to find proteins capable of interacting with CFTR and modifying its processing. We have identified COMMD1 as a new CFTR partner. COMMD1 is a regulator of copper homeostasis and sodium uptake through interaction with ENaC, it is also the prototype of a new protein family that plays a role in inhibiting NF-kappaB signalling

Co-immunoprecipitation experiments showed that COMMD1 associates with endogenous CFTR in HT29 cells and with F508del-CFTR in heterologously expressing epithelial cells. COMMD1 sub-cellular distribution is both nuclear and cytoplasmic, and precisely in vesicular cytoplasmic compartments, as assessed by immunocytochemical confocal microscopy. Further studies showed that COMMD1 colocalised with an early endosomal compartments (TfR).

COMMD1 is not involved in CFTR processing (C band) but wt-CFTR cell surface expression was half-reduced when COMMD1 expression was silenced. Unlike F508del-CFTR in temperature rescue, COMMD1 over-expression increased 15% wt-CFTR cell surface expression. Assessments of CFTR ubiquitination showed that COMMD1 over-expression strongly decreased CFTR ubiquitination therefore increasing CFTR cell surface expression. Finally, these data indicates that COMMD1 intracellular compartment is involved in CFTR trafficking through inhibition of CFTR ubiquitination.

Understanding how COMMD1 modulation modifies transepithelial transport and inflammation in CF versus non CF cells should give new therapeutic clues to reduce exaggerated inflammation and improve fluid secretion in CF patients.

Supported by INSERM and Vaincre La Mucoviscidose.

Poster # 32

GPact-11a: new CFTR activator

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One of the major therapeutic strategy in Cystic Fibrosis (CF) aims to develop modulators of CFTR (Cystic Fibrosis Transmembrane conductance Regulator) channels. Previously, we identified a new family of water-soluble and non-toxic CFTR inhibitors: the methylglyoxal- α -aminoazaheterocycle adducts (Routaboul *et al*; JPET 2007). Interestingly, in a structure-activity relationship study, some of them, like GPact-11a, were found positive as CFTR potentiator.

Using the iodide efflux technique, we demonstrated that GPact-11a potentiates the forskolin-induced efflux with an EC₅₀ of approximately 2 μ M in heterologous (CHO-CFTRwt) and endogenous (Calu-3) wt-CFTR expressing systems. Furthermore, we recorded short-circuit current measurements on colon from wild type (*cftr*^{+/+}) and knock-out (*cftr*^{-/-}) mice. GPact-11a induces a glibenclamide-inhibited chloride secretion in *cftr*^{+/+} mice (EC₅₀ of 134.9 μ M or 256.4 μ M in presence or absence of 1 μ M forskolin respectively) but not in *cftr*^{-/-} mice. Finally, we compared the salivary secretion induced by isoprenaline with or without increasing concentrations of GPact-11a. We observed an increase of the salivary secretion in the presence of GPact-11a (EC₅₀=7.2 μ M) in *cftr*^{+/+} mice, whereas no effect was observed in *cftr*^{-/-} mice.

To conclude, this work identifies, using *in vitro*, *ex vivo* and *in vivo* experiments, a novel CFTR potentiator; GPact-11a. This agent, non-toxic and water-soluble, represents a good candidate for the development of pharmacologic therapy in CF.

This work is supported by “Mucovie 66” and “Vaincre la mucoviscidose”.

Poster # 33

Endoplasmic reticulum stress (ER): implications for cystic fibrosis (CF)

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CF is an autosomal recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR functions as a chloride (Cl⁻) channel in the apical membrane of epithelial cells. The most common mutation is a deletion of a phenylalanine residue at position 508 (delF508). The delF508-CFTR protein is incorrectly folded and accumulates in the endoplasmic reticulum (ER). Because we previously showed that the unfolded protein response (UPR) may be triggered in CF (Kerbiriou *et al.* 2007) and because prolonged UPR activation may lead to apoptosis, our aim was to compare the ER stress-induced apoptosis pathway between wild type (Wt) and delF508-CFTR expressing cells. This work consists in comparing the Ca²⁺ - Calpain - Caspase 12 - Caspase 3 apoptotic pathway after thapsigargin (Tg) treatment between Wt (16HBE) and delF508-CFTR (CFBE) expressing cells. The expression of the different actors of this pathway was realized and we showed by western blotting a decrease of pro-caspase 3 expression in CFBE cells. The fura-2AM measurement will be done and the caspases activities will be realized. The results will provide new information regarding the pathophysiology of CF and contribute to establish a link between UPR and apoptosis in delF508-CFTR expressing cells.

Session 7:
Genetic and gene therapy

Poster # 34

Mechanisms of cftr pre-mRNA splicing

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Background:

CF is the most common autosomal recessive disorder in Caucasians, and it is caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene. One of the causes for milder forms of CF is the skipping of cftr exon 9, which results in a non functional protein. This skipping is under the control of a polymorphism in intron 8, which can be bound by the splicing protein TDP-43.

Aims:

The aims of this work were: 1- to elucidate the antagonist effect previously reported between TDP-43 and ETR-3 and 2- to look for other regulator(s) of CFTR exon 9 skipping, which could explain the differences in the observed phenotypes.

Methods:

We performed co-transfections of CF minigenes, which contained cftr exon 9 and its flanking introns with 5 different polymorphisms, and expression vectors of several splicing proteins.

Results:

We show that ETR-3, which was reported to decrease CFTR exon 9 exclusion, dramatically increases its exclusion in our minigenes. Moreover, its effect on exon 9 skipping is stronger than that of TDP-43. In addition, CUGBP-1, a structurally close member of the ETR-3 protein family, which binds CUG sequences, also has the opposite effect on CFTR exon 9 splicing. Hence CUGBP-1 reduces exon 9 exclusion. Finally, we identify MBNL-1 as an additional splicing factor that induces exon 9 skipping.

Conclusions:

In this study, we described 3 new splicing regulators of CFTR exon 9, ETR-3, CUGBP-1 and MBNL-1. All these factors are able to bind UG-repeats and are implicated in another genetic disease (Myotonic Dystrophy). The abundance and/or activity of each factor in different tissues could explain the variability in CFTR exon 9 skipping phenotypes.

Acknowledgments:

Thanks to: N. Charlet-Berguerand, A. Kornblihtt, E. Buratti, C. Le Jossic-Corcos, B. Simon and Y. Dréano.

Poster # 35

Functionnal interplay between *c/ebpb*, *yy1* and *usf* involved in transcriptional *cftr* regulation

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There are currently few data that throw light on the mechanisms of basal tissue-specific or temporal regulation of the *CFTR* gene. We previously reported a functional antagonism between YY1 and SRF and a cooperation between USF and Sp1 transcription factors (TF) on the *CFTR* promoter. Nevertheless, these data are not sufficient to account for basal *CFTR* transcriptional activation. Recently, we evidenced that the C/EBPb transcription factor binds the *CFTR* promoter and contributes to its basal transcriptional activity in epithelial cell lines. Because C/EBP functionality including its tissue-specificity may be regulated by interactions dependent on the additional factors, multiple transfection assays were performed. The data revealed a positive regulatory interaction between C/EBPb and USF on *CFTR* transcription. Using both reporter and ADN/protein interaction assays, we also evidenced a functional antagonism between C/EBPb and YY1 through DNA binding competition. Since phosphorylation plays a key role in the modulation of C/EBPb function including its DNA-binding capacity, we sought to evaluate whether phosphatase inhibitors might influence on this interplay. The results showed that phosphorylated C/EBPb is associated with an increase in its DNA occupancy, a *CFTR* activity and a decrease in YY1 DNA binding. These data abound with the notion that genes expression is regulated by an exquisite balance between various activators and/or repressors in a coordinated fashion. Characterization of different TF and their associated-cofactors will ultimately yield a rich source insight into regulation of *CFTR* gene transcription.

This work is supported by the association Vaincre la Mucoviscidose.

Poster # 36

Generation of CFTR mutant pig models for cystic fibrosis

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Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasians. The causative gene, CF transmembrane conductance regulator (CFTR), encodes a chloride channel. Deletion of a single residue ($\Delta F508$), which occurs in most patients with CF, impairs maturation and function of this protein leading to pathophysiological consequences in various organs including the respiratory, gastrointestinal and reproductive tract, pancreas and liver. Numerous CFTR deletions have been established in the mouse, however, the mutants lacked the severe phenotype in the respiratory tract. In contrast to mouse, the pig is supposed to represent a more convenient animal model, due to its anatomic and physiological similarities to man. The aim of this project is the generation of CFTR $\Delta F508$ transgenic pigs, as well as pigs completely lacking the CFTR-gene. The resulting genetic alterations are introduced into the porcine genome by homologous recombination using either conventionally designed targeting vectors or BAC-based targeting vectors, which show a higher recombination efficiency. The constructs are transfected into pig fetal fibroblasts by nucleofection followed by the screening for successful targeting of the CFTR gene by qPCR. Screening analysis follows the “loss of homozygosity” principle. After expansion of positive cell clones, fibroblasts will be introduced into enucleated oocytes and transferred into synchronized gilts after blastocyst maturation.

This study was founded by the Mukoviszidose e.V. and the Bayrische Forschungsstiftung (grant number DOK-69-06).

Poster # 37

Imaging tools for the study of gene transfer using synthetic vectors

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The safe and efficient delivery of therapeutic DNA to cells represents a fundamental obstacle to the clinical success of gene therapy. For the rational development of non-viral vectorisation methods (cationic lipids, polymers) there is a need for molecular imaging tools to follow their intracellular trafficking. However there is no simple method to visualize vectors in a cell at a molecular scale.

Methods :

We have developed a simple labeling strategy for those synthetic vectors that mimic physicochemical as well as transfection properties. The labeling strategy consists in the use of aminated nanoparticles that bind DNA via electrostatic interaction. We labeled lipoplexes (cationic lipids/DNA complexes) and copoplexes (block copolymers/DNA) and followed their cellular trafficking by electron-microscopy.

Results :

Block copolymers have recently been identified as a novel class of synthetic vectors for pulmonary epithelium gene transfer. Surprisingly they are inefficient *in vitro*. To better assess the mechanisms by which they deliver or not DNA to the nucleus, we performed *in vitro* transfection study using labeled copoplexes on two cell lines (human pulmonary epithelial cells H1299 and mice muscular cells C2C12). We show that copoplexes are internalized by the cells despite no transfection is detected, what was not suspected. To better understand this phenomenon we realised a comparative analysis using BGTC-DOPE/DNA lipoplexes that show good transfection properties *in vitro*. We were able to visualize different steps in their intracellular trafficking from the internalization to the endosomal escape.

Conclusion :

This work brings new imaging tools for the study of gene transfer using synthetic vectors as well as a better understanding of their transfection mechanisms.

Acknowledgements :

We are grateful to Vaincre La Mucoviscidose for financial support.

Poster # 38

In vitro screening model for viral vectors for optimal pulmonary transduction

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Background and Aims:

Extra- and intracellular barriers represent a major barrier to successful gene transfer of the conducting airways in vivo. When bioengineering viral vectors for optimal airway transduction the need for an elegant screening model arises. Screening of multiple optimized vectors in animal models is labour-intensive and time-consuming due to the need for large groups for statistical analysis. Testing in immortalized pulmonary cells is suboptimal due to the lack of apical differentiation. We investigated a culture model of polarized primary epithelial cells as an in vitro screening tool.

Methods:

Tracheal cells of adult NMRI mice were isolated and cultured at an air-liquid interface as described previously (Davidson et al. 2005). Lentiviral vectors with different envelopes from respiratory viruses were constructed and produced in addition to different AAV serotypes. The polarised primary cell cultures were transduced with these viral vectors encoding GFP as a transgene. After one week the cultures were assessed using confocal microscopy and FACS analysis.

Results:

Lentiviral vectors pseudotyped with the envelope of Ebola virus and AAV serotype 6.2 displayed a superior transduction efficiency compared to the other envelopes and serotypes.

Conclusions:

Primary tracheal cell cultures, grown at an air-liquid interface, mimic the in vivo polarization of the pseudostratified respiratory epithelium. It serves as a financially acceptable in vitro screening model for the optimized viral vectors.

Poster # 39

Long term pulmonary gene transfer with a lentiviral vector in a fetal mouse model

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Background and Aims:

Fetal pulmonary gene therapy is currently being evaluated for several genetic conditions such as cystic fibrosis, surfactant deficiencies and primary pulmonary hypertension. Fetal therapy has several advantages including small target volume, presence of progenitor and stem cells, long contact time between vector and target cells and reduced immune response. In the current study we examined the long-term expression of a reporter gene and subsequent immune response in a fetal mouse model.

Methods:

Time mated pregnant NMRI mice (term=19 days) underwent at E18 general anesthesia, laparotomy and exposition of both uterine horns. Fetuses were transthoracically injected under the right axilla with a lentiviral vector expressing firefly-luciferase (n=16). Control animals were injected using normal saline (n=10). After birth surviving pups were followed up using a bioluminescence (BLI) camera. Scans were performed at month 1,2,3,4, 5 and 6. Seroconversion for the transgene was assessed by Western blot analysis, serum from intravenous injected adult animals was used a positive control.

Results:

In both groups two fetuses did not survive the procedure. Of the remaining fetuses all were found positive on BLI scan after injection of lentiviral vector. The BLI signal stayed detectable upto 6 months. No signal was detected in the animals injected with saline.

No seroconversion against the transgene could be detected using Western blot analysis in the fetuses, adult animals did show seroconversion.

Conclusions:

In the mouse model fetal gene therapy into pulmonary tissue using a lentiviral vector results in long term gene expression with no detectable humoral immune response.

Session 8:
Ion channels in CF

Poster # 40

Differential expression of murine clca-gene family Members in cystic fibrosis mouse models

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Background: CLCA (chloride channels, calcium activated) proteins are thought to mediate an alternative, calcium-activated chloride conductance in Cystic Fibrosis (CF) patients and CF mouse models. An association between a CFTR-independent residual chloride conductance in gastrointestinal epithelia with the allele distribution within the human CLCA locus has previously been observed (Ritzka et al., 2004). Aim: This study aims on the characterization of the cellular expression pattern of murine CLCA on the RNA- and protein-levels with a special focus on mCLCA6. Methods: Experiments principally employ RT-qPCR-based detection of mCLCA in whole tissue samples and single cell samples gained by laser capture microdissection. mCLCA6-protein was localized using self-generated antibodies and co-localized with CFTR using confocal laser-scanning microscopy. Murine CF relevant tissues were investigated in two mouse models (cftrtm1Cam, cftrTgH(neoim)Hgu) and four mouse strains (NMRI, BALB/c, C57BL/6, DBA/2). Results: Murine CLCA show a gene-specific expression pattern. mCLCA6 is located in non-goblet cell enterocytes and co-localizes with the CFTR channel on the apical surface of enterocytes. mCLCA6-RNA has a significantly higher expression level in the caecum of DBA/2 cftrTgH(neoim)Hgu mice than in the wild type controls. Conclusion: Due to its co-localisation with the CFTR and its expression in the intestine of CF mice, the murine mCLCA6 may be of special interest as a mediator of an alternative chloride conductance in the intestine. Acknowledgements: We thank the Mukoviszidose e.V. for financial support, H. deJonge for the anti-CFTR-antibody and B. Tümmeler, H.-J. Hedrich and U. Seidler for the donation of the CF mouse models.

Poster # 41

Normal Ca²⁺-activated Cl⁻ channel (CaCC) activity in native airway tissues of mCLCA3 deficient mice

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Background:

Defective CFTR mediated Cl⁻ secretion plays a central role in the pathogenesis of cystic fibrosis (CF). Functional studies document that the airway epithelia express alternative Ca²⁺ activated Cl⁻ channels (CaCC), which have been proposed as a therapeutic target to compensate for lack of CFTR activity in CF patients. However the molecular identity of CaCC has not been identified.

Aims:

The goal of this study was to determine the role of mCLCA3 as a putative candidate for the endogenous CaCC.

Methods:

To determine the role of mCLCA3 in Ca²⁺-activated Cl⁻ secretion we performed trans-epithelial measurements of ion transport in freshly excised tracheal tissues from mCLCA3 deficient mice and wild-type littermates using perfused Ussing chambers. Further, we performed immunohistochemistry and histology to compare mCLCA3 localization and pulmonary morphology in mCLCA3 deficient and wild-type mice.

Results:

We demonstrate that basal (bumetanide-sensitive) Cl⁻ secretion and Ca²⁺-activated (UTP-mediated) Cl⁻ secretion are not altered in mCLCA3 deficient mice compared to wild-type controls. Further transepithelial Na⁺ absorption and cAMP induced Cl⁻ secretion were normal in mCLCA3 deficient mice. Lack of pulmonary expression of mCLCA3 was confirmed by immunohistochemistry, but did not result in a spontaneous pulmonary disease phenotype in mCLCA3 deficient mice.

Conclusions:

Our results argue against a role of mCLCA3 in Ca²⁺-activated Cl⁻ secretion in native murine airway epithelia.

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Poster # 42

Towards a rational therapy for Cystic Fibrosis Liver Disease

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Background:

Cystic Fibrosis Liver Disease (CFLD) is a major complication in Cystic Fibrosis (CF). In liver, the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein is found in bile duct epithelia. It is assumed that thickening and sludging of bile results in obstruction and secondary bile duct damage. However, solid data on CFLD pathophysiology are lacking.

Aims:

To identify a therapy for CFLD based upon its pathophysiology.

Methods:

Bile flow and composition were analyzed in CFTR- Δ F508 mice and two *Cftr* knockout models. The effect of the choleric bile salt ursodeoxycholic acid (UDCA) on bile flow and composition was determined in the presence and absence of *Cftr*.

Results:

Basal bile flow was not altered in Δ F508 and *Cftr* $-/-$ ^{tm1Cam} mice compared to controls. Surprisingly, bile flow was also not changed in *Cftr* $-/-$ ^{tm1Unc} mice that spontaneously develop liver disease. The phospholipid to bile salt ratio, a measure for bile cytotoxicity, was not altered in any of the CF models. UDCA administration to *Cftr* $-/-$ ^{tm1Cam} mice increased bile flow independently of *Cftr*. Cholate, however, induced less flow and a more cytotoxic bile composition in CF mice than in controls. *Cftr* $-/-$ ^{tm1Unc} mice had an altered bile salt profile.

Conclusions:

Under basal conditions, *Cftr* deficiency does not affect bile flow or cytotoxicity. Upon bile salt administration, however, CF-specific changes in bile flow and cytotoxicity develop. We speculate that an altered bile salt profile is related to liver disease in *Cftr* $-/-$ ^{tm1Unc} mice.

Poster # 43

Upregulated expression of ENaC subunits in human CF nasal epithelium

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The genetic defect that causes cystic fibrosis (CF) results in ion transport abnormalities, which are characterised by a reduced Cl^- secretion and a markedly increased Na^+ absorption mediated by the amiloride-sensitive epithelial sodium channel (ENaC). Since several years the Na^+ transport defect in CF patients due to ENaC has become focus of many studies. ENaC mediates the first step in Na^+ reabsorption in epithelia cells such as kidney, lung, and colon and may consist of four homologous subunits (α , β , γ , δ). Predominantly, the α -subunit is expressed in these epithelia and it usually forms functional channels with the β - and γ -subunit. The δ -subunit was first found in human brain and kidney but the expression was also detected in human cell lines of lung, pancreatic and colonic origin. Recently, we showed an enhanced expression of α -, β - and γ - subunits in human nasal tissue of CF patients compared to that of non-CF patients using qRT-PCR. By comparing the two groups on the protein level, we observed also differences in the abundance of ENaC. Afterwards, we were interested whether human nasal epithelium of CF and non-CF patients expresses the δ -ENaC subunit. After cloning the full length cDNA of the δ -subunit we carried out immunofluorescence experiments to detect this subunit at the apical surface of the cells. Thus, we could show that the expression of the δ -subunit is enhanced in the tested CF epithelia, too. So far, the physiological and pathological role of δ -ENaC in non-neuronal tissues is still unclear, but our findings raise the question if the δ -ENaC subunit possesses important regulatory functions and if it interacts with the other ENaC subunits or members of the DEG/ ENaC family in the human respiratory epithelium of CF and non-CF patients.

Poster # 44

Enduring inhibition of Na⁺-hyperabsorption in human CF nasal epithelia cells

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The genetic disease cystic fibrosis (CF) is caused by a defective chloride secretion mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) and excessively increased Na⁺-absorption via the amiloride-sensitive epithelial Na⁺ channel (ENaC). Since activation of Cl⁻ conductances failed and amiloride therapy gives only temporary relief, in this project the Na⁺ hyperabsorption is inhibited with ENaC specific antisense oligonucleotides (AON). These short synthetic DNA molecules are complementary to mRNA sequences encoding for ENaC and prevent its functional protein expression. From these studies we expect a long lasting suppression of the excessive Na⁺ absorption in CF by AON administration, thereby improving life quality of the patients. Human non-CF and CF nasal epithelial cells were seeded on permeable collagen filters and measured in modified Ussing chambers. Na⁺ absorption through ENaC was assessed as short-circuit current (I_{SC}) and conductance (G_i) before and after AON transfection. To analyze the inhibitory effects of AON treatment on the protein level, we carried out additionally Western blot analyses with a specific anti α-ENaC antibody. AON transfection inhibits the ENaC current by about 66% in non-CF cells and by about 74% in CF cells. Furthermore, we found in Western blot analyses a suppression of the α-ENaC protein in AON transfected human non-CF cells. The expression of the protein was reduced by about 47%. Thus, ENaC-specific AON effectively decreases Na⁺ hyperabsorption in CF epithelia. Administered via inhalation these AON could eliminate Na⁺ hyperabsorption in the airways of CF patients.

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Poster # 45

Recurrence of PSA after early treatment- a long-term follow-up study

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The chronic infection with *Pseudomonas aeruginosa* (PSA) is a key event in the clinical course of patients with cystic fibrosis (CF). A daily tobramycin inhalation leads to the eradication of the first detected PSA to about 80%. The purpose of the study is to examine, how long the patients remain free of PSA after the coming off the early therapy.

We followed retrospectively 26 patients (age: (mean \pm SD) $6,9 \pm 3,5$ years; Sex: male 14, female 12) where PSA was proved for the first time, for a period of at least 3 up to 8 years. 26 patients inhaled tobramycin (80mg twice daily; for 12 months) as early PSA eradication therapy. 12/26 got additional sporadic systemic antibiotics.

During treatment PSA disappeared (i.e. 3 negative swabs in a sequence with a least distance of 30 days) in all 26 patients. 11/26 remained without a new PSA proof for (mean \pm SD) 1962 ± 688 days. In 15/26 PSA was detected again during the follow-up period. 6/15 patients were tested PSA positive under the initial therapy, 9/15 after the end of therapy. The recurrence of PSA after stopping initial therapy was either early (<300 days; n=5) or late (>500 days; n=4). In four patients PSA isotypes were tested for the first and the new proof. In 3 patients with an early PSA recurrence the same PSA isotypes were detected. In 3 patients with late PSA recurrence different isotypes were identified. Our results confirm the success of the early PSA-treatment. However they also point to the fact that the disappearance of PSA under the early PSA therapy represents a temporary suppression and not a complete eradication in a relevant part of the patients.

Poster # 46

Own clinical observations with long-term tobramycin inhaled therapy in children with cystic fibrosis

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Background:

Respiratory disease is the major cause of mortality in cystic fibrosis (CF) patients. Colonisation with *Pseudomonas aeruginosa* in the airways causes deterioration in pulmonary function. Inhaled tobramycin is an antibiotic that can improve the pulmonary conditions.

Objectives:

The study was carried out in 17 children and adolescents with CF and chronic infection with *P. aeruginosa*. The patients were treated with inhaled tobramycin for 3 years. This was opened, uncontrolled, observational study of clinical effects of the therapy.

The aim of the study was to find if long-term therapy with inhaled tobramycin may contribute to better control of lung function.

Methods:

The clinical status was assessed by pulmonary function, measurements of somatic features (weight, height, BMI), chest X-ray and occurrence of adverse events. The results were compared with their previous results (three years period before treatment with tobramycin).

Results:

After three years of treatment we observed decline in FEV1% predicted (median value with lower and upper quartile) 2,87% with -10,13 and 3,67 from baseline values. During TOBI therapy the deterioration of lung function was significantly lower ($p=0,03$) than in the period before treatment. During 3 years period before TOBI therapy median BMI increased by 0,5 kg/m² with 0,11 and 0,62. After 3 years of treatment median BMI increased significantly ($p=0,02$) by 1,19 kg/m² with 0,31 and 1,89. TOBI treatment significantly delayed progression of pulmonary X-ray changes assessed by Brasfield score ($p=0,01$).

Conclusions:

We observed that long-term therapy with inhaled tobramycin reduced progress of pulmonary disease, delayed progression of pulmonary X-ray changes and improved nutritional status of CF patients.

Poster # 47

Exhaled leukotriene B4 and nitric oxide in cystic fibrosis

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Background Cystic fibrosis (CF) is the most commonly inherited lethal pulmonary disorder in Caucasians. Exhaled breath condensate (EBC) is emerging in the search for noninvasive biomarkers for airway diseases.

Aims The aim of this study is to search for inflammatory markers for CF.

Methods EBC samples were collected from 32 CF patients, of which 9 were chronically colonized with *Pseudomonas aeruginosa*, and 21 healthy controls (age 6-23 years). EBC was collected by tidal breathing during 15 minutes in a RTube (Respiratory Research). EBC pH was measured without deaeration, exactly 5 minutes after sample collection. NO was measured with a NIOX Mino (Aerocrine).

Results Exhaled LTB₄, measurable in 73% of the samples (EIA, Cayman), was significantly higher in CF patients compared to healthy controls (median: 5.71 vs 3.59 pg/ml; Mann-Whitney U test: p=0.0029). Values were especially elevated in CF patients with *P. aeruginosa* (10.05 pg/ml), but not significantly higher than in other CF patients. Gender, body weight, height or age didn't have an influence on exhaled LTB₄. EBC pH was lower in the CF patients than in the control group, but the difference was not significant (median: 5.37 vs 5.70). No correlation was observed between LTB₄ and EBC pH. Exhaled NO was significantly lower in CF patients compared to healthy controls (median: 8 vs 10 ppb; p=0.010). A small but significant negative correlation between LTB₄ and logNO was observed (p=0.049). **Conclusions** These data suggest that exhaled LTB₄, which has potent chemotactic activity for neutrophils, could be used to monitor airway inflammation in CF patients.

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Poster # 48

The effect of vitamin K supplementation on bone formation in children with Cystic Fibrosis

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Background:

Patients with Cystic Fibrosis (CF) are at risk for developing a poor bone status and its related complications in adult life. Vitamin K (VK) is the cofactor in the carboxylation of osteocalcin (OC). Carboxylated osteocalcin (c-OC) is the active form able to bind calcium.

Aim:

To evaluate the effect of different doses of VK supplementation on bone formation in children with CF.

Methods:

26 patients (16 boys, 10 girls, 4-15 years) were randomized in a double blind 2 years study. Subjects received either daily 0,1 mg VK (CF low, n=12) or 1 mg vitamin K (CF high, n=14) supplementation. At start (t=0), after one year (t=1) and after two years (t=2) bone markers undercarboxylated osteocalcin (u-OC), c-OC and bone alkaline phosphatase (BAP) and bone resorption marker N-term collagen type 1 (NTX) were determined in serum using ELISA type kits. Bone mineral density (BMD) was measured each year by dual energy X-ray absorptiometry (DEXA). Results will be interpreted using aged-matched controls (Z-scores). Non-parametric Wilcoxon's (Mann-Whitney) rank sum test was used to analyze the data. A $p < 0.05$ was considered significant.

Results:

Serum c-OC and u-OC were higher at t=1 and t=2 in CF treated with low and high VK. The u/c OC ratio increased in time. The difference in increase was not significant between the two treatments. There was no difference in serum BAP at t=1, however, serum BAP was higher in CF low compared to CF high at t=2, though not significant. Serum NTX was lower at t=1 and t=2 in CF low and CF high, however the difference in decrease was not significant.

Conclusions:

Even 0.1 mg/day VK was sufficient to improve OC carboxylation. The effect of VK administration on the other bone markers was less pronounced, but tended in the direction of improved bone formation and decreased bone resorption. Bone density as outcome parameter by DEXA, will be analyzed and might reveal its clinical importance. These data show the importance of supplementing VK in CF patients.

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