16th Scientific Meeting

22nd – 23rd September 2016

Schloss Waldthausen/Mainz

Fungi in CF: Where do we stand?

and results of research projects funded by the Mukoviszidose e.V.

Program and Abstracts

Chairs:

Anna-Maria Dittrich (Hannover)

Carsten Schwarz (Berlin)

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Thursday, September 22nd

11:45 am	**Get together + Lunch**
12:30 pm	**Opening of the meeting** Manfred Ballmann (Chairman of the supervisory board Mukoviszidose Institut) Michael Hogardt (Member of the scientific board Mukoviszidose e.V.)

Session 1: Fungal epidemiology and pathogenicity

Moderation:
Anna-Maria Dittrich (Hannover)
Carsten Schwarz (Berlin)

12:40 – 1:25 pm	**Opening Talk** **Fungal epidemiology and diversity in cystic fibrosis patients over a 5 year period in a national reference center** Ludwig Sedlacek (Hannover)
1:30 – 2:05 pm	**Fungal Colonization in Cystic Fibrosis** Andreas Hector (Tübingen)
2:10 – 2:45 pm	**Identification and localization of fungi in respiratory samples using Fluorescence in situ hybridization** Volker Rickerts (Berlin)
2:50 – 3:30 pm	**Coffee Break & Discussion**
3:30 – 4:05 pm	**Pathogen-specific T-cells as specific sensors for diagnosis of immune-related disease exacerbations in cystic fibrosis patients** Alexander Scheffold (Berlin)
4:10 – 4:30 pm	**The CF Foundation Fungal Project** Michael P. Boyle (Baltimore/USA)

Session 2: Short talks on submitted abstracts I

Moderation:
Dominik Hartl (Tübingen)
Andreas Jung (Zürich)

4:40 – 5:00 pm **Establishment of *Galleria mellonella* larvae as host organism in an in vivo infection model for testing virulence of *Exophiala dermatitidis***
Frederike Hoffmann (Essen)

5:05 – 5:25 pm **Expression of IL-17A and IL-22 by innate lymphoid cells from lymph node tissue from CF patients**
Melanie Albrecht (Hannover)

5:30 – 6:00 pm **Coffee Break & Discussion**

6:00 – 06:55 pm **Keynote Session**
Does cystic fibrosis predispose to fungal respiratory infections?
Jean-Philippe Bouchara (Angers/France)

8:00 pm **Dinner**

Friday, September 23rd

Session 3: Diagnosis and treatment of fungi in CF

Moderation:
Helge Hebestreit (Würzburg)
Olaf Eickmeier (Frankfurt)

8:00 – 8:35 am **Detection of fungi by electronic nose technology in CF patients**
Els Weersink (Amsterdam/Netherlands)

8:40 – 9:15 am **Azole-resistant *Aspergillus fumigatus* in CF patients**
Jörg Steinmann (Essen)

9:20 – 9:55 am	**Fungicidal activity of *N-chlorotaurine* in artificial sputum medium** Michaela Lackner (Innsbruck/Austria)
10:00 – 10:35 am	**Fungal Biofilms in Cystic Fibrosis; do they matter?** Craig Williams (Paisley/UK)
10:40 – 11:10 am	**Coffee Break & Discussion**

Session 4: Short talks on submitted abstracts II

	Moderation: Olaf Sommerburg (Heidelberg) Michael Hogardt (Frankfurt)
11:10 – 11:30 am	**Improved Lung Function and Hepatosteatosis after oral Choline Substitution in CF Patients** Wolfgang Bernhard (Tübingen)
11:35 – 11:55 am	**Diversity and dynamics of *Staphylococcus aureus* during chronic airway infection of cystic fibrosis patients** Barbara Kahl (Münster)
12:00 – 12:20 pm	**Novel treatment strategies to common strain rapid antibiotic resistance evolution in cystic fibrosis pathogens** Hinrich Schulenburg (Kiel)
12:25-12:45 pm	**Non-allergic Aspergillus bronchitis in cystic fibrosis** Andreas Jung (Zürich)
12:50 – 1:25 pm	**Closing Talk** **Treatment of pulmonary non-aspergillus infections** Carsten Schwarz (Berlin)
1:30 pm	**Closing of the meeting + Lunch**

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Fungi in CF: Where do we stand?

Session 1: Fungal epidemiology and pathogenicity

Fungal epidemiology and diversity in cystic fibrosis patients over a 5 year period in a national reference center

Authors: S. Ziesing, S. Suerbaum, L. Sedlacek

Introduction: Fungal colonization of the respiratory tract is frequently found in cystic fibrosis (CF) patients and seems to increase. The aim of this report is to give an overview for Germany on the diversity and epidemiology of fungal species in CF patients.

Methods: Over a 5-year period, all fungal isolates cultured from microbiological specimen from CF patients were recorded (2009-2013). Beside standard bacteriological culture media two fungal media were used for cultivation. Species were identified by microscopy, biochemical profiling, MALDI-TOF analysis or DNA sequencing methods.

Results: 25,975 clinical samples from CF patients were analyzed. About 75 % of CF patients were colonized by yeasts, mainly *Candida albicans* (38 %) and *Candida dubliniensis* (12 %). In 35 % of the patients Aspergillus spp. (*Aspergillus fumigatus*: 29 %) were detected, followed by *Exophiala dermatitidis* and *Scedosporium/Lomentospora* complex isolates (4 % each). The occurrence of rare fungi in CF patient, like *Ramsamsonia argillacea* and *Trichosporon mycotoxinivorans* showed no variation over time.

Conclusion: Over a 5-year period, the epidemiology of fungal species detected in CF patients was relatively constant.

Fungal colonization in cystic fibrosis – a retrospective study

Authors: A. Hector, T. Kirn, A. Ralhan, U. Graepler-Mainka, S. Berenbrinker, J. Riethmueller, M. Hogardt, M. Wagner, A. Pfleger, I. Autenrieth, M. Kappler, M. Griese, E. Eber, P. Martus, D. Hartl

Introduction: The airways are directly exposed to airborne pathogens such as bacteria or fungi. As a result of the chronic lung disease in CF, the airways are inable to effectively clear these pathogens, thus, leading to their persistence, colonization and potential infections. Moreover, intensified antibiotic therapy and longer survival of CF patients has been associated with the isolation of a more complex pattern of bacteria and fungi. However, the clinical relevance of these emerging pathogens for lung function remains poorly defined. The aim of this study was to assess the association of bacterial and fungal colonization patterns with lung function in adolescent patients with CF.

Methods: Microbial colonization patterns and lung function parameters were assessed in 770 adolescent CF patients in a retrospective European (German/Austrian) patient cohort (median follow-up time: 10 years)

Results: Infection/Colonization with *Pseudomonas aeruginosa*, *MRSA*, *Aspergillus fumigatus*, *Candida glabrata* were associated with loss of lung function, while mainly colonization with *Haemophilus influenzae* was associated with preserved lung function. *Aspergillus fumigatus* was the only species that was associated with an increased risk for infection with *Pseudomonas aeruginosa*.

Conclusion: Collectively, this study identified potentially protective and harmful microbial colonization patterns in adolescent CF patients. The results suggest that there might be relevant pathogen-pathogen-interactions that have to be considered in the treatment of CF patients. Further studies in different patient cohorts are required to evaluate these microbial patterns and to assess their clinical relevance.

Identification and localization of fungi in respiratory samples using Fluorescence in situ hybridization

Author: V. Rickerts

Introduction: A diverse array of microorganisms can be cultivated from the respiratory tract of patients with cystic fibrosis, including yeasts and molds. Factors shaping such microbial communities are challenging to understand but likely include interactions between microbes in these communities. Such interactions have been described to alter phenotypic characteristics of fungi including virulence factors, suggesting that they may influence host pathogen interactions.

Sequencing techniques have been successfully used to identify the constituents of microbial communities. In addition, Fluorescence in situ hybridization (FISH) targeting microbes aims at deciphering the spatial organization of microbial communities. This has been successfully used to identify the etiology of invasive infections in the context of polymicrobial colonization. In addition, it has been used to understand the function of microbes within a community and may identify microbes possibly interacting by identification of closely linked ones.

Methods: We develop FISH probes targeting the ribosomal RNA of fungi in order visualize them in clinical samples of cystic fibrosis (CF) patients. Probes targeting *Candida, Aspergillus, Scedosporium* and the Mucorales have been developed. In addition, broadrange PCR with sequencing is used to confirm the presence of fungi.

Results: Preliminary data suggest that FISH identifies fungi in clinical samples from patients with invasive mycoses. When using differentially labeled probes, species differentiation is possible among morphologically similar fungal elements in tissue.

Conclusion: The combination of sequencing techniques and FISH may lead to improved identification of fungi in clinical specimens, leading to superior identification of etiologic agents of invasive infections allowing for targeted treatment. In addition, by identification of spatially linked microbes, potentially interacting species may be identified. This may form the basis to decipher mechanisms of interaction to identify potential interventions.

Pathogen-specific T-cells as specific sensors for diagnosis of immune-related disease exacerbations in cystic fibrosis patients

Author: A. Scheffold

Introduction: Pathogen-driven disease exacerbations are a major complication and risk factor for cystic fibrosis (CF) patients. Rapid and timely diagnosis of the critical pathogen(s) is highly demanding for selection of the optimal treatment strategy. For many pathogens, including fungi, microbe-directed diagnostics cannot differentiate between colonization or sensibilization or infectious complications. We are testing the hypothesis that pathogen-specific T cells as the primary specific immune sensors and amplifiers of in vivo confrontation with the pathogen, provide unique diagnostic potential. We are investigating inasmuch the quantity or quality of pathogen-specific T cells indicates the actual status of host pathogen interactions or may allow to predict the individual risk to experience clinical complications with a certain pathogen.

Methods: We have developed a sensitive assay allowing to analyse the rare pathogen-specific CD4 T cells from periperal blood of patients using Antigen-Reactive T cell Enrichment (ARTE). ARTE allows collecting sufficient quantities of pathogen-specific T cells to perform a precise detailed phenotypic and molecular characterization.

Results: ARTE provides a sensitive system for analysing pathogen-specific CD4 T cells from human blood. We show that the analysis of the characteristics of pathogen-specific T cells allows early and specific diagnosis of invasive mycoses in hematologic patients and allows to identify patients with active Mycobacterium tuberculosis infection. In particular, we are analyzing whether the T cell reaction allows discrimination between different fungal pathogens, such as *A. fumigatus*, *Mucorales spp*, *Scedosporium spp*, *Fusarium spp* or *C. albicans*, to improve targeted therapy of the patients. ARTE also allows further classification of CF patients according to the quality or quantity of pathogen-specific T cells, which may have prognostic potential. Our results provide first indications that the specific T cell reaction also identifies mechanisms of underlying immunopathology (allergy) and may have diagnostic potential for fungal sensitization and invasive infection.

Conclusion: Pathogen-specific T cells can be used as specific sensors of microbial infections reporting the actual host pathogen interaction status beyond quantitative analysis of microbial colonization. T cell based assays may provide a diagnostic tool for discrimination of fungal pathogens and may allow identification of individual immunological risk factors regarding the involvement of fungi in CF disease exacerbations.

The U.S CF Foundation Fungal Project

Author: M. Boyle

Over the last decade there has been a steady increase in the prevalence of fungal organisms detected in respiratory cultures of US CF patients. How much of this increase is due to a true increase in prevalence and how much is due to improved fungal detection techniques is not clear. What is clear is that even these data may underrepresent the true prevalence of fungal organisms found in CF sputa, as even higher prevalence rates are found when fungal selective media are utilized. While there is strong desire to perform a study to determine how frequently fungal organisms are pathogenic and contribute to decline in lung function in CF, initial standardization of optimal fungal microbiologic techniques may be required to enable subsequent fungal clinical research. Results from a fungal microbiologic technique study, a CF registry analysis of risk factors for fungal acquisition, and future plans for fungal research will be presented.

Session 2: Short talks on submitted abstracts I

Establishment of *Galleria mellonella* larvae as host organism in an in vivo infection model for testing virulence of *Exophiala dermatitidis*

Authors : F. Hoffmann, M. Olsowski, P.-M. Rath, J. Steinmann

Introduction: To determine the virulence of pathogens, in vivo infection models are indispensable. Until recently, mice were used as test animals, but they are expensive and subject to strict ethical controls. The larvae of the greater wax moth *Galleria mellonella* are inexpensive to house, simple to handle and are more ethically accepted. We present *G. mellonella* as an invertebrate host organism for infection with *Exophiala dermatitidis.*

Methods: After purchase, larvae were kept 48 h in the dark with Haydan medium at room temperature for recovery. Only larvae with a uniform colour and a weight of 250 – 350 mg were chosen for infection. *E. dermatitidis* and *Candida albicans* (ATCC® 90028) were cultured in yeast form (liquid culture: 35°C, 200 rpm, 48 h). To determine an adequate inoculum, different concentrations were tested (1 x 10^6 – 1 x 10^9). The infection model was established using *C. albicans* as an infection control, PBS as a negative control of infection and a non-injected group of larvae in each experiment as a negative control of injection. For infection, we used three different invasive *E. dermatitidis* isolates (PA2, PA3 and PA5) and injected 10 µl/larvae using a syringe pump. Each infection group contained 5 - 10 larvae. The experiment spanned 8 d and the larvae were examined daily. Larvae without movement were assumed to be dead. Larval haemolymphs were extracted and cultured at 35°C on candida chrom agar (Oxoid). Statistical analyses were performed using GraphPad PRISM®.

Results: *G. mellonella* larvae only need darkness and food, and are temperature tolerant between 15 - 37° C, making them an ideal in vivo infection model for virulence testing of fungi. The results demonstrate that a higher inoculum (1 x 10^8 cells/ml) of *E. dermatitidis* is needed to kill larvae than that of *C. albicans* (1 x 10^6 cells/ml), which indicates a lower virulence of *E. dermatitidis* compared to *C. albicans*. The results also state that PA5 is less virulent than PA2 and PA3.

Conclusion: The established in vivo infection model using *G. mellonella* larvae as a host organism is suitable for virulence testing of *E. dermatitidis*. The black yeast-like fungus showed a lower virulence than *C. albicans.*

Expression of IL-17A and IL-22 by innate lymphoid cells from lymph node tissue from CF patients

Authors: M. Albrecht, S. Pallenberg, M. Cases, D. Jonigk, G. Warnecke, A.-M. Dittrich

Introduction: Studies describing IL-17 producing T cells in CF lung tissue independent of infection as well as increased numbers of IL-17A/IL-22 co-producing memory T cells from lymph nodes (LN) and lung tissue and a preferential development of T cells towards a Th17 phenotype in CF patients , suggest that the lung micro-environment in CF lung disease is shaped by IL-17A-producing cells. Although CD4+ IL-17A- and IL-22-producing cells in tissue from CF patients were already identified, several other lymphocytes are capable of IL-17A-production, some of which might represent early sources of these cytokines even before IL-17A and/or IL-22 production by the adaptive immune system. Hence we were aiming at

a)delineating the expression of IL-17A and/or IL-22 producing lymphocytes in CF lymph node tissue and

b)analyzing the soluble mediators secreted by these cells.

Methods: We identified IL-17A and IL-22 expressing lymphocytes by flow cytometry in restimulatedbronchial lymph node cells obtained from lung explants from CF patients undergoing lung transplantation. Multiplex analyses of supernatants and RNA analyses of genes implicated in the IL-17A signaling pathway and tissue injury processes from lung and lymph node cells complemented these analyses. Expression was compared to cells from healthy controls (lymph node cells from donor lungs) and cells from non CF patients prior to lung transplantation.

Results: Our analyses revealed expression of IL-17A and IL-22 in all innate lymphocyte cell populations studied with differences between cells of CF origin compared to control lymphocyte populations. Similarly, differences were noted by comparison of cyto- and chemokine profiles secreted by restimulated lymphocytes. Moreover, we observed different matrixmetalloproteases to be expressed in the supernatants suggesting a role for these cells and their mediators in lung tissue injury.

Conclusion: Our analyses identified IL-17A and IL-22 expression in different innate lymphoid subpopulations. Differential expression between cells from CF patients vs. controls was noted for the majority of innate lymphoid subpopulations studied, suggesting a specific implication of IL-17A and/or IL-22 production by innate lymphoid cells for CF lung disease.

Keynote Session

Does cystic fibrosis predispose to fungal respiratory infections?

Authors : P. Vandeputte, M. Fleury, S. Giraud, M. Pihet, T. Dugé de Bernonville, N. Papon, J.-P. Bouchara

Respiratory infections are the major cause of morbidity and mortality in patients with cystic fibrosis (CF). Bacteria, especially *Pseudomonas aeruginosa*, are primarily responsible for these infections. A lot of works therefore have been performed during the past decades aiming to improve the treatment and prevention of bacterial infections. Progress in this field, together with the development of an early diagnosis of CF and with the improvement of the nutritional status of the patients, has led to a marked increase in life expectancy. Nevertheless, later in age, the respiratory tract of the patients may also be colonized by filamentous fungi. This fungal colonization of the airways which is facilitated by the repeated cures of antibiotics and the use of inhaled corticosteroids may also lead to respiratory infections, the frequency of which regularly increased along with the increase in life expectancy.

Among the filamentous fungi colonizing the CF airways, *Aspergillus fumigatus* is by far the most common, but many other fungal species may also be encountered in this clinical context, some of them being almost exclusively associated with cystic fibrosis.

All the fungal species associated with CF are environmental molds for which the contamination of the patients is very likely to occur through inhalation of some airborne conidia. Nevertheless, these fungi are not the most common among the airborne conidia and in this talk, we will show that the fungal species associated with CF have all the equipment needed to establish within the host and to cause a chronic colonization of the airways. In addition, we will propose an hypothesis to explain the relative frequency of these fungi in CF.

Session 3: Diagnosis and treatment of fungi in CF

Detection of fungi by elctronic nose technology in CF patients

Authors: K. de Heer, MGM Kok, N. Fens, EJM Weersink, AH Zwinderman, MPC van der Schee, CE Visser, MHJ van Oers, PJ Sterk.

Introduction: Evidence is mounting that fungal colonisation, especially *Aspergillus fumigatus*, in Cystic Fibrosis (CF) is associated with lower lung function. Variability in the screening tests can complicate diagnosis and estimates of prevalence. The detection of *A. fumigatus* and other fungi from clinical specimens such as sputum is highly dependent upon the methodology used, and a more sensitive approach that is standardised and universally adopted is a definite need. A new non-invasive and culture independent method can be the eNose, a cheap and realtime measurement instrument. A numerous disease states now, as asthma COPD and lung cancer, can be recognized by their unique exhaled breath profil.

Hypothesize: It is unknown whether colonization by *A. fumigates* in CF patients results in a change in exhaled breath profile. We hypothesized that *A. fumigates* colonization in CF patients leads to a distinct exhaled breath profile.

Methods: Exhaled breath of 27 CF patients was analyzed with a Cyranose 320. Culture of sputum defined the *A.fumigatus* colonization status. eNose data were classified using canonical discriminant analysis after principal component reduction. Our primary outcome was the cross-validated accuracy, defined as the percentage of correctly classified subjects using the leave-one out method. The p-value was calculated by generation of 100.000 random alternative classifications.

Results: 9 of the 27 CF patients were colonized by *A.fumigatus*. In total 3 subjects were misclassified, resulting in a cross-validated accuracy of a Cyranose in detecting *A. fumigatus* of 89 % ($p = 0.004$, sensitivity 78 % and specificity 94 %). Receiver Operating Characteristic (ROC) curve analysis showed an AUC of 0.89.

Conclusion: The results indicate that *A.fumigatus* colonization leads to a distinct breathprint in CF patients. The present proof-of-concept data merit external validation of the ability of an eNose, to diagnose colonization by *A. fumigates*.

Prevalence and characterization of azole-resistant *Aspergillus fumigatus* in German CF-patients

Authors: Steinmann J and the ARAF CF study group

Introduction: *Aspergillus fumigatus* is the most prevalent fungus in the respiratory tract of patients with CF, ranging from 6 % to 60 %. Recent studies reported the emergence of acquired resistance of *A. fumigatus* to azole compounds in different patient populations, including CF patients. The objective of the present study was to analyse the prevalence of azole resistant *A. fumigatus* in German CF patients.

Methods: The prevalence of azole-resistant isolates was investigated by prospectively screening *A. fumigatus* isolates found in respiratory secretions from German CF patients. Twelf university-based centers across Germany participated in the study. For all isolates that were sent from external laboratories screening was performed by culturing *A. fumigatus* isolates on agar containing 4 mg/L itraconazole. In case of growth on the screening agar or elevated itraconazole MIC a PCR for the detection of mutations in the cyp51A gene was performed. With all azole-resistant isolates antifungal susceptibility testing (itraconazole, voriconazole, posaconazole and isavuconazole) by broth microdilution method according to EUCAST was done.

Results: Twelf centers across Germany participated in the study. In total, 2882 *A. fumigatus* isolates from 968 patients were analysed for azole resistance. Overall, we found 107 *A. fumigatus* isolates being resistant at least against one azole. The prevalence of azole resistance per patient was 5.2 %. The resistance rate varied between the centers. The Essen center had the highest prevalence (10.1 %), than Munich (7.8 %) and than Muenster and Hannover (6.0 % and 5.2 %), respectively. Molecular characterization of the isolates revealed that most of the isolates (n=87) carried a TR34/LH98 mutation.

Conclusion: In conclusion, susceptibility testing of *A. fumigatus* isolates from CF patients who are receiving of azole-based treatment is recommended. More epidemiological surveillance projects, studies on the relevance of azole-resistant Aspergillus in CF and on different treatment options are urgently needed.

Fungicidal activity of N-chlorotaurine in artificial sputum medium

Authors: M. Lackner, I. Moser, M. Gruber, M. Nagl

Introduction: *N-chlorotaurine* (NCT) is a well-tolerated endogenous antiinfective. Due to its good tolerability, it potentially can be used for inhalation. As *Scedosporium spp*. frequently colonizes the lung of patients suffering from cystic fibrosis (CF), the fungicidal activity of NCT against Scedosporium and Lomentospora was tested in a medium (ASM), whose composition mimics the bronchial mucus of CF patients.

Results: A previous study found already that in quantitative killing assays, both hyphae and conidia of *Scedosporium apiospermum*, *Scedosporium boydii*, and *Lomentospora prolificans* (former Scedosporium prolificans) were killed by 55 mM (1.0 %) NCT at pH 7.1 and 37 °C with a log10 reduction in CFU of 1 - 4 after 4 h and of 4 to > 6 after 24 h. Addition of ammonium chloride to NCT markedly increased this activity. The present study found that NCT at a concentration of 0.3 % - 1.0 % showed a microbicidal effect on all *Scedosporium spp*. The impact of 0.5 % and 1 % NCT against these fungi was significantly stronger in ASM medium than in buffer solution. *Scedosporium spp*. were killed after 15 minutes to the limit of detection. Measurements by means of iodometric titration showed oxidizing activity over 30 min (60 min) at a concentration of 0.3 % (1.0 %) NCT, what matches the killing tests.

Conclusion: NCT demonstrates fungicidal activity against different Scedosporium and Lomentospora species at concentrations ideal for clinical use. Microbicidal activity of NCT in CF medium was better than in buffer solution, which can be explained by formation of monochloramine by transhalogenation, which rapidly penetrates fungi.

Fungal Biofilms in Cystic Fibrosis; do they matter?

Author: C. Williams

Introduction: For a long time the microbiology of cystic fibrosis has been focussed on *Pseudomonas aeruginosa* and associated Gram-negative pathogens. An increasing body of evidence is available which demonstrates an important role for moulds and yeasts within this complex patient group. Whether or not fungi are active participants, spectators or transient passer-by's remains to be elucidated. However, functionally they do appear to play a contributory role in pathogenesis, albeit we do not know if this is a direct or indirect effect. In addition although it has been recognised for a number of years that *Aspergillus fumigatus* has the ability of to form biofilms, that is multicellular populations of filamentous intertwined hyphae attached to surfaces or one another and enclosed within a dense extracellular matrix, the clinical relevance of these biofilms remains unclear.

Methods: We have examined a range of in vitro biofilm systems to elucidate mechanisms of antifungal resistance and interkingdom interactions within biofilms.

Results: *P. aeruginosa* is able to selectively form biofilms on C. albicans, hyphae but not the yeast form which results in the death of the Candida through the release of a phenazine toxin. It has also been shown to inhibit the morphological transition through a 3-oxo-C12 homoserine lactone a phenomenon replicated in studies of *A. fumigatus* biofilm. In a murine model lung tissue injury caused by *P. aeruginosa* infection is alleviated if preceded by a short term **C. albicans** colonisation due to *C. albicans* activating IL-22 producing innate lymphoid cells, which provided protection *from P. aeruginosa* induced injury.

C. albicans quorum sensing molecule farnesol impacts upon *P. aeurginosa* by inhibiting its quinolone signalling, which controls pyocyanin production. The release of the metalloprotease elastase from *P. Aeruginosa* is significantly increased in the presence of *A. fumigatus* during biofilm co-culture. Intersetingly interactions between *A. fumigatus* and *P. aeruginosa* are related to the source and phenotype of the *P. aeruginosa* isolate with CF isolates being more inhibitory than non-CF isolates, and non-mucoid CF isolates the most most inhibitory of all.

Conclusion: Although our knowledge regarding the role of fungi in the pathogenesis of CF is improving, many questions remain. Are certain fungi pathogenic, and if so, is the mechanism of pathogenicity direct or mediated by complex interactions within the lung microbiome? If pathogenicity is accepted, should attempts be made to eradicate fungi along with antibacterial treatments? If so, what drugs should be used and for what duration and how important is the biofilm phenotype in promoting resistance? These questions are difficult to answer on the basis of existing knowledge and further studies are needed both in individual fungi and in the context of a complex interkingdom microbiome.

Session 4: Short talks on submitted abstracts II

Improved Lung Function and Hepatosteatosis after oral Choline Substitution in CF Patients

Authors: W. Bernhard, R. Lange, U. Graepler-Mainka, J. Riethmüller

Introduction: Choline is an essential nutrient, and a component of phosphati-dylcholine (PC), a phospholipid in all cell membranes and many secretions, particularly bile. In adults, hepatic PC turnover via bile amounts 20-45 % of total organ pool size per day, equaling 0.7-1.5g choline. Moreover, PC is essential for hepatic triglyceride export via lipoproteins, and for clearing organs from pro-apoptotic ceramides via sphingomyelin synthase. Due to pancreatic phospholipase A2 deficiency, fecal choline loss is high in CF, resulting in choline deficiency and low plasma PC. In CF patients, plasma levels of choline and PC correlate with lung function. We therefore investigated the effect of choline substitution on lung function in adult CF patients. Additionally, we investigated the effect on liver fat concentrations.

Methods: Unblinded intervention study (N=10, male). 3x1g choline chloride for 90 days. Pre-/post-intervention assessment of lung function (FEV_1, FVC, MEF 25-75). Quantification of liver fat by magnetic resonance spectroscopy and mass spectrometric determination of plasma choline.

Results: Choline substitution increased plasma levels in study patients from 6.3±1 to 15.3±2.8µmol/L ($p<0.05$). When hepatosteatosis was present, this was abolished after 90d choline substitution. In 9 out of 10 patients lung function was improved within 90 days: FEV_1 from 67+/-19 % to 74+/-19 %; $p=0.0028$). FVC and MEF25-75 were significantly improved as well ($p=0.0259$ and 0.0198, respectively). Several patients reported effects on subjective well-being, like improved performance, digestion and flatulence.

Conclusion: Correcting for the impaired choline status in CF patients by substitution may improve clinically relevant parameters like lung function and hepatosteatosis. Future studies will have to define the mechanisms involved, optimal dosage and galenics, applicability to pediatric CF patients, and the long-term efficiency of such treatment.

Diversity and dynamics of *Staphylococcus aureus* during chronic airway infection of cystic fibrosis patients

Authors: B. Husmann, T. Thissen, S. Deiwick, H. Rengbers, A. Dübbers, C. Kessler, J. Große-Onnebrink, P. Küster, H. Schültingkemper, B. C. Kahl

Introduction: Cystic fibrosis patients suffer from chronic recurrent bacterial airway infections which ultimatively lead to lung insufficiency and decreased life expectancy. *Staphylococcus aureus* is one of the earliest and one of the most common pathogens isolated from the airways of CF patients. The diversity of *S. aureus* during chronic airway infection is not known. Therefore, we conducted a prospective study to determine the dynamics of diverse phenotypes in the lung habitat of patients chronically infected by *S. aureus* in a one-year period.

Methods: We selected 14 patients of 2 CF-centers in Münster, who were persistently infected by *S. aureus* and regularly expectorate sputum. From every sputum we isolated 40 colonies and determined diversity by evaluating size (normal/SCV/intermediate), pigmentation (yellow, not yellow), hemolysis (?-hemolysis/?-toxin/non-hemolytic) on Columbia blood (normal atmosphere) or Schaedler agar (5% CO2), mucoidity on Kongored agar (non-mucoid/intermediate/mucoid) and resistance profiles.

Results: The median age of patients was 24 years (range 16, 45). Nine patients (70 %) were co-infected by *Pseudomonas aeruginosa*. Preliminary results of 29 sputa (n=1160 isolates) from 14 patients revealed a high diversity of phenotypes within individual sputa (1 -29 phenotypes, mean 8). The number of phenotypes varied during sequential visits. 277 (24 %) isolates were stable or dynamic SCVs, 1101/1160 (95 %) were hemolytic, 249 (21 %) were ?-toxin positive; 303 (26 %) isolates displayed a mucoid phenotype on blood or Kongored agar. 171 (15 %) isolates were PSSA, 771 (66%) MSSA and 218 (19%) MRSA, respectively. Most patients were infected by a single clonal lineage.

Conclusion: Our preliminary results revealed a high diversity of *S. aureus* phenotypes during persistent airway infection which varies within and also between patients. Associations of changing phenotypes with bacterial density, exacerbation, co-infection with *P. aeruginosa* or antibiotic therapy will be further evaluated.

Novel treatment strategies to conmstrain rapid antibiotic resistance evolution in cystic fibrosis pathogens

Author: H. Schulenburg

Introduction: Evolution is at the core of the current antibiotic crisis. The enormous potential of bacteria to adapt also applies to pathogens infecting the cystic fibrosis lung, including *Pseudomonas aeruginosa*, which shows an increasing number of resistances against diverse antibiotic drugs. Therefore, evolutionary principles should be considered for the development of novel sustainable antibiotic treatment strategies.

Methods: We here use the approach of experimental evolution under controlled laboratory conditions in order to assess the efficacy of alternative antibiotic treatment strategies to minimize resistance evolution. These include combination and also sequential treatment protocols.

Results: Fast sequential protocols show very high efficacy in both constraining bacterial growth and also antibotic resistance evolution. These protocols often lead to extinction of bacterial populations, even if used at sub-lethal concentrations.

Conclusion: Fast sequential protocol produce highly dynamic selective environments, which make it very difficult for bacteria to adapt. Therefore, they are able to limit resistance evolution to a larger extent than alternative protocols such as monotherapies or combination treatments. In consequence, they offer a promising treatment option to constrain Pseudomonas infections in cystic fibrosis patients in vivo.

Non-allergic Aspergillus bronchitis in cystic fibrosis

Author: A. Jung

Introduction: A 11 year-old girl with classical CF (Q525X homozygos, pancreatic insufficient, mild purulent pneumopathy, PA neg.) and a stable lung function over years showed prolonged loss of FEV_1 from >100 % to 66 % over a period of several months without clinical sings of pulmonary exacerbation and only very mild increase in sputum and cough. FEV1 did not recover substantially after i.v. antibiotivs, intensified secretolytic inhalation and pyhsiotherapy. Serology and CT scan for ABPA was negative.

Methods: The patient was subjected to an explorative bronchosyopy with BAL.

Results: Bronchoscopy showed no signes of inflammation and no purulent secretion. However, bilaterally in the peripheral airways, extremely sticky mucus plugs were observed. The plugs were very difficult to remove by recurrent lavage and suction, and showed configuration of typical bronchial casts. Microbiology showed growth of *S. aureus* and *A. fumigatus*. Histology of the casts confirmed *aspergillus mycelia*, leading to the diagnosis of aspergillus bronchitis. After a second bronchoscopic session 2 weeks later with removal of the remainign casts and a six weeks antimycotic treatment with voriconazole, FEV1 was fully recovered to 110% pred.

Conclusion: Aspreguillus bronchitis without sensitization or ABPA can occur in CF patients. Unclear loss of lung function unresposnive to antibiotics should induce bronchoscopic evaluation. Evidence of *aspergillus mycelia* in the BAL fluid confirms diagnosis.

Scedosporium/Lomentospora infections of the lung in Cystic Fibrosis – therapeutic strategies

Authors: C. Schwarz, C. Brandt, P. Bouchara, K. Tintelnot

Background Cystic fibrosis (CF) is a rare disease, characterised by chronic airway infection with bacteria and fungi. *Scedosporium* species and *Lomentospora prolificans* are the most detected molds in respiratory samples in patients with CF next to *Aspergillus* spp.. Little is known about pathogeniceity of fungal species colonizing the airway in patients with CF and how to treat patients with highly probable invasive scedosporiosis.

Methods In this prospective multicentre study all patients (n=31) with fungal lung infections due to *Scedosporium* and *Lomentospora* spp. (n=31) were registered in the „German Network on *Scedosporium* spp. in CF" from January 2008, to December 2014. 12 centres took part in this study. A modified definition of probable fungal infection according to European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria was used/applied for including patients.

Results Pulmonary fungal infection with *Scedosporium* and *Lomentospora* spp. was found in 31 patients. *Scedosporium apiospermum* was isolated in 19, *Scedosporium boydii* in 10, *Scedosporium aurantiacum* in 3, *Lomentospora prolificans* in 3 cases and *Scedosporium minutispora* in 1 case. Single antifungal treatment was given in 7/31, double in 10/31 and triple in 14/31 patients. The median duration of the antifungal treatment was 3.9 month (range 4-28 month). In 20 from 31 antifungal courses a therapeutic response was achieved (regress in radiology, symptoms, or FEV1). All up to one responding patients had a combined treatment with a minimum of two antimycotic drugs.

Conclusions *Scedosporium* and *Lomentospora spp.* are resistant fungi, a combined anti-fungal treatment should be started for at least >4 weeks. In some cases a much longer therapy is required, e.g. 6 month.

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