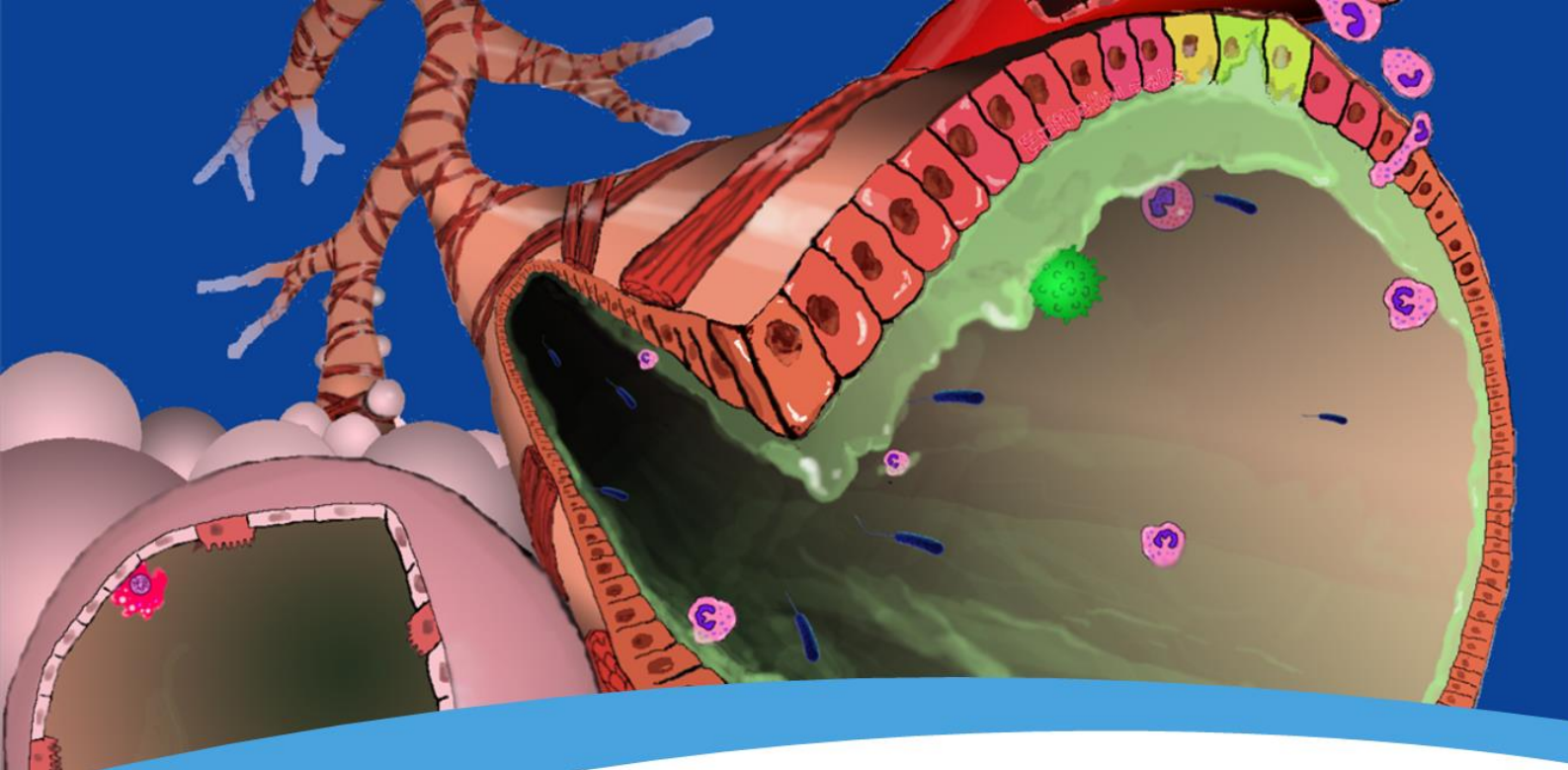

MUKOVISZIDOSE
Mukoviszidose Institut gGmbH

18th Scientific Meeting

Anti-infective and anti-inflammatory strategies to modulate the CF-microbiome

27th – 28th September 2018

Hotel Badehof in Bad Salzschlirf/Fulda (Germany)

Organization

Scientific advisory board of the German Research Community for Cystic Fibrosis (FGM) & Mukoviszidose Institute gGmbH (MI)

Chairs

Olaf Eickmeier (DE/Frankfurt)

Barbara Kahl (DE/Münster)

Burkhard Tümmler (DE/Hannover)

Table of content

*Not all talks have a published abstract.

Program

Thursday, September 27th

11:30-12:20 pm	*Get together + Lunch*
12:30-12:40 pm	**Opening of the meeting + short introduction**
	Manfred Ballmann (Chairman of the supervisory board Mukoviszidose Institute)
12:40-2:35 pm	**Session I** *Moderation: Olaf Eickmeier (DE/Frankfurt), Burkhard Tümmler (DE/Hannover)*
12:40-1:30 pm	**Lucas Hoffman (USA/Seattle)** From stem to stern: How a pulmonologist came to admire the CF fecal microbiome
1:30-2:20 pm	**Michael Tunney (GB/Belfast)** Manipulating the airway microbiota in people with CF
2:20-2:35 pm	**Katarzyna Pienkowska (DE/Hannover)** – *submitted abstract* The cystic fibrosis airways metagenome
2:35-3:00 pm	*Coffee break + free discussion*
3:00-5:00 pm	**Session II** *Moderation: Manfred Ballmann (DE/Rostock), Michael Hogardt (DE/Frankfurt)*
3:00-3:50 pm	**Rolf Müller (DE/Saarbrücken)** Post-genomic approaches towards innovative microbial natural product antibiotics
3:50-4:30 pm	**Michaela Lackner (AT/Innsbruck)** New insight in the antimicrobial efficacy of *N-chlorotaurin*
4:30-4:45 pm	**Claudia Neumann (DE/Münster)** – *submitted abstract* Unraveling antibiotic resistance mechanisms and dynamics of resistant *Staphylococcus aureus* isolates during chronic airway infection in CF patients
4:45-5:00 pm	**Mirjam Stahl (DE/Heidelberg)** – *submitted abstract* Influence of a preventive inhalation therapy on the airway microbiome in CF
5:00-5:30 pm	*Coffee break + free discussion*
5:30-7:00 pm	**Keynote-Session** *Moderation: Anna-Maria Dittrich (DE/Hannover), Barbara Kahl (DE/Münster)*
	Stefanie Widder (AT/Wien) The complex lung microbiome in persons with CF
8:00 pm	*Dinner*

Friday, September 28th

8:00- 9:10 am	**Session III** *Moderation: Barbara Kahl (DE/Münster), Helge Hebestreit (DE/Würzburg)*
8:00-8:40 am	**Susanne Häußler (DE/Hannover)** Application of "Omics" to treat *Pseudomonas aeruginosa*
8:40-8:55 am	**Sébastian Boutin (DE/Heidelberg)** – *submitted abstract* Opposing effects of cystic fibrosis airway microbiota dominated by *Pseudomonas aeruginosa* or *Prevotella melaninogenica* on airway inflammation and neutrophil activity
8:55-9:10 pm	**Julian Pott (DE/Homburg)** – *submitted abstract* Serum endotoxin levels are increased in cystic fibrosis
9:10-9:30 am	*Coffee break + free discussion*
9:30-11:30 am	**Session IV** *Moderation: Olaf Eickmeier (DE/Frankfurt), Michael Hogardt (DE/Frankfurt)*
9:30-10:20 am	**Marcus Mall (DE/Berlin**) Modulation of inflammation in CF
10:20-11:10 am	**Valerie Urbach (FR/Paris)** Specialised Proresolving Mediators regulate epithelial ion transport and inflammation in cystic fibrosis airways
11:10-11:30 am	**Burkhard Tümmler (DE/Hannover)** Ibuprofen - never forget!
11:30-12:00 am	*Coffee break + free discussion*
12:00-1:25 pm	**Session V** *Moderation: Helge Hebestreit (DE/Würzburg), Burkhard Tümmler (DE/Hannover)*
12:00-12:40 pm	**Petra Bacher (DE/Berlin)** Fungus-reactive CD4+ T cells as specific diagnostic sensors for fungus-mediated diseases in CF patients
12:40-12:55 pm	**Dario Frey (DE/Heidelberg)** – *submitted abstract* Monitoring of free and surface-bound protease activity in airway secretions from patients with cystic fibrosis using FRET sensors
12:55-1:10 pm	**Susanne Dittrich (DE/Heidelberg)** – *submitted abstract* Relationship of interleukin-1 with inflammation, structural lung damage and bacterial infection in early CF lung disease
1:10-1:25 pm	**Matteo Guerra (DE/Heidelberg)** – *submitted abstract* Cathepsin G activity reporters detect lung inflammation by microscopy and flow cytometry
1:25 pm	**Closing of the Meeting**
1:40 pm	*Lunch*

Session I

From stem to stern: How a pulmonologist came to admire the CF fecal microbiome
Lucas Hoffman

Introduction: Compared with the respiratory tract, few studies have focused on the microbiology of the CF gastrointestinal (GI) tract. GI symptoms, including nutrient malabsorption, growth failure, and GI obstruction, are among the earliest and most severe CF manifestations. The CF and non-CF GI luminal environments likely differ, potentially impacting the microbiota. Most CF GI microbiome studies have focused on adults, who often receive antibiotics and other confounding therapies. By comparison, children with CF, particularly infants, are likely less impacted by these issues. We hypothesized that infants with CF have GI dysbioses compared with infants without CF that are (1) due to CFTR dysfunction and are (2) independent of confounders such as diet and antibiotics. As the GI microbiota play important roles in nutrient harvest, vitamin levels, GI health, and growth, all of which relate to long-term health, the CF GI microbiota represents a potential, modifiable target for improving diverse early outcomes.
Methods: We performed shotgun metagenomic sequencing of fecal samples from two studies of infant nutrition: The 28-center Baby Observational and NUtrition Study (BONUS, 231 infants with CF studied during the first year of life), and the companion Healthy Infants Study (25 infants without CF with study visit schedule paralleling BONUS). Samples were collected at 2-3, 4, 6, 9-10, and 12 months. Taxonomic profiles were inferred computationally using Metagenomic Phylogenetic ANalysis (MetaPhlAN).
Results: While differences between the control and CF infant fecal microbiota were small at 3 months of age, the average microbiota of the two groups became progressively distinct. These differences were largely characterized by the relative depletion of Bacteroidetes and Firmicutes spp., and the enrichment of Proteobacteria, in CF microbiota. Development of CF microbiota appeared to lag behind that of the controls, resulting in predicted differences in health-associated microbiota functions. These taxonomic differences could not be explained by differences in diet (including breast versus formula feeding or table food), treatments (including antibiotics) or geographical location.
Conclusion: These findings indicate that infants with CF have progressive fecal dysbioses impacting the abundances of important bacterial taxa that are likely due to CFTR dysfunction rather than influences such as diet or medications. Many taxa depleted in the CF microbiota, such as Bifidobacterium, Roseburia, Faecalibacterium, Eubacterium and Bacteroides spp., are known to be important for GI health, nutrient harvest from undigested food, and somatic growth. Conversely, several of the taxa enriched in CF samples, particularly Proteobacteria, are associated with GI inflammation and are potential pathogens. These results provide a critical baseline upon which the relationships between CF GI microbiomes and clinical outcomes, including somatic growth and intestinal function, can be determined.
This study was supported by the National Institutes of Health and the Cystic Fibrosis Foundation.

Manipulating the airway microbiota in people with CF

Michael Tunney

Introduction: It has been suggested that disruption of the 'normal' airway microbiota and dominance of recognised pathogens is responsible for progression of lung disease in people with CF.

Methods: We have performed next generation sequence analysis on samples from a range of clinical studies, which demonstrate the effect of treatment on the airway microbiota. Data will be presented to demonstrate the effect of age, antibiotic treatment and treatment with the CFTR potentiatior, ivacaftor, on the airway microbiota.

Results: For patients with an exacerbation, significant changes in lung function and microbial community composition were observed following antibiotic treatment. However, overall community structures were resilient over time, generally returning to pre-treatment status by day 28 and 3 months post-treatment. Treatment with ivacaftor was associated with a reduction in the relative abundance of Pseudomonas species and an increase in the relative abundance of bacteria associated with more stable community structures.

Conclusion: The airway microbiota is resilient over time but does change with treatment, which suggests that strategies could be employed to prevent or reverse microbiota evolution.

The cystic fibrosis airways metagenome

Katarzyna Pienkowska, Margaux Gessner, Patricia Morán Losada, Rebecca Hyde, Christin Arnold, Silke Hedtfeld, Marie Dorda, Lutz Wiehlmann, Jochen G. Mainz, Burkhard Tümmler

Introduction: Chronic bacterial airway infections determine the outcome in cystic fibrosis (CF). Although upper and lower airways are inhabited by polymicrobial communities, clinical practice is driven by the identification of aerobic pathogens, mainly *Staphylococcus aureus* and *Pseudomonas aeruginosa*. We analysed the composition of the bacterial metagenome in respiratory secretions to unravel associations between the bacterial microbiome and CF disease status.

Methods: The bacterial metagenome was analysed by sequencing of total DNA isolated from nasal lavage, oropharyngeal swab and induced sputum collected from CF children and adults. The patient's clinical status was represented by age- and gender-corrected disease centiles. Associations between metagenome composition, inflammatory and clinical parameters were assessed by unsupervised analyses.

Results: 194 metagenome datasets obtained from 71 CF patients identified disease-associated bacterial communities in upper and lower airways, but a normal bacterial flora in the oral cavity. Microbial load in the three habitats was not associated with disease severity. Firmicutes were the dominant phylum in pancreatic sufficient patients whereas Proteobacteria had a larger share in pancreatic insufficient patients. Four distinct groups distinguished by either *S. aureus*, *P. aeruginosa*, *Haemophilus influenzae* or anaerobes as the prevailing bacteria, could be discerned in the sputum metagenome of all age groups. *Prevotella melaninogenica* was recovered more often and at higher abundance in nasal lavage and sputum from CF patients with mild disease. According to random forest analysis the two most common known CF pathogens *S. aureus* and *P. aeruginosa* on one hand and *Veillonella parvula*, *Rothia mucilaginosa* and oral streptococci on the other were decisive for branches and nodes of the tree. Airway levels of interleukins and neutrophilic enzymes were associated with metagenome composition of samples from PI, but not from PS patients.

Conclusion: This so far most comprehensive metagenome study in three cystic fibrosis airway habitats provided insights into the association of microbiome with disease status suggesting that in-depth metagenome analysis of respiratory secretions should be implemented into clinical practice.

Session II

New insight in the antimicrobial efficacy of N-chlorotaurine

H. Leiter, M. Nagl, S. Dollinger, J. Gostner, M. Lackner

Introduction: N-chlorotaurine (NCT) is an endogenous long-lived oxidant that can be used as an antiseptic in different body regions. Recently, tolerability of inhaled NCT has been demonstrated in humans so that it is of interest for future treatment of cystic fibrosis. In the present study, we tested the bactericidal and fungicidal activity of NCT in a lung cell culture model.
Methods: Bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa*) and fungi (*Candida albicans, Exophiala sp.*) were added to monolayers of lung epithelial cells, and after 4 h NCT was added. After different incubation times, aliquots from extracellular fluid as well as from intracellular one after lysis of the cells with NP-40 were removed and quantitative cultures were performed.
Results: NCT at the therapeutically applied concentration of 1% (55 mM) killed the test pathogens within 15-30 min. Killing by 0.3% NCT lasted up to 4h dependent on the pathogen. 0.1% NCT was the threshold concentration for killing since this amount of oxidation capacity was consumed by reactions with the organic compounds of the medium within 30 min.
Conclusion: NCT in therapeutic concentration demonstrated its microbicidal activity in the presence of lung epithelial cells.

Unraveling antibiotic resistance mechanisms and dynamics of resistant *Staphylococcus aureus* isolates during chronic airway infection in CF patients

Claudia Neumann, Lena Ueberhorst, Theo Tißen, Björn Husmann, Barbara Grünastel, Johanna Sobocinski, Susanne Deiwick, Heike Rengbers, Barbara C. Kahl

Introduction: *Staphylococcus aureus* is one of the first and most frequent pathogens recovered from the airways of cystic fibrosis (CF) patients with increasing prevalence rates due to early eradication strategies of *Pseudomonas aeruginosa*. A rising number of multidrug resistant bacteria has been observed and antimicrobial resistance pattern of the bacteria involved in respiratory infections of CF patients is of increasing concern. In CF lungs, the bacteria can cause long-term infections despite appropriate antibiotic therapy through adaption to this specific niche by various mechanisms including phage insertions and/or exclusions, genome rearrangements, loss of agr activity, emergence of small colony variants (SCVs) and mucoid isolates.
Methods: We conducted a prospective one-year observational study, including 14 CF patients with chronic *S. aureus* airway infection with the aim to investigate the diversity and resistance pattern of *S. aureus* in this special habitat by randomly picking 40 *S. aureus* isolates, which were characterised by phenotypic evaluation (size, hemolysis, mucoidy), genotyping (spa-sequence typing) and susceptibility testing.
Results: We were able to analyze 2.320 *S. aureus* isolates from 58 visits of 14 CF patients. We observed a high diversity of phenotypes and resistotypes of *S. aureus* within single sputa, which changed during the observation period partly dependent on clinical status and antibiotic treatment. We noticed that in patients, which were infected only by susceptible *S. aureus* strains up to 4 different clonal lineages co-existed and all these different clonal lineages persisted. In contrast, during the observation period in some patients resistant *S. aureus* isolates outcompeted isolates with other spa-types and these more resistant *S. aureus* strains persisted over the study period.
Conclusion: A high diversity of *S. aureus* phenotypes and resistotypes could be observed during our prospective one-year observational study. In the future, we plan to analyze the dynamics and mechanisms of resistance and the association of different *S. aureus* strains with distinct clinical manifestations.

Keynote-Session

The complex lung microbiome in persons with CF

Stefanie Widder

Introduction: The lung microbiome in CF is a dynamical, evolving ecosystem that displays classic features of a complex system. It shows temporal changes in composition, is spatially stratified with regard to the alveolar microenvironment and microbial interactions generate an ecological dependency structure in the community. Therefore, emergent disease dynamics of lung inflammation are complex and despite highest importance for the individual disease trajectory, exacerbations are difficult to predict. We want to identify the most informative level, whether compositional, functional or interactional and try to better understand the role of interactions within the microbiota and with the host during exacerbation episodes.

Methods: We use 16S and metagenomic sequencing data derived from large sputum collections and apply complex systems theory, network science and modeling techniques.

Results: Comparing the airway microbiome during clinical baseline symptoms or acute exacerbation, reveals shifts in taxa dominance, interaction architecture and importantly - metabolic activity of the community. In particular, fermentative products and other acids are generated that likely cause a local pH decrease promoting competitive dynamics. We identified temporally changing dysbiosis patterns that are characterized by distinct metabolic profiles. We and others hypothesize that stable microbiota communicate with host immunity by small metabolites and that shifts in the metabolic profiles effectively trigger overshooting immune response such as occurring in exacerbation.

We furthermore show that knowing the interaction structure can from the basis for the prediction of drug targets and that recurrent microbiome patterns correlate with clinical parameters such as FEV_1, disease state or stage. Based on these insights we are furthering computational models for the prediction of disease aggravation.

Conclusion: Interactions, both among microbiota and with the human host, are crucial for understanding emergent disease dynamics that lead to inflammation exacerbation and irreversible lung function decline in CF. Theoretical and clinical expertise is required to identify the principles of microbiome-host dynamics to avoid exacerbations and prolong patients' lives.

Session III

Application of "Omics" to treat *Pseudomonas aeruginosa*

Ariane Khaledi, Janne Thöming, Matthias Preuße, Susanne Häussler

Introduction: Since diagnosis of resistance prior to antibiotic treatment is essential to guide clinicians in their choice of anti-infective therapy, rapid results on antibiotic susceptibility are mandatory. Increasing rates of biofilm associated infections, emergence of multi-drug resistance and the urgent need of a timely reporting of antibiotic resistance calls for the development of modern molecular diagnostics for rapid detection of resistance.

Methods: With the aim to identify novel genetic determinants of antibiotic resistance and biofilm formation in *Pseudomonas aeruginosa* we have recently screened a large panel of clinical isolates for resistance profiles towards several antibiotics and the capability to produce biofilms.

Results: From these studies it has become clear that many previously unidentified genes play a role in antimicrobial resistance and biofilm formation and that, depending on the antibiotic; there are many modifiers of the expression of resistance in *P. aeruginosa*. We will use extensive whole-genome sequencing approaches with the aim to identify novel genetic markers of antibiotic resistance and biofilm associated phenotypes via genome-wide association studies in clinical isolates.

Opposing effects of cystic fibrosis airway microbiota dominated by *Pseudomonas aeruginosa* or *Prevotella melaninogenica* on airway inflammation and neutrophil activity

Sébastien Boutin, Susanne A. Dittrich, Dario L. Frey, Sabine Wege, Marcus Mall, Alexander Dalpke

Introduction: In the last decades, the complexity of cystic fibrosis (CF) airways microbiota just started to get explored. Diversity of the microbiome is correlated with lung function, aging and severity of the disease. The aim of this study was to explore the relationship between the microbiome of CF adult patients and pro-inflammatory cytokines and neutrophils recruitment.

Methods: Using 16S amplicon sequencing targeting V4, we analyzed sputum from 77 adult patients during periods of clinical stability. Sequences were processed with the package DADA2 to ensure a good estimation of the error rate of each run, to trim the reads for good quality (1 estimate error allowed per read) and to remove chimeras. Each ribosomal sequence variant (RSV) was then classified at the taxonomic level using the silva database v132. In parallel to the microbiome exploration, sputum samples were used for cytokine measurements (IL8, TNFalpha, IL1beta) by ELISA, neutrophils were counted and neutrophil activity was measured by a novel technology employing the FRET probe 'nemo1'.

Results: We observed a significant negative correlation between IL-8 concentration and the alpha-diversity (p-value<0.05) as well as community's evenness (p-value<0.05). The diversity of the microbiome was also negatively correlated with the neutrophils elastase activity measured in the supernatant of the sputum. Furthermore, bacterial richness was negatively correlated with the absolute number of neutrophils/mL measured in the sample. We also correlated the most abundant RSV with the cytokines levels and observed that only two RSV were statistically significantly associated with cytokines levels: One RSV classified as *Pseudomonas aeruginosa* was positively correlated with IL-8 while the other RSV classified as *Prevotella melaninogenica* was negatively correlated with TNFalpha levels. To evaluate the relationship between microbiome's structure and cytokine levels we clustered the population based on hierarchical clustering method. We obtained 7 different microbiome types (ecotypes). One ecotype was characterized by a rich microbiome and the high prevalence of anaerobic bacteria including *Prevotella melaninogenica* while the others were characterized by the dominance of typical (*Pseudomonas aeruginosa,* Staphylococcus) or atypical pathogens (Mycoplasma, Stenotrophomonas). The ecotype showing the highest abundance of *Pseudomonas aeruginosa* was associated with a higher level of IL8 and IL1beta as well as a higher neutrophils elastase activity compared to the ecotype characterized by the presence of anaerobes.

Conclusion: Our results show that the high abundance of *P. aeruginosa* in the sputum correlates to more pro-inflammatory cytokines and more neutrophils recruitment and activity. This phenomenon was not observed with *S. aureus* dominance indicating a Pseudomonas specific impact. Our results also argue in favor of a decrease of the inflammatory cytokine secretion in presence of high abundance of anaerobes, especially from the genus Prevotella.

Serum endotoxin levels are increased in cystic fibrosis

Julian Pott, Eva Papatsanis, Christian Herr, Christoph Beißwenger, Angelika Krill, Heinrike Wilkens, Robert Bals

Introduction: Lung disease in cystic fibrosis (CF) is a chronic infectious disorder. The composition of the lung microbiota / microbiome has been studied in detail. It is common belief that the microorganisms are restricted to the lung in a chronic stage and bacteraemia or translocation of bacterial components is a rare event. The aim of the study was to measure the levels of LPS in serum in patients with CF.
Methods: 27 patients with CF and 32 healthy individuals were recruited within the PULMOHOM study, a prospective, observational cohort study in inflammatory lung disease. Clinical data and serum was samples and the serum LPS concentration was measured by a commercial Limulus Amebocyte Lysate assay (GenScript, USA) that was modified to detect LPS in serum from human and murine source.
Results: LPS levels were significantly increased in the serum of patients with CF as compared to healthy controls (0,851 vs. 0,100 EU/ml, P = 0.000). LPS was not associated with other parameters of systemic inflammation such as CRP, IL-8 or fibrinogen.
Conclusion: LPS levels are significantly increased in patients with CF. This highlights the systemic nature of CF lung disease and provides a novel mechanisms how pulmonary colonization related to systemic inflammation.

Session IV

Specialised Proresolving Mediators regulate epithelial ion transport and inflammation in cystic fibrosis

Valerie Urbach

The acute inflammatory response is host-protective to contain foreign invaders, which is normally self-limited to avoid tissue damage. The resolution of acute inflammation was initially thought to be a passive process and that inflammatory mediators would just simply dilute and dissipate to stop the infiltration of leukocytes into the tissues. However, studies performed on mice inflammatory exudates revealed that self-limited acute inflammatory responses involves an active resolution phase designed to restore tissue homeostasis, carried out by the actions of specialized pro-resolving mediators (SPMs) such as lipoxin, resolvins and maresins. The abnormal production and/or action of SPM is now considered as a pathophysiologic basis associated with widely occurring inflammatory diseases including cystic fibrosis.

In Cystic Fibrosis, the altered hydration of the airway surface (ASL) and mucociliary clearance that favors chronic bacterial colonization, persistent inflammation and progressive lung destruction is classically explained by ion transport abnormalities directly related to CFTR mutation. However, intrinsic abnormalities of the inflammatory response in CF has been suggested. Several groups including ours have reported an abnormal production of Lipoxin A4 (LXA4) in the airways of individual with CF that could play a central role in the pathophysiology of CF airway disease.

We demonstrated that LXA4 and resolvin D1 (RvD1) regulate airway epithelial function that are altered in CF. These SPMs stimulate CFTR-independent chloride secretion in vitro, in human CF airway epithelial cells, and in vivo, in CF mice, and restores the ASL high in human CF airway epithelium. LXA4 also delays colonisation of airway epithelium by *Pseudomonas aeruginosa*. LXA4 stimulates epithelial repair and tight junction formation of CF airway epithelium. RvD1 decreased TNFalpha induced IL-8 secretion and enhanced the phagocytic and bacterial killing capacity of human CF alveolar macrophages enhanced macrophages.

Session V

Fungus-reactive CD4+ T cells as specific diagnostic sensors for fungus-mediated diseases in CF patients

Petra Bacher, Thordis Hohnstein, Eva Beerbaum, Marie Röcker, Svenja Kaufmann, Claudia Brandt, Jobst Röhmel, Ulrik Stervbo, Mikalai Nienen, Nina Babel, Julia Milleck, Mario Assenmacher, Oliver A. Cornely, Guido Heine, Margitta Worm, Petra Creutz, Christoph Ruwwe-Glösenkamp, Leif E. Sander, Olaf Kniemeyer, Axel A. Brakhage, Carsten Schwarz, Alexander Scheffold

Introduction: Patients with cystic fibrosis (CF) suffer from chronic infections of the lung, which determine morbidity and mortality. The contribution of individual pathogens to chronic disease and acute lung exacerbations are often difficult to determine due to the complex composition of the lung microbiome of CF patients. In particular, the relevance of fungal pathogens in CF airways remains poorly understood due to limitations of current diagnostics to identify the presence of fungal pathogens and to resolve the individual host-pathogen interaction status. T lymphocytes play an essential role in host defense against pathogens, but also in inappropriate immune reactions such as allergies. They have the capacity to specifically recognize, discriminate and react with a diverse array of effector functions against the different pathogens. Thus, the analysis of the antigen-specific T cell status of an individual can in principle provide detailed information about the actual antigen-host interaction status and may allow to sub-classify patients according to appropriate (protective) or inappropriate (pathology-associated) immune reactions.

Methods: Due to technical limitations, comprehensive information about the frequency and phenotype of rare antigen-specific CD4+ T cells against many disease-relevant antigens is missing. We used a highly sensitive enrichment system (Antigen-reactive T cell enrichment, ARTE) based on antigen-induced CD154 (CD40L) versus CD137 (4-1BB) expression, to detect human fungus-reactive conventional (Tcons) and regulatory (Tregs) T cells directly ex vivo in peripheral blood.

Results: Fungus-reactive CD4+ T cells are present in all healthy donors at low but conserved frequency ranges. In patients with CF, effector functions of fungus-reactive T cells are strongly altered, allowing to identify distinct response patterns according to fungus-reactive T cell cytokine production. In particular, a subgroup of patients with sensitization to *A. fumigatus* is not only characterized by Th2 cytokine production, but also increased Th17 responses, which strongly correlated with acute allergic bronchopulmonary aspergillosis (ABPA). Our data further identify innocuous airborne antigens, including airborne fungi, as a target of human Tregs and provide direct evidence that antigen-specific Tregs are potent suppressors of allergy development. We provide an explanation how allergen-specific Th2 responses can escape Treg control due to selective targeting of allergenic proteins not protected by a specific Treg response.

Conclusion: Our data show that antigen-specific CD4+ T cell responses can be used as a diagnostic read-out to define more precisely the interaction status of a patient with a specific pathogen and to enable an adapted therapeutic intervention.

Longitudinal monitoring of protease activity in airway inflammatory cells of Cystic Fibrosis patients using FRET sensors

Dario L. Frey, Susanne A. Dittrich, Iris Kühbandner, Aliaksandr Halavatyi, Matteo Guerra, Sabine Wege, Olaf Sommerburg, Carsten Schultz, Marcus A. Mall

Introduction: Neutrophil Elastase (NE) is a hallmark for the development and progression of cystic fibrosis (CF) lung disease. Free NE activity in sputum and bronchoalveolar lavage (BAL) fluid is identified as major risk factor causing decline in lung function and early stage bronchiectasis. Nowadays research focuses mainly to measure free NE activity in supernatants and BAL fluids. Within this study measurement of several clinical parameters like forced expiratory volume in 1 second (FEV_1) should be longitudinal compared to free and membrane bound protease activity as well as cytokine levels. Recently, lipidated FRET (Förster resonance energy transfer) reporters were developed which allow a detection of NE activity on cell surfaces.

Methods: So far the 156 spontaneous sputum samples of 83 CF patients (17 treated with CFTR modulator) and induced sputum of 7 healthy controls could be collected. Cells were isolated and separated from the supernatants; the membrane-associated NE activity was quantified with the FRET reporter NEmo-2E within 2h after cough up. The activity was calculated as donor/acceptor ratio and normalized to the samples treated with Sivelestat (an NE inhibitor). For cytokine measurements CBA and ELISA assays were used.

Results: A first subset of patients, not discriminating between exacerbated and stable patients, was correlated with cytokine measurements and clinical data. Soluble NE activity weakly correlates to Neutrophil elastase a-1 antitrypsin complex levels (NEAAT, rho=0.22, p=0.01, n=132), as well as strongly to the proteinase 3 and cathepsin G levels (PR3, rho=0.68, p<0.0001, n=60; CTSG, rho=0.74, p<0.0001, n=31). Besides that CTSG shows moderate correlations to several inhibitors (NEAAT, rho=0.39, p=0.026, n=33; SLPI, rho=-0.36, p=0.040, n=33). Proteinase 3 strongly correlates to inflammation markers IL-1ß and IL-8 (IL-1ß, rho=0.63, p=0.002, n=31; IL-8, rho=0.61, p=0.0002, n=31). Membrane-associated NE activity of a subset of CF patients moderately correlates with airflow limitation (FEV_1 predicted, rho=-0.48, p<0.01, n=37) and highly with air trapping (FRCpleth predicted, rho=0.61, p<0.01, n=23). First experiments measuring the membrane bound NE activity with the flow cytometer are strongly correlating with confocal analysis (rho=0.74, p=0.006, n=12). At the FACS the reporter showed an increased dynamic range, compared to microscopic measurement techniques.

Conclusion: The analysis of the first subset of patients is very promising. 41 patients were already measured more than once (2-6 times), we are aiming to get at least 3 longitudinal measurements of at least 40 different patients. A detailed analysis of the comparison between PR3, CTSG and NE has not been described for a comparable sized cohort in CF so far. The experimental set up of the membrane bound activity at the flow is more convenient and can easily been transferred either to other labs or even a clinical environment. The analysis is faster and the results are more comprehensive.

Relationship of interleukin-1 with inflammation, structural lung damage and bacterial infection in early CF lung disease

A. Susanne Dittrich, Samuel T. Montgomery, Luke W. Garratt, Lidija Turkovic, Dario L. Frey, Stephen M. Stick, Anthony Kicic, Marcus A. Mall, AREST CF

Introduction: Cystic fibrosis (CF) lung disease starts in the first month of live and is associated with mucus plugging and neutrophilic airway inflammation, even in absence of bacterial infection. Preclinical studies demonstrated that Interleukin-1 alpha (IL-1alpha) is released during hypoxic necrosis of airway epithelial cells and maintains sterile inflammation. In this study, we hypothesized that IL-1alpha is elevated in bronchoalveolar lavage fluid (BALf) from young children with CF prior to respiratory infection and associated with markers of neutrophilic inflammation and structural lung damage determined by computed tomography (CT).

Methods: Our study investigated 102 clinically stable children with CF (110 visits, mean age 3.8, range 0.16-7.81 years) participating in the Australian Respiratory Surveillance Team for CF (AREST CF). Respiratory infection status was determined by culture and BALf from uninfected and infected infants (n=51 and n=59) was assessed for IL-1alpha, IL-1beta, IL-8, absolute neutrophil counts per mL and neutrophil elastase (NE) activity. Extent of structural lung disease on CT was measured via the PRAGMA-CF score and associations with IL-1alpha, IL-1beta, IL-8, neutrophils and NE activity were investigated via multiple linear regression (adjusted for age and sex).

Results : IL-1alpha and IL-1beta were detectable in BALf from young children with CF in absence of detectable infection and were increased in presence of bacterial infection ($p<0.05$ and $p<0.01$). IL-1alpha and IL-1beta were correlated independent from bacterial airway infection (uninfected: $r=0.83$, $p<0.0001$ and infected: $r=0.62$, $p<0.0001$). Both, IL-1alpha and IL-1beta, were associated with IL-8 ($r=0.64$, $p<0.0001$ and $r=0.64$, $p<0.0001$), absolute neutrophil counts ($r=0.71$, $p<0.0001$ and $r=0.67$, $p<0.0001$) and NE activity ($r=0.26$, $p<0.01$ and $r=0.32$, $p<0.001$). When stratified by respiratory infection status, IL-1alpha, IL-1beta, IL-8, neutrophil count and NE activity were associated with the extent of CT-morphologic structural lung disease in children without respiratory infection. Of note, the association between IL-1alpha and the extent of structural lung disease on CT was the strongest association discovered (1.20 [0.33, 2.06], $p=0.008$).

Conclusion: Our results demonstrate that IL-1alpha is detectable in airways of young children with CF and associated with neutrophilic inflammation and structural lung disease in absence of detectable airway infection. This indicates that IL-1alpha is an important modulator of sterile inflammation in early CF lung disease and might serve as target for anti-inflammatory therapies.

Cathepsin G activity reporters detect lung inflammation by microscopy and flow cytometry

Matteo Guerra, Dario Frey, Matthias Hagner, Marcus Mall, Carsten Schultz

Introduction: Cystic fibrosis (CF) elicits a massive neutrophilic infiltration into the lungs. This is also promoted by neutrophil serine proteases (NSP) such as cathepsin G (CG), neutrophil elastase (NE) and proteinase 3 (P3), versatile enzymes secreted for permitting penetration of the extracellular matrix. However, on the surface of the secreting neutrophil, NSPs are shielded from antiproteases and are able to do major damage to the connective tissue. So far, little is known about the function of CG in CF, especially regarding its membrane-bound fractions. Hence, the development of activity reporters as well as clinically applicable tools to interrogate patient sputum samples is necessary.
Methods: We synthetized two new ratiometric FRET reporters via solid phase peptide synthesis (SPPS). The probes have been fully characterized in vitro by fluorescence spectroscopy and in vivo via confocal microscopy. Furthermore, we used the reporters to assess CG activity in sputum from patients with CF. Finally, we translated the application of a small molecule FRET reporter into the flow cytometry technology.
Results: We present a new pair of FRET reporters: Sol-SAM measures CG activity in patient sputum supernatants, while Lip-SAM is a lipidated CG reporter that binds to cell membranes where reports on membrane-bound protease activity. We analyzed the sputum of 21 patients (7 healthy non-smokers donors, 14 CF) by microscopy and showed that surface-bound CG is highly activated in lung inflammation. While we did not detect any enzymatic activity in the control cohort, CF neutrophils showed a 1.4- to 4.9- fold increase in CG activity. We also employed Sol-SAM to monitor CG activity present in patients fluids where it resulted highly activated compared to healthy ones. Finally, we applied Lip-SAM in flow cytometric energy transfer (FCET). We quantified the average donor/acceptor ratio for 6 subjects (5 CF and 1 healthy) measured by FCET and obtained a significant correlation ($rho = 0.93$; $p = 0.0076$) with the same samples analyzed by microscopy.
Conclusion: Our findings point at cathepsin G as a new possible player in the pathogenesis of CF. We envision that the introduction of small molecule FCET technology will speed up medical studies and promote the use of FRET reporters into the clinical routine.

Speakers

Bacher, Petra
Charité - University Hospital
Berlin (Germany)
petra.bacher@charite.de

Boutin, Sébastien
University Hospital Heidelberg
Heidelberg (Germany)
sebastien.boutin@med.uni-heidelberg.de

Dittrich, Susanne
University Hospital Heidelberg
Heidelberg (Germany)
susanne.dittrich@med.uni-heidelberg.de

Frey, Dario L.
University Hospital Heidelberg
Heidelberg (Germany)
dariolucas.frey@med.uni-heidelberg.de

Guerra, Matteo
University Hospital Heidelberg
Heidelberg (Germany)
matteo.guerra@embl.de

Häußler, Susanne
Helmholtz Centre für Infection Research
Braunschweig (Germany)
susanne.haeussler@helmholtz-hzi.de

Hoffman, Lucas
Seattle Childrens's Hospital
Seattle (USA)
lhoffm@uw.edu

Lackner, Michaela
Medical University of Innsbruck
Innsbruck (Austria)
michaela.lackner@i-med.ac.at

Mall, Marcus
Charité - University Hospital Berlin
(Germany)
marcus.mall@charite.de

Müller, Rolf
Helmholtz-Institute for Pharmaceutical
Research Saarland (HIPS)
Saarbrücken (Germany)
rolf.mueller@helmholtz-hzi.de

Neumann, Claudia
Münster University Hospital
Münster (Germany)
cneumann@uni-muenster.de

Pienkowska, Katarzyna
Hannover Medical Scool
Hannover (Germany)
pienkowska.katarzyna@mh-hannover.de

Pott, Julian
Saarland University Medical Center
Homburg (Germany)
julian-pott@web.de

Stahl, Mirjam
University Hospital Heidelberg
Heidelberg (Germany)
mirjam.stahl@med.uni-heidelberg.de

Tunney, Michael
Queens University Belfast
Belfast (Great Britian)
m.tunney@qub.ac.uk

Urbach, Valérie
INSERM
Paris Cedex (France)
valerie.urbach@inserm.fr

Widder, Stefanie
Vienna General Hospital
Wien (Austria)
stefanie.widder@univie.ac.at

Chairs

Eickmeier, Olaf
University Hospital Frankfurt
Frankfurt/Main (Germany)
olaf.eickmeier@kgu.de

Kahl, Barbara
Münster University Hospital
Münster (Germany)
kahl@uni-muenster.de

Tümmler, Burkhard
Hannover Medical Scool
Hannover (Germany)
tuemmler.burkhard@mh-hannover.de

Moderators

Ballmann, Manfred
University Hospital Rostock
Rostock (Germany)
manfred.ballmann@rub.de

Dittrich, Anna-Maria
Hannover Medical School
Hannover (Germany)
dittrich.anna-maria@mh-hannover.de

Hebestreit, Helge
University Hospital Würzburg
Würzburg (Germany)
hebestreit_h@klinik.uni-wuerzburg.de

Hogardt, Michael
University Hospital Frankfurt
Frankfurt/Main (Germany)
michael.hogardt@kgu.de

The Meeting is supported by the
DFG (Deutsche Forschungsgemeinschaft)

and sponsored by:

Chiesi GmbH/3.000€; Novartis Pharma GmbH/3.000€; Teva GmbH/3.000€;
Vertex Pharmaceuticals (Germany) GmbH/3.000€